

Dysfonction vasculaire et conditions environnementales dans des modèles expérimentaux chez l'homme et l'animal

Asmaa Alameddine

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Dysfonction vasculaire et conditions environnementales dans des modèles expérimentaux chez l'homme et l'animal

JURY

Rapporteurs :	**Ramez CHAHINE**, Professeur d'Université, Université Libanaise, Beyrouth, Liban
	Claude GHARIB, Professeur d'Université – Praticien Hospitalier, Université Claude Bernard, Lyon
Examinateurs :	**Daniel HENRION,** Directeur de Recherche, Université d'Angers
	Guillemette GAUQUELIN-KOCH, PhD, HDR, CNES, Paris
Directeur de Thèse :	**Marc-Antoine CUSTAUD**, Professeur d'Université – Praticien Hospitalier, Université d'Angers
Co-directeur de Thèse :	**Ziad FAJLOUN**, Professeur d'Université, Centre Azm, Tripoli, Liban

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LISTE DES ABREVIATIONS

Ach: Acéthylcholine

AGEs: Advanced glycation end products

AHA: American heart association

AMP: Adenosine monophosphate

AngII: Angiotensin II

AT2R: Angiotensin type 2 receptor

BK: Bradykinin

BP: Blood pressure

$CaCl_2$: Calcium chloride

$CaCO_3$: Carbonate de sodium

CD3OD: Deuterated methanol

CEC: Circulating endothelial cells

cGMP: Guanosine cyclic 3′,5′-monophosphate

CHM: Chinese herbal medicine

CO_2: Carbone dioxide

CRP: C-reactive protein

CRC: Concentration response curve

CVD: Cardiovascular disease

COX2: Cyclo-oxygenase

DBP: Diastolic blood pressure

DI: Dry immersion

DM: Diabetic mellitus

DT2: Diabetic mice

EDHF: Endothelium-Derived Hyperpolarizing Factor

ER α: Estrogen receptor alpha

ER β: Estrogen receptor beta

E2: 17-βoestradiol

eNOS: Endothelial nitric oxide synthase

EMP: Electromagnetic pulse

EMPs: Endothelium-derived microparticles

FMD: Flow mediated dilation

GK: GotoKakizaki rat model

GLUT-4: Glucose transporter type 4

HDBR: Head down bed rest

HDL: High-density lipoprotein

HFD: Hight fat diet

HPLC: Hight performance liquid chromatography

HR: Heart rate

ICAM-1: IntercellularAdhesionMolecule 1

IL1B: Interleukin 1 beta

IL-6: Interleukin-6

IMT: Intima-media thickness

KCl: Potassium chloride

KH_2PO_4: Monobasic potassium phosphate

LBNP: Lower body negative pressure

LDL: Low-density lipoprotein

$MgSO_4$: Magnesium sulfate

$NaHCO_3$: Sodium bicarbonate

NADPH oxidase: Nicotinamide adenine dinucleotide phosphate-oxidase

NA^+: Sodium ion

NaCl: Sodium chloride

NO: Nitric oxide

OGTT: Oral glucose tolerance tests

OVX: Ovariectomized mice

PGI2: Prostacyclin

PKc: Protein kinase C

PPS: Physiological salt solution

RAS: Renin-Angiotensin system

RVE: Resistive vibration exercise

ROS: Reactive oxygen species

SBP: Systolic blood pressure

SCI: Spinal cord-injured

SE: Standard error

SNP: Sodium nitroprusside

TNFα: Tumor necrosis factor alpha

ULLS: Unilateral lower limb suspension

VD: Vasodilation

VEGF: Vascular endothelium growth factor

VO_2 max: Maximal oxygen consumption

VSMC: Vascular smooth muscle cells

WBV: Whole body vibration

Wky: Wsitar Kyoto rat

Le contexte général

L'homme par sa nature cherche à découvrir le caché, le mystérieux d'où son intérêt à explorer l'espace. Le vol spatial prend son essor à la fin de la Seconde Guerre mondiale grâce aux avancées allemandes dans le domaine des fusées et il donne lieu à plusieurs évènements retentissants durant la seconde moitié du xx^{e} siècle. En 1957, le programme Vostok visant l'envoi de l'homme dans l'espace, fut démarré permettant le premier voyage de l'homme dans l'espace par Youri Gagarine le 12 avril 1961.

Jusqu'à présent, beaucoup de missions spatiales habitées avec des objectifs scientifiques mais aussi politiques, ont eu lieu ou sont en cours de réalisation. Ces missions habitées font aussi l'objet d'une coopération internationale nécessaire.

Les études menées aujourd'hui ont pour but de comprendre et d'affronter les conséquences négatives de l'absence de gravité sur la physiologie des astronautes durant leur séjour dans l'espace, d'où l'importance de bien comprendre l'environnement spatial. La gravité qui agit sur l'homme lorsqu'il est sur terre est la résultante de l'interaction entre la terre et le corps humain. Dans l'espace, la gravité diminue jusqu'à presque s'annuler en s'éloignant de la terre et le corps de l'astronaute se trouve en état de microgravité.

Le vol spatial procure un environnement d'isolement où plusieurs facteurs se combinent pour agir sur les différentes fonctions de l'organisme créant des risques physiologiques et psychiques divers. Les principaux facteurs liés à l'environnement spatial et induisant une adaptation physiologique sont l'absence de gravité, le confinement et l'effet stress lié à la mission ainsi que l'absence de zone d'appui et la privation sensorielle en général. Les radiations dans l'environnement spatial, par leur dangerosité à court et à long terme, doivent faire l'objet d'une protection particulièrement efficace pour les astronautes.

Parmi ces facteurs intéressons nous tout particulièrement à l'absence de gravité et plus précisément à la microgravité (les astronautes sont soumis à une gravité extrêmement réduite mais non nulle). Cette microgravité va être à l'origine de modifications très importantes au niveau de l'organisme de l'astronaute touchant tous ses systèmes de régulation (Lane et al., 1998). D'une façon générale cette microgravité va induire des transferts liquidiens vers la partie thoraco-céphalique, une diminution des contraintes physiques appliquées à l'organisme et une diminution de l'activité physique combinant une hypokinésie (restriction des mouvements du fait de l'espace réduit) et une hypodynamie (diminution de l'activité musculaire tonique liée au maintien de la posture) (in Hypokinesia and

Weightlessness, Clinical and Physiologic Aspects 1992, Atkov and Bednenko). La microgravité est ainsi accompagnée par une inactivité physique poussée qui participe aux changements physiologiques touchant les astronautes.

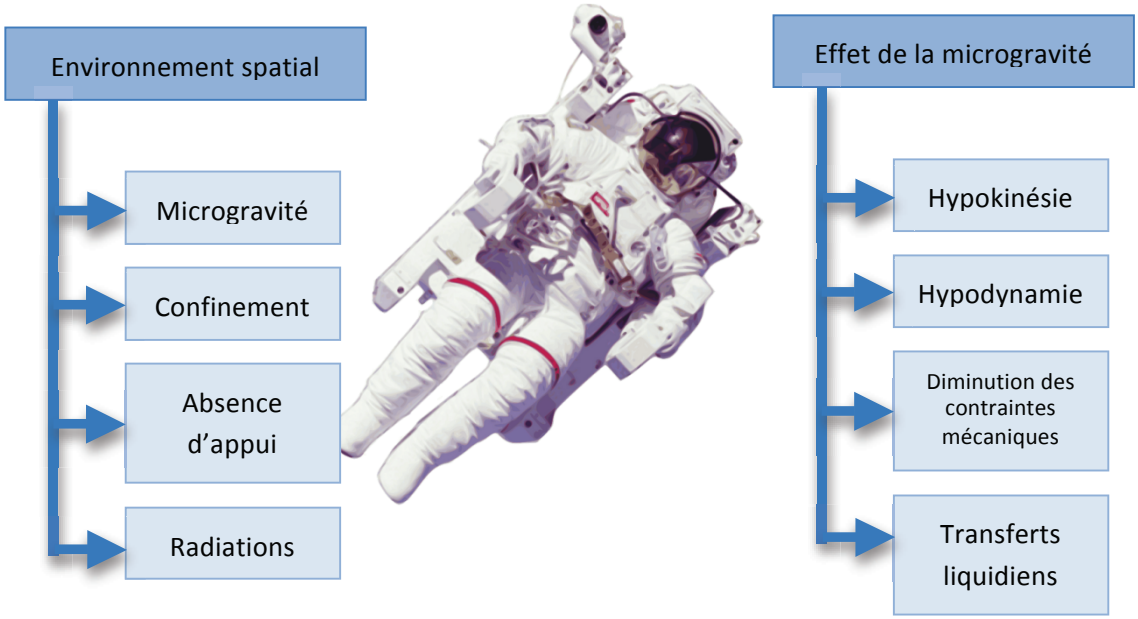


Figure 1 : *Environnement spatial, microgravité et conséquences sur l'astronaute aboutissant à une modification des grandes fonctions de l'organisme*

Beaucoup de modèles humains et animaux sont utilisés pour comprendre les altérations physiologiques liées à l'environnement spatial, de ces modèles nous pouvons citer :
Les modèles humains : modèle de décharge unilatérale d'un membre inférieur (Berg et al., 1991) , alitement anti orthostatique (Pavy-Le Traon et al., 2007), immersion sèche (Navasiolava et al., 2011), confinement (Tafforin, 2015) et bien sûr la microgravité réelle (Adams et al., 2003). Les modèles animaux sont basés sur la mise en décharge du train posterieur (Morey-Holton and Globus, 2002).

Dans cette introduction nous ferons une brève synthèse des atteintes liées à la microgravité et à l'inactivité physique. Dans la première partie de ma thèse nous exposerons une revue plus détaillée des effets de l'inactivité physique et de la microgravité sur les dysfonctions vasculaires et sur les contremesures qui peuvent être proposées.

Perte osseuse

L'exposition du tissu osseux à la microgravité cause une perte osseuse, et exerce un effet considérable sur l'homéostasie minérale suite à une perte de balance entre la résorption des ostéoclastes et la formation des ostéoblastes (Bucaro et al., 2007; Dai et al., 2007; Tamma et al., 2009; Vico et al., 2000) La perte osseuse débute rapidement dès les premiers jours d'exposition à la microgravité, suite à une augmentation de la fonction et du nombre des ostéoclastes et à une diminution de la fonction et du nombre des ostéoblastes, ainsi qu'une diminution du nombre et de la capacité des cellules souches mésanchymales (MSCs) (Dai et al., 2007). La diminution des contraintes physiques ainsi que des impacts mécaniques est le principal mécanisme conduisant à cette perte osseuse. Celle-ci va conduire à une fragilisation osseuse. Les contremesures contre cette perte osseuse ont pour objectif pour la plupart de reproduire ces contraintes physiques et les impacts mécaniques.

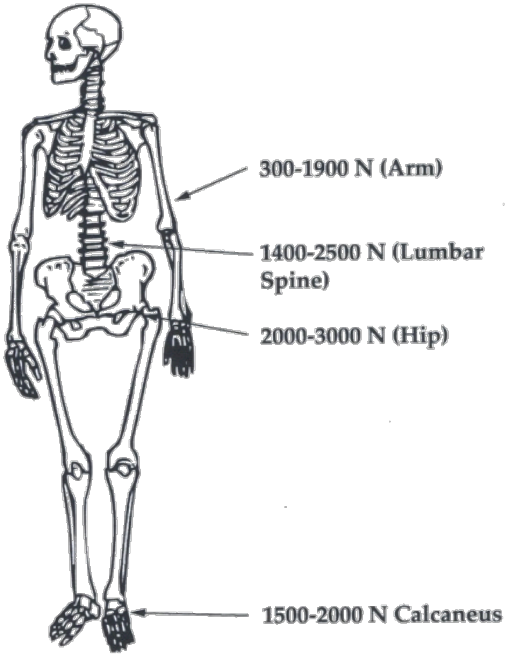


Figure 2 : *Contraintes osseuses estimées en Newton (N) chez une personne de 70 kg en position debout. (in Space Physiology, Buckey 2006)*

Troubles de la fonction motrice et neuromusculaire

L'absence de gravité induit une diminution très importante de l'activité musculaire par hypokinésie et hypodynamie. Tous les muscles ne sont pas affectés de la même façon, ce sont surtout les muscles de la posture qui vont avoir les modifications les plus importantes. (in Space Physiology, Buckey 2006)

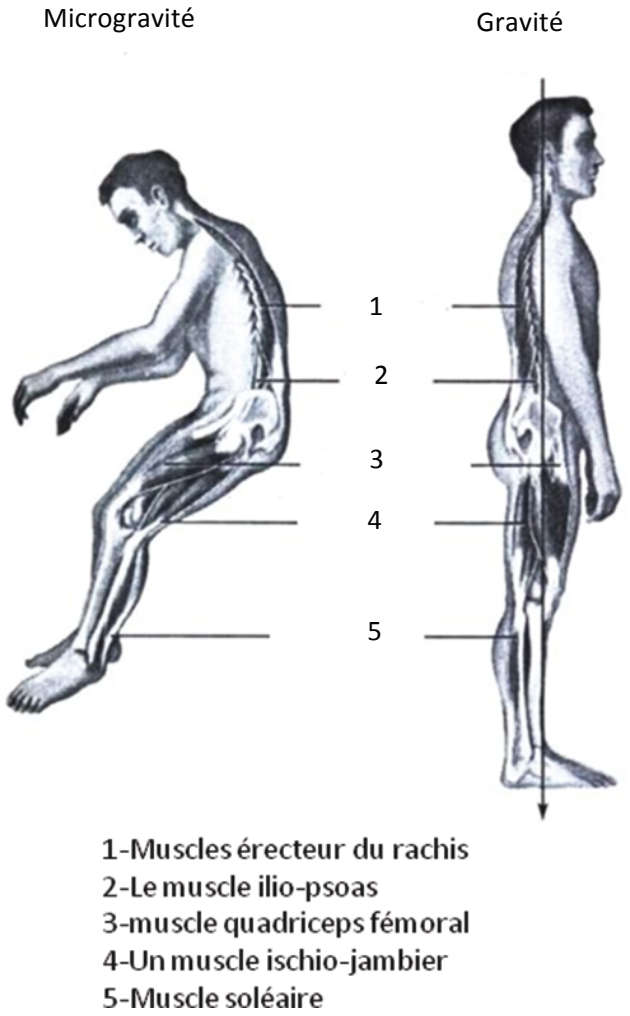


Figure 3 : *Principaux muscles du tonus postural très impactés par la microgravité (in Space Physiology, Buckey 2006)*

L'exposition à la microgravité induit une atrophie musculaire (Pavy-Le Traon et al., 2007), principalement des muscles posturaux avec une modification du type de fibres musculaires (passage de fibres de type « lents » vers des fibres de type « rapides »). La diminution d'activité physique est la principale cause de cette modification musculaire, mais il faut aussi prendre en compte un apport calorique et protéique inadéquat, l'effet du stress et les modifications hormonales.

Pour comprendre la fonction motrice, il faut s'intéresser non seulement au muscle lui même mais aussi à l'intégration neuromusculaire. Les astronautes souffrent de troubles de la proprioception et d'une dégradation de la perception de la position des membres (Viguier et al., 2009). Ces troubles retentissent sur la fonction motrice. Ces atteintes seraient dues au changement des interprétations perceptives des signaux proprioceptives plus qu'à la diminution de la sensibilité des fuseaux neuromusculaires.

En plus, la microgravité entraîne une perte du fonctionnement normal de l'appareil vestibulaire («équilibre») dans l'oreille interne. La fonction normale des otolithes (cristaux de carbonate de calcium ($CaCO_3$) associés aux cellules sensorielles ciliées

du vestibule, est de permettre de percevoir les mouvements en trois dimensions (in Medical Physiology, Ganong 2007) est perturbée de sorte que le corps perd son sens inné de haut en bas. Un déséquilibre entre le système visuel et le système vestibulaire survient en microgravité, les yeux 'voient' un mouvement, l'oreille en 'perçoit' un autre. Ceci a des conséquences sur la coordination des mouvements (Clément et al., 2005).

Altérations cardiovasculaires

D'importants changements au niveau du système cardiovasculaire ont lieu après une exposition, même brève, à la microgravité. Le cœur, le sang circulant et l'ensemble de l'arbre vasculaire sont concernés (Coupé et al., 2009; Pavy-Le Traon et al., 2007).

- Nouvelle répartition immédiatement du sang et des liquides de l'organisme vers la partie thoraco-céphalique du corps
- Diminution du volume sanguin circulant avec aussi une réduction du débit cardiaque et du volume d'éjection systolique adaptée à la microgravité et à la diminution de l'activité physique
- Diminution de la masse du ventricule gauche
- Remodelage au niveau de la macrocirculation
- Dysfonction endothéliale au niveau de la microcirculation
- Modification des propriétés veineuses des membres inférieurs
- Atteintes des boucles nerveuses et hormonales de régulation de la pression artérielle.

Tous ces facteurs participent au syndrome de déconditionnement cardiovasculaire avec une intolérance orthostatique (Blomqvist, 1996), une réduction de la capacité d'exercice aérobique (Hargens and Richardson, 2009), et une augmentation de la fréquence cardiaque. L'atteinte vasculaire ainsi que celle des fonctions endothéliales nous laisse penser que l'exposition à la microgravité à long terme, au même titre que la sédentarité, est un facteur de risque cardiovasculaire (Coupé et al., 2009).

Le syndrome de déconditionnement cardiovasculaire survient très rapidement après exposition à la microgravité vraie ou simulée. La tolérance orthostatique est définie par la capacité du corps à maintenir la conscience et la perfusion cérébrale suite à un changement de position (passage de la position allongée ou assise vers la position verticale). Dans ce cas les systèmes cardiovasculaire et neuro-hormonal sont responsables du maintien de la pression artérielle normale. En cas de défaillance de ces boucles de régulation, l'intolérance à l'orthostatisme survient avec le risque de la syncope.

Le remodelage vasculaire observé lors de la microgravité simulé est celui que l'on observe suite à une inactivité physique poussée. Il est vraisemblable que la

diminution des contraintes hémodynamiques locales au niveau vasculaire joue un rôle prédominent dans ce remodelage vasculaire à côté de facteurs nerveux, hormonaux et autres facteurs circulants (Navasiolava et al., 2010; Thijssen et al., 2010; Yuan et al., 2015). Au niveau de l'artère fémorale, son diamètre diminue avec l'importante de l'inactivité physique alors que le diamètre carotidien est peu modifié. Au cours d'un confinement de longue durée (500 jours) une augmentation de l'épaisseur intima-média a été observée au niveau de la carotide et de la fémorale sans que les mécanismes impliqués soient encore clarifiés (Arbeille et al., 2014). Au niveau de la microcirculation une atteinte de l'endothélium est observée dans des modèles de microgravité (Navasiolava et al., 2010).

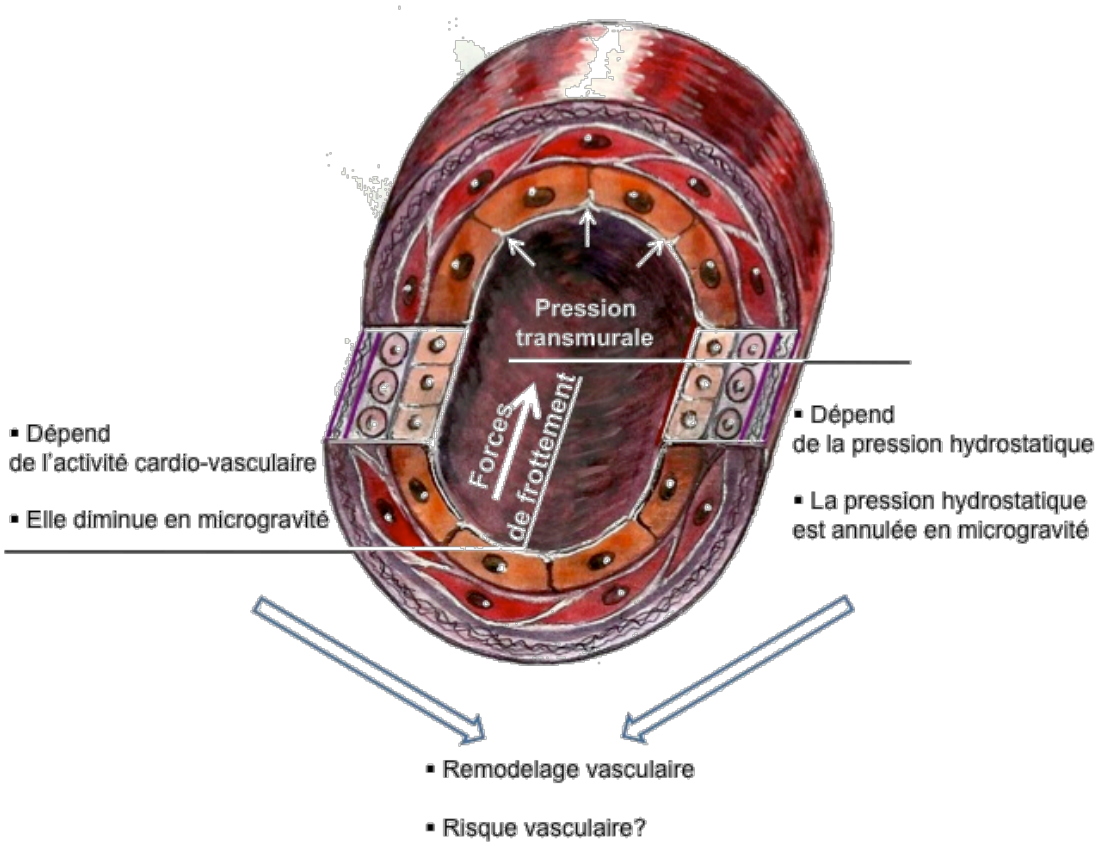


Figure 4 *: Coupe transversale d'un vaisseau et forces physiques en présence*

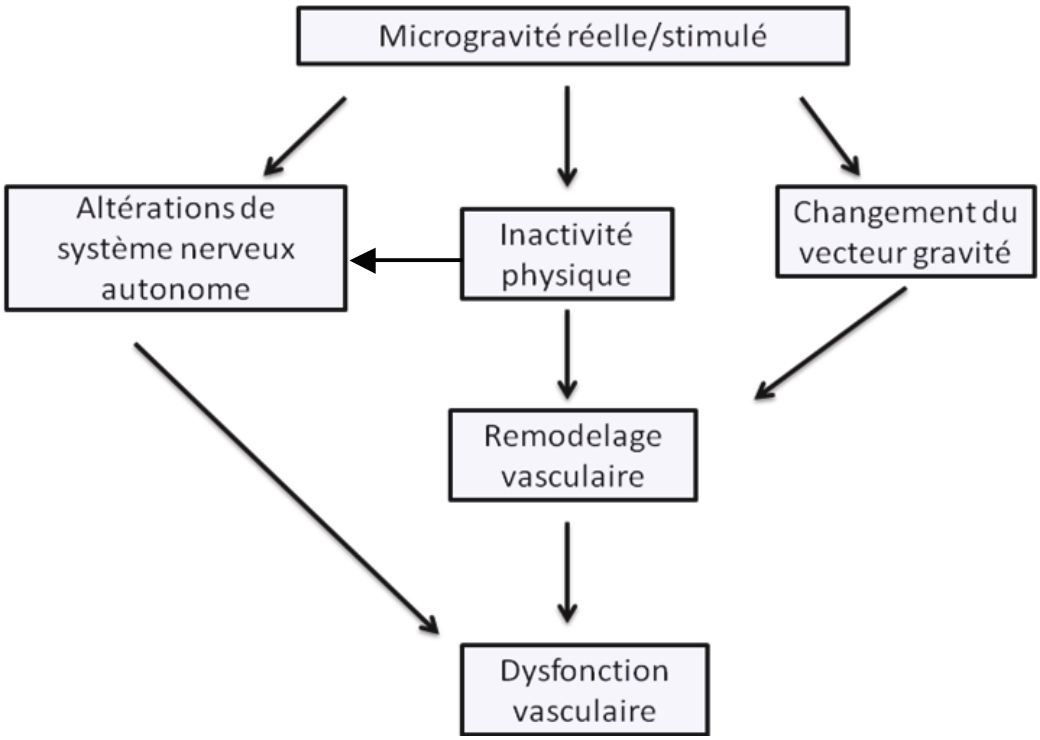


Figure 5: *Résumé des modifications physiologiques liées à la microgravité et conduisant à la dysfonction vasculaire*

Atteinte du métabolisme

L'exposition à la microgravité induit une atteinte du métabolisme énergétique. Les problématiques nutritionnelles sont majeures lors d'un vol spatial et doivent respecter l'ensemble des besoins nutritionnels pendant toute la mission sans induire de déséquilibre. Le plaisir lié à l'alimentation doit autant que possible être respecté. L'alimentation optimisée, comme un « alicament » est aussi actuellement un moyen de contremesure pour prévenir les atteintes de l'organisme. Les atteintes du métabolisme énergétique en microgravité sont pour la plupart dues à l'inactivité physique liée à l'environnement spatial. Il a été démontré que 3 jours d'alitement suffisent à une réduction de la sensibilité des muscles à l'insuline (Smorawiński et al., 2000). En plus, l'inactivité physique est associée à une réduction de l'action de l'insuline, lié à une diminution de GLUT-4 au niveau des muscles (Vukovich et al., 1996). Nous observons aussi dans ce contexte une augmentation de 7 % de la masse de la graisse viscérale, une intolérance au glucose, une altération du profil lipidique post-prandial, et une dyslipidémie (Hamburg et al., 2007). Ces atteintes métaboliques vont aussi contribuer aux atteintes vasculaires et en particulier à la dysfonction endothéliale observée. Pour résumer, l'inactivité physique induite par l'alitement induit, indépendamment des changements dans la balance énergétique, une réduction de la capacité à utiliser la graisse comme substrat énergétique, une atrophie musculaire, une résistance à l'insuline, une hypertriglycéridémie et un

changement de type des fibres musculaires au profit des fibres musculaires à contraction rapide (Bergouignan et al., 2011).

L'une des principales conditions de l'environnement spatial est l'annulation des forces physiques principalement du à l'absence de la gravité, ce qui fait que l'astronaute n'est pas obligé de faire un effort physique pour bouger. Les troubles dont souffrent les astronautes rejoignent les maladies liées à l'inactivité physique et à la sédentarité.

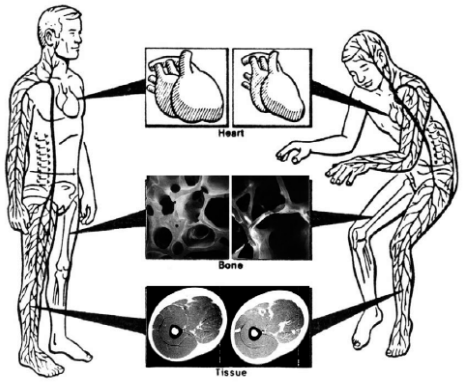


Figure 6 : *illustrant les changements structuraux avant et après un vol spatial, au niveau du cœur (atrophie myocardique), de l'os (résorption osseuse) et des muscles (atrophie musculaire)*

Le but de ce travail de thèse est d'étudier les altérations vasculaires liées d'une part à l'inactivité physique dans le contexte de la microgravité, d'autre part à un régime alimentaire inadapté.

Le premier travail est mené chez l'homme afin de tester le Taikong Yangxin, un composé issu de divers extraits végétaux, sur le dysfonctionnement vasculaire induit par la microgravité simulée par alitement anti-orthostatique.

Ma deuxième étude est menée chez l'animal pour tester le Salidroside, un composé fréquent trouvé dans des extraits végétaux, sur le dysfonctionnement vasculaire induit par le diabète de type II dans le modèle du rat GK diabétique spontanément.

La troisième partie de cette thèse est menée chez l'animal afin de tester l'effet d'un apport alimentaire hypercalorique chez la souris C57BL6. Nous étudierons l'implication du récepteur AT2R et aux oestrogènes dans l'atteinte métabolique et vasculaire. Ceci permettrait de trouver de nouvelles voies ciblées pour des contremesures.

Atteinte vasculaire et microcirculatoire induite par l'inactivité physique et la microgravité

Mécanismes et méthodes prophylactiques

Manuscrit en préparation :

"How to prevent vascular and microvascular dysfunction induced by physical inactivity and microgravity?"

L'inactivité physique est un facteur de risque majeur des maladies cardiovasculaires. Tout l'effort physique que nous réalisons quotidiennement se fait contre la force de la gravité. Ainsi l'exposition à la microgravité réelle ou ses simulations au sol impliquent une inactivité physique poussée. Ce sont des modèles particulièrement pertinents pour étudier spécifiquement les mécanismes liant l'inactivité physique et le remodelage au niveau de la macrocirculation et de la microcirculation sans prendre en compte d'autres facteurs de risque associés. Les modèles de microgravité au sol sont: l'alitement, l'alitement anti-orthostatique et l'immersion sèche. D'autres modèles impliquent une inactivité physique: le confinement, la réduction volontaire du nombre de pas quotidiens en dessous de 5000 pas/jour, le maintien de la position assise plusieurs heures, et la décharge unilatérale d'un membre supérieur ou d'un membre inférieur.

L'objectif de cette revue, basée sur les publications utilisant tous ces modèles d'inactivité, est de déterminer les caractéristiques du remodelage induit par l'inactivité physique, les mécanismes impliqués, et quelles sont les méthodes prophylactiques qui peuvent être envisagées.

Au niveau de la macrocirculation un remodelage eutrophique survient au niveau des artères des membres inférieurs alors que le diamètre carotidien ne change pas. Les capacités de vasodilatation endothéliale dépendantes ne sont en général pas ou peu altérées au niveau de la macrocirculation mais sont significativement atteintes au niveau de la microcirculation. De nombreux arguments fonctionnels et biologiques indiquent la survenue d'une dysfonction endothéliale significative même après quelques jours d'inactivité physique.

De nombreux facteurs sont impliqués dans ce remodelage au niveau de la macrocirculation et dans la survenue de cette dysfonction endothéliale: diminution des forces de cisaillement, modification de la pression transmurale, modification du métabolisme énergétique et lipidique, et le possible survenu d'un stress oxydatif local au niveau local.

Les contre-mesures potentielles pour prévenir ces atteintes vasculaires peuvent être classées en 2 groupes: les contre-mesures physiques (exercice aérobique, exercice résistif, mise en dépression de la partie inférieure du corps, bracelets veino-constrictifs, plateaux vibrants et enfin gravité artificielle) et les contre-mesures nutritionnelles et pharmacologiques (restriction calorique, compléments nutritionnels avec Resvératrol ou d'autres extraits végétaux comme le TaiKongYangXin). Nous ne disposons pas encore de contre-mesures pharmacologiques ciblées qu'il faudrait développer.

How to prevent vascular and microvascular dysfunctions induced by physical inactivity and microgravity?

Authors: M. Coupé[1, 4], N. Navasiolava[1, 4, 5], A. Alameddine[1], R. Murphy[6], G. Gauquelin-Koch[2], C. Gharib[3], M.A. Custaud[1, 4, 5]

Affiliations:

[1]UMR CNRS6214 – INSERM1083, Université d'Angers, France

[2]Centre National d'Etudes Spatiales (CNES), Paris, France,

[3]Laboratory of Environmental Physiology, Claude Bernard University Lyon 1, France,

[4]CaDyWEC International Laboratory, Angers Faculty of Medicine, France & Institute of Biomedical Problems, Russia,

[5]Clinical Research Center, CHU d'Angers, France,

[6] School of Health and Human Performance, Dublin City University, Ireland.

Corresponding author: M.A. Custaud

Email: macustaud@chu-angers.fr

Address: Clinical Research Center, CHU d'Angers, France.

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Abstract

Physical inactivity is known to have deleterious cardiovascular effects. Space environment and its ground-based models offer conditions to study cardiovascular effects of enhanced physical inactivity without other vascular risk factors, and more particularly at the macro- and microcirculatory levels. Mechanisms involved in vascular dysfunction and remodeling are not sufficiently studied. In this paper we aimed to summarize macro- and microvascular changes induced by models of physical inactivity, and to review the effects of prophylactic strategies (countermeasures) on vascular and microvascular functions. We discussed physical countermeasures (exercise, vibration, lower body negative pressure, thigh cuffs, and artificial gravity) and nutritional and pharmacological ones (caloric restriction, resveratrol and other polyphenols, Chinese herbs).

Aerobic exercise appears until now the most effective countermeasure to protect vascular function, nevertheless resistive exercise should also be taken into account. Pharmacological countermeasures are not considered now in the context of microgravity. However, nutritional countermeasures are very promising. Dietary supplements/natural health products, especially plant extracts, should be extensively studied. Finally, the ideal prophylactic strategy is a combination of countermeasures effective not only at the cardiovascular level but for the organism as a whole.

INTRODUCTION

Cardiovascular diseases represent actually the major mortality cause. Physical inactivity is one of the most important behavioural risk factors of cardiovascular diseases. For cardiovascular system, risk of being unfit exceeds the risks associated with smoking, elevated blood pressure, hypercholesterolemia or obesity, whereas regular exercise is associated with reduction in vascular events (Thijssen *et al.,* 2010). Studying specifically physical inactivity, in specific models performed with healthy subjects, without interference from other vascular risk factors provides valuable data in the general context of vascular diseases. Physical inactivity effects could be studied in different situations. Fighting gravity requires daily physical exercise, thus exposure to microgravity is associated with enhanced inactivity (Hughson 2009). Microgravity and its long-term simulations – with bed rest, head down bed rest (HDBR) (Pavy-Le Traon *et al.,* 2007) and dry immersion (Navasiolava *et al.,* 2011) - provide unique models to study the effects of global enhanced physical inactivity imposed to healthy subjects (Widlansky 2010). Other models of pure physical inactivity should be considered such as the sitting position for few hours (Restaino *et al.,* 2015; Thosar *et al.,* 2015). Low daily physical activity by reducing walking below 5000 steps/day is easy to implement and have show to impair vascular and metabolic functions (Boyle *et al.,* 2013; Renolds *et al.,* 2015). Besides, segmental inactivity models are also proposed. Spinal cord injury (SCI) in patients provides a model to assess peripheral vascular adaptations to extreme inactivity (De Groot et al. 2003). Unilateral lower limb suspension (ULLS) (Berg et al. 1991) and limb casting (Ingemann-Hansen and Halkjaer-Kristensen 1977; Johnston et al. 2009) are less extreme segmental models with a limited duration.

Physical inactivity and reduced gravity situation modify mechanical forces applied to the biological systems, alter mechanotransduction and lead to important physiological changes. A host of physiological alterations is observed in people who experience actual or simulated microgravity, including fluid changes, hormonal changes, muscle atrophy and force reduction, bone loss, autonomic dysregulation, cardiac atrophy, vascular impairment and microcirculatory dysfunctions (Coupe *et al.,* 2009). Surprisingly, cardiovascular system of astronauts adapts well to microgravity. However, the price of this adaptation is a rapid cardiovascular deconditioning - a syndrome combining orthostatic intolerance, heart rate increase and exercise capacity decrease - accompanied by vascular disorders. Similar cardiovascular deconditioning is also observed on Earth, and was firstly described in bedridden patients in 1945 (Keys 1945). Along with fluid transfer, physical inactivity is likely to play specific role in this cardiovascular deconditioning.

Several countermeasures such as exercise, whole body vibration, lower body negative pressure (LBNP), venoconstrictive cuffs, centrifugation, caloric restriction, nutritional supplements, natural health products and medications were evaluated to prevent microgravity- and inactivity-induced cardiovascular changes. Astronaut health protection is an essential issue of space medicine. However, knowledge acquired in this area could be extended to the general sedentary population.

In this paper we summarize the effects of microgravity and physical inactivity on macro- and micro-circulation and review the effects of different physical, nutritional and pharmacological countermeasures on vascular and micro-vascular functions.

1. VASCULAR AND MICROVASCULAR CHANGES INDUCED BY PHYSICAL INACTIVITY AND MICROGRAVITY

Large arteries on the one hand and resistive arteries and microcirculation on the other hand have different functions. The main role of large arteries is conduction, though they are also involved in blood pressure regulation because of their compliance. Large arteries are the vessels affected by atherosclerosis responsible for cardiovascular events. Resistive arteries and microcirculation determine total vascular resistance and blood pressure as well as downstream supply of nutritive blood flow (Thijssen *et al.,* 2011). Microcirculation is thus intimately associated to organs functioning and is deeply involved in the mechanisms of cardiovascular diseases. For vascular functions, the endothelium is of special interest. This inner layer plays a crucial role in the regulation of vascular homeostasis and local blood flow. Under normal conditions, the endothelium maintains optimal balance between vasodilation and vasoconstriction, limits vascular inflammation, and maintains blood fluidity. Endothelium is very sensitive and endothelial dysfunction appears as an early event in the pathogenesis of vascular disease. The dysfunctional endothelium adopts a phenotype that facilitates vasoconstriction, thrombosis and inflammation. In conditions with low level of physical activity, vascular functions, especially endothelial ones, are very likely to be modified.

1.1. Changes in macro- and micro-vessels under physical inactivity and microgravity

Variables used to characterize in vivo vessel structure are diameter/cross-sectional area, intima-media thickness (IMT). Functional testing of the vessels concerns mainly endothelial function, smooth muscle function, and compliance/elasticity measurements. The available data are summarized in **Table 1**.

1.1.1. Conduit arteries

Structure changes

Physical inactivity has a marked effect on structure of conduit arteries, which differ for lower and upper body. In general, for large arteries of lower limbs, which are particularly unloaded in our models, physical inactivity is associated with inward remodeling and decrease in luminal diameter of different extent (Bleeker *et al.,* 2005b, Bleeker *et al.,* 2005a, de Groot *et al.,* 2006b, de Groot *et al.,* 2006b; de Groot *et al.,* 2004, Palombo *et al.,* 2014; Yuan *et al.,* 2015), depending on intensity and duration of inactivity (Thijssen *et al.,* 2010). IMT at the femoral artery level remains unmodified after

short-term inactivity (7-day leg casting) (Sugawara et al., 2004) but increase with longer inactivity (60-day HDBR) (van Duijnhoven *et al.,* 2010a).

At carotid level, diameter remains unmodified (Arbeille *et al.,* 1999, van Duijnhoven *et al.,* 2010a, Yuan *et al.,* 2015). For the arteries at the arm level (which are almost not unloaded in our models) the diameter remains stable (Hughson *et al.,* 2007, de Groot *et al.,* 2004) or can even be increased (Bonnin et al. 2001). IMT at the carotid level is unmodified (Yuan *et al.,* 2015) or increased (van Duijnhoven *et al.* 2010a) after long term head down bed rest.

Functional changes

Brief episode of physical inactivity such as induced by few hours of sitting position induces decrease in the endothelium-dependent vasodilation capacity at the leg level estimated by flow mediated dilation (FMD) (Restaino *et al.,* 2015; Thosar *et al.,* 2015). Reduction of daily physical activity by taking <5000 steps/day decrease also the FMD response at the popliteal level whereas basal popliteal diameter did not change (Boyle *et al.,* 2013). However, at femoral level advanced but prolonged inactivity preserves endothelium-dependent vasodilation (FMD) (Bleeker *et al.,* 2005c, de Groot *et al.,* 2004) or even increases it (Bleeker *et al.,* 2005a, de Groot *et al.,* 2006, van Duijnhoven *et al.,* 2010b). Inward remodeling of the arterial vessels at the lower limb level induced by prolonged physical inactivity probably explain the discrepancy with results observed after very short and mild inactivity. At the brachial level also, 7 days of HDBR enhance flow-dependent vasodilation (Bonnin et al., 2001), suggesting that not only local factors are involved but also systemic mechanisms. It has been shown that low FMD values are predictive of future cardiovascular events (Green *et al.*, 2011; Yeboah *et al.,* 2007), so we could draw as preliminary conclusion that large vessels seem protected in situation of enhanced physical inactivity. Concerning smooth muscle functions at the large artery level, no significant changes in sensitivity of smooth muscle to NO were observed after spinal cord injury (Thijssen *et al.* 2008) or after 60 days of HDBR in women (Zuj *et al.,* 2012). Concerning macrovascular elasticity, prolonged inactivity decreases compliance at femoral level, but has no effect on carotid compliance (Yuan *et al.,* 2015). Birk et al. studied the effect of 8 days of unilateral forearm inactivity. Flow mediated dilation and basal diameter was unchanged but post ischemic peak blood flow occurs suggesting a remodeling of forearm resistance vessels (Birk *et al.,* 2013). Initial remodeling induced by physical inactivity occurs first at distal ends of the vascular tree.

1.1.2. Resistance vessels and microcirculation

Structure changes

Studies that have examined reactive hyperemic responses after enhanced physical inactivity suggest time- and intensity- dependent decrease in global resistance artery cross-sectional area (Bleeker *et al.,* 2005a; de Groot *et al.,* 2006a; de Groot *et al.,* 2006b). Endothelial integrity markers are also impaired by physical inactivity. HDBR for 2 months (in women) increased circulating endothelial cells number (Demiot *et al.,* 2007). Dry immersion for 7 days was sufficient to increase circulating endothelial microparticles level (Navasiolava *et al.,* 2010). Even mild inactivity such as induced by few days with less than 5000 steps/ day induces increase of endothelial microparticles of apoptotic phenotype (Boyle *et al.,* 2013). Those markers probably represent mainly endothelium from the microcirculation, taking into account that it is quantitatively much more important, and - basing on vasodilative function assessment, - more affected than endothelium from large vessels. Moreover, a reduction of physical activity in healthy subjects for 10 days induces a decrease in some circulating angiogenic cell populations (Guhanarayan *et al.,* 2014).

Functional changes

Endothelium-dependent VD

Many studies have shown an impairment of the endothelium-dependent vasodilation at the microcirculatory level contrary to what observed for conduit arteries. At calf skin level, an attenuation of endothelium-dependent vasodilation, measured using laser Doppler coupled with ACh iontophoresis, was observed after 2-month bed rest in men (Yuan *et al.,* 2015) and women (Demiot et *al.,* 2007), and after 7-day dry immersion in men (Navasiolava *et al.,* 2010). Similarly, Hesse et al. (Hesse *et al.,* 2005) found a decrease in dilation to ACh assessed by plethysmography at the forearm level, following 2-week bed rest. A significant reduction in vasodilative response to shear (reactive hyperemia) in the upper and lower limbs was observed in healthy men after 5 days of bed rest (Bleeker *et al.,* 2005b).

Endothelium-independent VD

Smooth muscle function seems preserved. Endothelium-independent vasodilation to SNP remained unchanged at arm resistance vessels level after 13 days of HDBR (Hesse *et al.,* 2005) and at skin microcirculatory level after 7 days of dry immersion (Navasiolava *et al.,* 2010).

Basal tone

Many evidence indicate increased basal vascular resistance after physical inactivity (Bleeker *et al.,* 2005a; Christ *et al.,* 2001). Even 5-day of bed rest (Hamburg *et al.,* 2007) or dry immersion (Navasiolava *et al.,* 2011) are sufficient to induce this increase in basal vascular resistance. Interestingly, NO-mediated dilation and NO bioavailability at the level of leg resistance vessels are not altered in SCI individuals and in men after 4 wk of limb suspension (Bleeker *et al.,* 2005c). In another study, forearm resistance responses to NO blockade were not altered by 6-week forearm cast (Green *et al.,* 1997). These studies suggest that the increased vascular resistance induced by physical inactivity is not necessarily explained by impairment of the NO dilator pathways. For resistive vessels, unlike conduit arteries with endothelium-dependent vasodilation predominantly by NO-pathway, three vasodilative pathways (NO-, PG- and EDHF-) are of equal importance. Prostaglandin or EDHF pathways together with neurovascular interactions at the microcirculatory levels should thus be considered.

1.2. Mechanical factors as mechanism of vascular and microvascular changes

The mechanical forces implemented by blood towards vascular wall are shear stress - exerted in the same axis as flow, and transmural pressure - exerted radially to flow (Bevan and Laher, 1991). There is a great difference in these forces between small and large vessels. Indeed, shear stress is higher in arterioles (40-60 dynes/cm^2), than in large arteries (10-30 dynes/cm^2) (Boisseau, 2005), whereas transmural pressure and its pulsatile oscillations are lower in small vessels.

Shear Stress

Shear stress is essential for endothelial cell survival and well-being. It is the most powerful physiological stimulus for NO production. It also promotes the release of factors inhibiting coagulation, leukocyte migration and smooth muscle cells proliferation. By assuming laminar flow conditions, shear stress can be calculated with the Hagen-Poiseuille approximation: $4\,\mu Q/\pi r^3$, where μ is the blood viscosity, Q is the blood flow, and r is the radius of the vessel.

Advanced physical inactivity decreases tissue demands and is associated with a general decrease in blood flow and, hence, decrease in shear stress (this is particularly true for lower limbs, extremely unloaded compared to their normal daily activity). Langille and O'Donnell (Langille and O'Donnell, 1986) were the first to show, using a model of unilateral external carotid artery ligation in rabbit, that a decrease in blood flow for 2 wk mediates an inward remodeling. Moreover, this response was abolished when the endothelium was removed. This indicates that chronic changes in shear stress mediate endothelium-dependent vascular remodeling. It is believed that inward remodeling homeostatically regulates wall shear (Tronc *et al.,* 1996). Inactivity-induced inward remodeling thus preserves shear stress rates of conduit vessels (Thijssen *et al.,* 2010). This explains why in advanced physical inactivity conditions, most of the studies report an unaffected or increased FMD in conduit arteries. However, resistance vessels, unlike conduit vessels, seem to have fewer possibilities for this inward remodeling, taking into account their modest lumen diameter compared to their wall thickness. Meanwhile, a chronic decrease in shear stress during physical inactivity is especially harmful for microcirculation (Boisseau, 2005). The differences in adaptation to initial decrease in shear stress might be the main reason of different dysfunction degree for macro- and microcirculation. While large vessels function re-adapts by remodeling, small vessels undergo functional impairment.

Transmural Pressure

Vascular morphology and functions, especially for smooth muscle cells, are sensitive to transmural pressure, itself depending on blood pressure and hydrostatic pressure. Apparently systemic blood pressure is not significantly modified by long-term bed rest (van Duijnhoven *et al.,* 2010a; van Duijnhoven *et al.,* 2010b; Fortney *et al.,* 1996), though chronic physical training is associated with a decrease in blood pressure (Pescatello et al., 2004). However, changes in hydrostatic pressure should be considered when analyzing vascular remodeling in actual or simulated microgravity. Daily orthostatic stimulation induces large variation of hydrostatic pressure in the upper part (up to -40 mmHg) and in the lower part (up to +100 mmHg) of the body (Rowell 1993). Those changes are suppressed by bed rest, dry immersion or microgravity conditions. These variations in hydrostatic pressure increase with the distance from hydrostatic indifferent point. So, during supine inactivity transmural pressure is significantly diminished at the femoral level, but not at the carotid level. Along with different shear stress level, different modifications in hydrostatic pressure contribute to differential effect of supine inactivity on upper and lower body.

1.3. Metabolic factors as mechanism of vascular and microvascular changes

Vascular and metabolic functions are closely linked. It is known that metabolic circulatory factors (i.e. in case of hyperglycemia or dyslipidemia) could impair vascular function.

Several studies have demonstrated that even short periods of physical inactivity (bed rest for 3-7 days, dry immersion for 5-7 days) increase fasting blood insulin (Hamburg *et al.,* 2007, Blanc *et al.,* 2000, Afonin and Sedova 2009, Coupé *et al.,* 2013), impair glucose tolerance (Smorawinski *et al.,* 2000, Hamburg *et al.,* 2007, Blanc *et al.,* 2000), and alter lipid profile (Hamburg *et al.,* 2007, Navasiolava *et al.,* 2011, Coupé *et al.,* 2013). These metabolic abnormalities are associated with endothelial dysfunction, as it was shown for microvascular level after 5-day bed rest (Hamburg et al. 2007). They might participate to endothelial dysfunction by triggering several oxidative and pro-inflammatory pathways (increased ROS production, activation of PKCs- and AGE-induced pro-inflammatory signaling…) leading to unbalanced release of endothelial mediators. Among these mechanisms, increased oxidative stress seems to be the pivotal alteration (Potenza *et al.,* 2009).

Thus, to interpret vascular changes induced by physical inactivity, especially endothelial dysfunction, it is necessary to take into account metabolic changes.

1.4. Inflammatory factors and oxidative stress as mechanisms of vascular and microvascular changes

Vessels, and once again endothelium, are sensitive to inflammatory circulating factors, such as cytokines (i.e. IL-1β, IL-6, TNF-α), adhesion molecules (i.e. E-selectin, P-selectin, ICAM-1), acute-phase reactants (i.e. CRP, serum amyloid A), lipopolysaccharide, etc. These factors are able to “activate” endothelium. An essential outcome of such activation is reduced bioavailability of endothelium-derived nitric oxide. Thus, inflammation is associated with an impairment of nitric oxide-dependent responses (Huang and Vita 2006). It was shown that inflammatory markers levels positively correlate with cardiovascular events risk (Pearson *et al.,* 2003). The question if physical inactivity by itself induces inflammation and increased oxidative state remains open. While acute exercise is able to induce an oxidative stress, this same exercise stimulus appears necessary for an upregulation in endogenous antioxidant defenses (Bloomer *et al.,* 2008). Physical inactivity might promote inflammatory state indirectly, via metabolic changes. For example, lipids modifications are associated with altered levels of circulating cytokines and adipocytokines (Petersen and Pedersen, 2005). However in the context of acute physical inactivity, in general there is no change in circulating inflammatory markers, arguing against systemic inflammation in these models. Hamburg et al.

noticed that after a 5-day non-strict bed rest, the metabolic changes (insulin resistance and dyslipidemia) were not accompanied by changes in systemic inflammatory markers (CRP, IL-6, and TNF receptor-II) (Hamburg *et al.*, 2007). Dry immersion for 5-7 days did not modify CRP level (Coupé *et al.,* 2013) or leukocytes number, a global marker of inflammation (Kozinets *et al.,* 1983; Navasiolava *et al.,* 2010).

However, physical inactivity induces muscle atrophy, which might be associated with muscle ROS production. Indeed, studies performed after 35-day bed rest (Agostini *et al.,* 2010; Dalla Libera *et al.,* 2009) or after 2 weeks of one-leg immobilization (Gram *et al.,* 2015) demonstrate increased local oxidative stress at the muscle level. Though it is not clear whether chronic physical inactivity increases general inflammatory factors, this mechanism should not be discarded when vascular functions are studied. At the microcirculatory level (and especially for skeletal muscles), the local oxidative stress mechanism should certainly be taken into account in the context of physical inactivity.

In conclusion, physical inactivity induces both large vessels and microcirculation impairment. Endothelial impairment for microcirculation seems more generalized (both lower and upper limbs), than structural modifications/remodeling for conduit vessels (mostly legs), suggesting involvement of some systemic mechanisms (EMPs? cytokines?...) for microvessels and rather local mechanisms (lumen adaptation to maintain shear stress) for macrovessels. Dysfunction at the microcirculatory level might contribute to pathological states related to physical inactivity, such as altered blood pressure regulation, changes in energy metabolism, muscle atrophy, and even skin ulcer formation. Macro- and especially microvascular properties should be a specific target for countermeasures designed to minimize or reverse the deleterious effects of chronic physical inactivity in human.

2. COUNTERMEASURES AND THEIR EFFECTS ON MACRO- AND MICROCIRCULATION

Countermeasures were originally designed to prevent symptomatic troubles induced by microgravity such as orthostatic intolerance, decrease in exercise capacity, muscle atrophy and weakness, osteopenia, neuro-vestibular troubles, etc. Their specific effects on macro- and microvascular functions were studied more recently. First, because vascular function study is methodologically difficult, but today it is facilitated by tools improvement (high resolution ultrasound, iontophoresis coupled with laser Doppler, assessment of new biological variables). Second, because vascular troubles finally appear as a full part of the general deconditioning syndrome.

2.1. Physical countermeasures

2.1.1. Exercise

Physical exercises can be classified into stretching, aerobic and anaerobic types. Stretching exercises improve flexibility and muscle elasticity. Aerobic exercises (walking, running, swimming, cycling, etc) involve large muscle groups and are especially effective to increase cardiorespiratory endurance/fitness. Anaerobic exercises can be defined as a rapid burst of hard exercise (resistive exercise, weight training, isometrics, sprinting, etc), and aim to increase strength, speed and muscle mass. Exercise beneficial effect on cardiovascular events rate is well-known (Abramson and Vaccarino 2002; Green *et al.,* 2011, Thijssen *et al.,* 2010). In addition to favorably modifying traditional risk factors such as blood pressure, lipid profile or glycemic homeostasis, a possible direct protective mechanism of regular exercise is via its effect on endothelial function (Niebauer and Cooke 1996). Exercise training is typically associated with enhanced vasodilator activity that is ultimately followed by outward remodeling and angiogenesis and consequent re-normalization of vasodilator function. These effects occur in vasculature of the active muscle beds, but also seem to be generalized to the arteries supplying skeletal muscles that are not directly involved in training (for review see Thijssen *et al.,* 2010).

Aerobic exercise

Several studies have shown that daily physical activity and arterial stiffness are inverse correlated (O'Donovan *et al.,* 2014) and that aerobic exercises specifically decrease arterial stiffness (Hayashi *et al.,* 2005; Tanaka et al., 1998). Regular aerobic exercise improves endothelial vasodilatory capacity, impaired by aging, metabolic troubles or hypertension. Thus, DeSouza et al. demonstrated that

endurance-trained men show no age-related decline in endothelium-dependent vasodilation. Moreover, in middle-aged people, aerobic exercise training (walking) for 3 mo restored vasodilatory function loss compared to sedentary peers (DeSouza *et al.,* 2000). In a more recent study, daily 40-min aerobic exercise for 3 mo (home-based aerobic exercise-training program) appeared to improve endothelium-dependent vasodilation in overweighed adults, independently of changes in body mass or composition (Mestek *et al.,* 2010). Regular aerobic exercise program for 3 mo (5-7 times a week) was also effective to improve endothelium-dependent vasodilation in both normotensive and hypertensive subjects (Higashi *et al.,* 1999). After 13-day HDBR, cutaneous vasodilation during exercise, as measured with laser Doppler, is impaired (Lee *et al.,* 2002). It was shown that aerobic exercise (daily supine cycling for 90 min) prevents this skin microcirculatory impairment after 13 days of HDBR (Shibasaki *et al.,* 2003).

Resistive exercise

Resistive exercise is another type of physical exercise tested and also proposed in patients with cardiovascular disease (Braith and Beck, 2008). If the beneficial effect of aerobic exercise on vascular functions is obvious, the effect of resistive exercise is more controversial. Resistive training first contraindicated for patients with coronary artery disease appears nowadays safe for clinically stable patients. Resistive training prevents decline in skeletal muscle mass and function associated with aging (Hurley and Roth, 2000). The effect of resistive training on exercise capacity is more disputed. Some studies have shown an increase in VO_2max after resistive training in patients with chronic heart failure, whereas other reported a non-improvement. A recent study in patients with type 2 diabetes (Kadoglou *et al.,* 2012) had shown that resistive training had no effect on VO_2max, lipid profile and body fat, though it improved glycemic control and basal insulin level. To date, there are no reports of arterial stiffness decrease induced by resistive training. High intensity resistive training might even increase central arterial stiffness (Miyachi *et al.,* 2004).

Combined resistive + aerobic exercise and endothelium

There is no report on the effects of resistive training by itself on the endothelium. But it was shown that a combination of resistive and aerobic training improves endothelium-dependent vasodilation (Maiorana *et al.,* 2000). In the context of inactivity, Demiot et al. studied the effects of combined aerobic (treadmill) and resistive (flywheel) exercise in a LBNP chamber during 60-day HDBR. They showed that endothelium-dependent vasodilation and the number of circulating endothelial cells

were preserved in the group with this combined countermeasure, indicating a protection of endothelial function (Demiot *et al.,* 2007).

2.2. Whole body Vibration

During all the activities we perform daily, our body interacts with the environment. While walking, the impacts of the feet on the floor induce vibrations all along the body. Vibration, characterized by an oscillatory motion, is an important mechanical stimulus. Since many years, whole body vibration (WBV) is proposed as therapeutic tool. A lot of studies have shown beneficial effects of WBV on bone structure (Gomez-Cabello *et al.,* 2012), on neuromuscular response (Fontana *et al.,* 2005; Torvinen *et al.,* 2002), on maximal muscular force and tone (Bosco *et al.,* 1999) and on endocrine system (Di Loreto *et al.,* 2004). When we are jumping, walking, running, the induced pattern of vibration is very different. Thus it is logical to test different procedures for WBV. WBV countermeasure is mostly practiced in standing position in combination with a resistive exercise such as active squat, but it can also be practiced in supine position with a harness. Vibration conditions are very important for data interpretation. To facilitate the comparison between studies, a recent consortium recommended a uniform WBV terminology and a checklist for methodological description of WBV protocols (Rauch *et al.,* 2010). Some results suggest that WBV acutely decreases arterial stiffness. Thus, Otsuki et al. (Otsuki *et al.,* 2008) showed that 10 sets of vibration (frequency 26 Hz) for 60 seconds in static squat position decreased brachial-ankle pulse wave velocity, an index of arterial stiffness, immediately after the WBV trials, with return to baseline within 60 min. With inactivity models, a combination of vibration and exercise has a beneficial vascular effect. Thus, in the experiment with 60-day HDBR, van Duijnhoven et al. (van Duijnhoven *et al.,* 2010b) compared the effects of resistive exercise alone and resistive exercise + WBV. They have shown that combined countermeasure preserved superficial femoral artery diameter and FMD, whereas resistive exercise alone was not sufficient to counteract vascular changes. Those results are in accordance with the data from Berlin Bed Rest study, where resistive exercise + WBV attenuated the decrease in leg conduit arteries diameter, induced by 52 days of horizontal bed rest (Bleeker *et al.,* 2005b).

Mechanisms involved in vascular protection by WBV remain unknown. WBV has been reported to immediately increase femoral and popliteal artery blood flow and shear rate, and thus could protect vascular properties (Kerschan-Schindl *et al.,* 2001). A more systemic hypothesis is that WBV might also prevent autonomic alterations associated with cardiovascular deconditioning. As demonstrated by Coupé et al. with a 60-day HDBR, daily low magnitude WBV (frequency 30 Hz) + resistive exercise

countermeasure preserves sympathetic index (reflecting sympathovagal balance of cardiac autonomic control) and spontaneous baroreflex sensitivity (Coupe *et al.,* 2011).

2.3.LBNP

Lower body negative pressure (LBNP) is intended to stimulate the venous system of the lower limbs. The subject's legs and pelvis are enclosed in the chamber from the iliac crest. The levels of negative pressure of about -40 to -50 mmHg are considered to produce in supine subject a downward fluid shift very close to that induced by standing position. LBNP appeared to reduce the development of orthostatic hypotension in HDBR experiments (Güell *et al.,* 1991), and is widely used at the end of long-term spaceflight to prepare for the return to Earth.

The effects of LBNP as a countermeasure were first tested alone, without combination, but in those early studies vascular function were not studied extensively. Today, LBNP is used in association with many countermeasures such as fluid loading (salt and water) and exercise. A combination of LBNP with aerobic (treadmill) and resistive exercise was shown to prevent endothelial impairment induced by a 2-mo HDBR in women (Demiot *et al.,* 2007).

2.4.Thigh cuffs

Venoconstrictive thigh cuffs are elastic strips placed tightly around each thigh to create a pressure equivalent to about 30 mmHg. They partially reproduce the effect of standing position on body fluid distribution. Since 1990 the thigh cuffs are used onboard as a routine countermeasure. Russian cosmonauts wear them for a daytime. This has been shown to reduce facial edema, headache and nasal congestion and significantly improve the comfort (Lindgren *et al.,* 1998). One study evaluated vascular effects of thigh cuffs used for 10 hrs daily during a 7-day HDBR (Arbeille *et al.,* 1999). The influence on arterial parameters was modest, without effect on carotid diameter and total peripheral resistance. Lower limb vascular resistance decreased more in the cuff group, probably related to blood stagnation induced by thigh cuffs. These combined results are in agreement with the level of orthostatic intolerance, the most serious symptom of cardiovascular deconditioning after HDBR, which was not improved by this countermeasure (Custaud *et al.,* 2000).

2.5. Artificial gravity

Artificial gravity (long arm centrifuge and now short arm centrifuge) is a promising countermeasure to reproduce Earth conditions and it could be combined with exercise or vibration (Clement and Pavy-Le Traon, 2004). The effect of centrifugation on vascular functions is very interesting to study. On one side, we could hypothesize that gravity reproduction might be of benefice for cardiovascular system. However, for the short arm centrifuge, the gravity at the leg level is much higher and might impair the microcirculation. Specific studies on vascular and microcirculatory consequences of artificial gravity are still lacking but are now in process.

2.6. Nutritional and pharmacological countermeasures

2.6.1. Caloric restriction

Caloric restriction is a dietary intervention maintaining proper nutrition, but low in calories. Caloric restriction might be beneficial for cardiovascular system even for healthy non-obese subjects. Thus, 25% caloric restriction for 6 months (CALERIE trial) decreased estimated 10-year CVD risk by 29%, though the effect on endothelial functions, as assessed by FMD at brachial artery level, was not found (Lefevre *et al.,* 2009). However, Hesse et al. demonstrated that 25% caloric restriction (mainly achieved by reduction of fat intake to a minimum recommended level of 60 g/day) for 13 days improved response of forearm resistance vessels to ACh (Hesse *et al.,* 2005). The effects of hypo energetic diet has been studied extensively in obese patients (Bergholm *et al.,* 2003; Sasaki *et al.,* 2002; Sciacqua *et al.,* 2003). These studies expose the beneficial effects of caloric restriction and weight loss on different pathways that modulate vascular responsiveness: lipid metabolism, blood pressure control, oxidative reactive species regulation. In astronauts, reduced food intake is often observed, but its influence on vascular functions remains unclear. However with 13-day HDBR it was shown that hypo-energetic low-fat diet prevents the impairment of endothelium-dependent vasodilation of forearm resistance vessels (Hesse *et al.,* 2005). Significant reductions in serum lipids, induced by low fat diet, might have contributed to endothelial protection. However, long-term caloric restriction regimen seems much too hard for most people (the situation similar to that with exercise), taking into account the actual lifestyle so inviting to excessive food intake. The possible solution may be recourse to pharmacological compounds imitating the effects of caloric restriction ("caloric restriction mimetics").

2.5.2 Resveratrol and other polyphenols

Polyphenols are organic substanses, mainly natural, characterized by the presence of several phenol structural units. They include simple phenols, flavonoids, and non-flavonoids such as stilbenes (resveratrol), saponin, curcumin and tannins. Epidemiological studies show an inverse relationship between dietary polyphenol consumption and mortality from cardiovascular diseases (Middleton *et al.,* 2000). Polyphenols exert numerous biological effects that might participate in protection of cardiovascular system: vasodilatory, antioxidant (Andriantsitohaina *et al.,* 2012), anti-aggregatory, cholesterol-lowering (Ngamukote *et al.,* 2011). It was shown that polyphenols may improve endothelial functions. Polyphenol-rich products at relatively low doses (corresponding to two glasses of red wine or to daily consumption of 46 g of dark chocolate for 2 wks) increase FMD in healthy subjects. Similarly, polyphenol-rich products, such as black tea, green tea extract, and red grape extract, improve FMD in patients with coronaropathy (Andriantsitohaina *et al.,* 2012).

Resveratrol is a natural polyphenol extracted from red wine. It has been the topic of numerous animal studies due to its action as a hormetic factor in metabolic pathways. In rodent models, resveratrol was shown to protect endothelial and cardiac functions (Rimbaud et al. 2011). Resveratrol might protect most of the physiological systems impaired by exposure to microgravity. It was tested as countermeasure against deconditioning induced by mechanical unloading (a model of microgravity) in rat. The results have shown that resveratrol (400mg/kg/day) acts as physical exercise or caloric restriction mimetic, preventing inactivity-induced insulin resistance, as well as metabolic, muscle and bone deconditioning (Momken *et al.,* 2011). In human studies, acute resveratrol prescription improved, as soon as one hour later, FMD in overweighed and mildly hypertensive individuals (Wong *et al.,* 2011). Prescription of a modified resveratrol for 3 months improved endothelial functions in adults with metabolic syndrome (Fujitaka *et al.,* 2011). Thanks to its pleiotropic effects, resveratrol may appear a good candidate to correct cardiovascular alterations, and should now be tested in human in the context of enhanced physical inactivity and microgravity.

2.5.3 TaikongYangxin and other Chinese herbs

Chinese Herbal Medicine (CHM) is one of the most important modalities of traditional Chinese medical care. Among about 500 Chinese herbs currently used, a number of herbs have primarily cardiovascular indications (Mashour *et al.,* 1998, Tang *et al.,* 2009). The special interest of CHM countermeasure against vascular deconditioning is its pleiotropic effect, not limited to one single

mechanism or to single application point (Lu *et al.,* 2004). TaikongYangxin ("outer space heart-nourishing") prescription is an herbal formula proposed by Chinese space agency. It is composed of over 10 herbs including *Panax ginseng*, *Astragalus membranaceus*, *Ligusticum wallichii*, *Schisandra chinensis,* etc. The study of Mi *et al.,* (2008) using rat microgravity model (tail suspension for 28 days) suggests that TaiKongYangXin may protect heart pump function. In the protocol with a 60-day HDBR Vandeput *et al.,* (2010) had shown that TaikongYangxin restricted, though only partially, the influence of HDBR on the cardiovascular regulation (HRV parameters). It was also shown that TaikongYangxin contributed to prevent the loss of vasoconstriction in leg and splanchnic areas following 60-day HDBR (Yuan *et al.,* 2012). After 60-day HDBR TaikongYangxin improved microvascular endothelial functions and preserved endothelial integrity, but had no beneficial effect for macrovascular diameter and compliance (Yuan *et al.,* 2015). Several active components of TaikongYangxin might be capable to ameliorate endothelium-dependent vasodilation with potential synergic interactions.

CONCLUSION AND PERSPECTIVES

We can classify the countermeasures into two groups: physical countermeasures that aim to reproduce the effects of gravity or daily physical activity, and nutritional or pharmacological countermeasures that target to protect specific functions. The strategy of countermeasures evaluation in space physiology responds to a practical approach (figure 1). Combined countermeasures are privileged and mostly tested, so it becomes difficult to analyze the effectiveness of individual countermeasure. However it would be useful as a mechanistic approach to design studies to test individually each countermeasure. To date, aerobic exercise remains the most effective countermeasure against vascular dysfunction induced by physical inactivity and space environment. Resistive exercise and vibration could give additional benefice. Actions on food and nutritional supplements are very promising. Lipid-depleted diet might preserve endothelial functions. Supplements with plant extracts (traditional Chinese medicine as example) also appear to have beneficial effects on endothelial functions. Animal and human studies in situations with vascular risk suggest that resveratrol is a potentially effective countermeasure, and not only for vascular functions. Other nutritional supplements are also proposed for their potential effects on blood vessels, such as chocolate (Fernandez-Murga *et al.,* 2011) and wallnut extract (Papoutsi *et al.,* 2008), rich in polyphenols, or whey protein (Ballard *et al.,* 2009, Pal & Ellis 2010). Surprisingly, pharmacological countermeasures for vascular functions are almost not used. If midodrine (Platts *et al.,* 2004) has been proposed to promote vasoconstriction after spaceflight and avoid orthostatic hypotension, its interactions with anti-emetics used in this context have interrupted the studies with this compound. Clinically, statins are widely used to improve and protect vascular functions in case of persistent dyslipidemia (Stone 2012). Vitamine C, for its anti-oxidant property, has been proposed to protect the endothelium impaired by 3 hours of sitting position (Thosar *et al.,* 2015). C21, an Angiotensin II type 2 receptor agonist is under development and evaluation now with the aim to protect the endothelium and the microcirculation (Henrion, 2012). These molecules could also be proposed during spaceflight or prolonged physical inactivity, provided that expected vascular risk exceeds their possible side effects. We must note that it is important to specifically evaluate the effects of all countermeasures on vascular functions. The study of vascular properties, although well developed (diameter, compliance and flow rate measurements for large vessels; vasodilation capacity assessment using ischemic stimulus, ACh and donors of NO; some biological assays), explores the vessels only partially. There are a lot of unresolved problems in the context of the effects of physical inactivity on vascular functions. Vasodilation by prostaglandin pathways is not sufficiently investigated. Microcirculatory neurovascular interactions are poorly understood. The

endothelial changes on muscle biopsy for example should also be studied, especially their potential link with local oxidative stress. Permeability at the microcirculatory level is exceptionally mentioned. These are just some ideas that suggest that the study of vascular functions in the context of physical inactivity and microgravity offers long-term prospects.

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Figure 1:

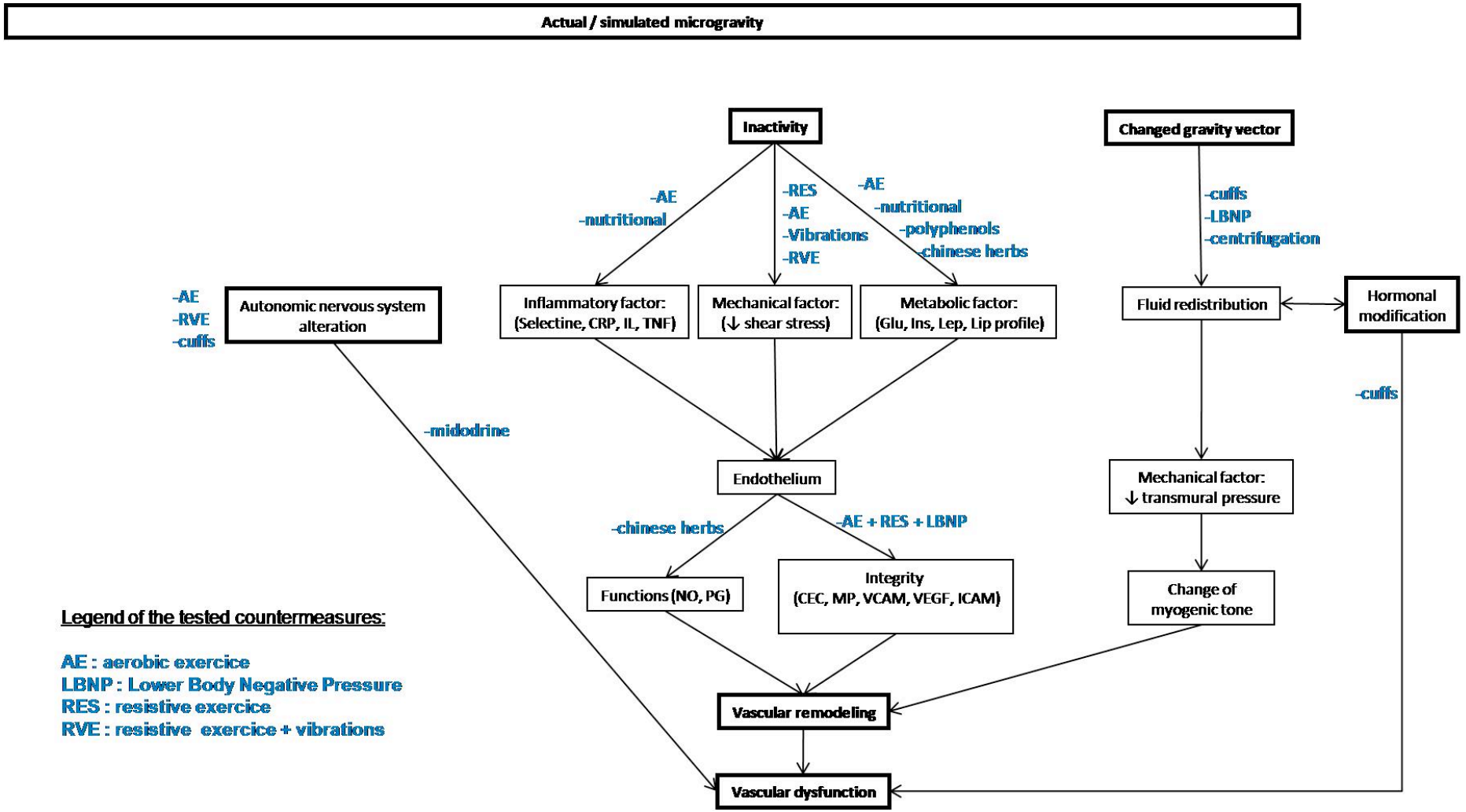

Actual / simulated microgravity
Inactivity
Changed gravity vector
-AE
-nutritional
-RES
-AE
-Vibrations
-RVE
-AE
-nutritional
-polyphenols
-chinese herbs
-cuffs
-LBNP
-centrifugation
-AE
-RVE
-cuffs
Autonomic nervous system alteration
Inflammatory factor: (Selectine, CRP, IL, TNF)
Mechanical factor: (↓ shear stress)
Metabolic factor: (Glu, Ins, Lep, Lip profile)
Fluid redistribution
Hormonal modification
-cuffs
-midodrine
Endothelium
Mechanical factor: ↓ transmural pressure
-chinese herbs
-AE + RES + LBNP
Functions (NO, PG)
Integrity (CEC, MP, VCAM, VEGF, ICAM)
Change of myogenic tone
Legend of the tested countermeasures:
AE : aerobic exercice
LBNP : Lower Body Negative Pressure
RES : resistive exercice
RVE : resistive exercice + vibrations
Vascular remodeling
Vascular dysfunction

Table 1: Effect of experimental physical inactivity of varying intensity and duration to vascular variables, and the effect of countermeasures.

Vascular level	Variable	Inactivity model	Duration	Inactivity effect (group without countermeasure)	Countermeasure tested	Countermeasure effects	Reference
Carotid	Compliance	HDBR	60 days	None	Chinese herbs	None	(Yuan et al., 2015)
	Diameter	HDBR	7 days	None (↓5%,NS)	Thigh cuffs	None	(Arbeille et al. 1999)
			60 days	None	Resistive exercise	Beneficial	(van Duijnhoven et al. 2010a)
				None	RVE	Beneficial	
				None	Chinese herbs	None	(Yuan et al., 2015)
	IMT	HDBR	60 days	None	Chinese herbs	None	(Yuan et al., 2015)
				↑ (17%)	Resistive exercise	Beneficial	(van Duijnhoven et al. 2010a)
					RVE	Beneficial	
Brachial	Diameter	HDBR	60 days	None	LBNP+aerobic+resistive exercise	None	(Hughson et al. 2007)
		HDBR	7 days	↑	Usual daily activity	Beneficial	(Bonnin et al. 2001)
		SCI (paraplegic)	11.6 ± 7.9 years	None	Usual daily activity (healthy volunteers)	None	(de Groot et al. 2004)
	FMD	HDBR	7 days	↑	Usual daily activity	Beneficial	(Bonnin et al. 2001)
		Bed rest	5 days	None	N/A	N/A	Hamburg et al. 2007
		SCI (paraplegic)	11.6 ± 7.9 year	None	Usual daily activity (healthy volunteers)	None	(de Groot et al. 2004)
Femoral	Compliance	HDBR	60 days	↓	Chinese herbs	None	(Yuan et al., 2015)
	Diameter	HDBR	60 days	↓	LBNP+aerobic+resistive exercise	Beneficial	Hughson et al. 2007
		HDBR	60 days	↓	Resistive exercise	None	Van Duijnhoven et al. 2010a
		ULLS	28 days	None	Usual daily activity	None	(Bleeker et al. 2005a)
		HDBR	60 days	↓	Chinese herbs	None	(Yuan et al., 2015)

					RVE	Beneficial	(van Duijnhoven et al. 2010b)
		Leg casting	7 days	↓ (6%)	Usual daily activity	Beneficial	(Sugawara et al. 2004)
		ULLS	28 days	↓ (13%)	Usual daily activity	Beneficial	(Bleeker et al. 2005a)
		HDBR	25 days	↓ (13%)	RVE	Beneficial	
			52 days	↓ (17%)			
		SCI (paraplegic)	21 days	↓ (25%)	None	N/A	(de Groot et al. 2006b)
		SCI (paraplegic)	11.6 ± 7.9 years	↓ (30%)	Usual daily activity (healthy volunteers)	N/A	(de Groot et al. 2004)
	FMD	HDBR	25 days	None	None	N/A	(Bleeker et al. 2005c)
			52 days	None	RVE	N/A	
			60 days	↑	RVE	Beneficial	(van Duijnhoven et al. 2010b)
					Resistive exercise	None	
		ULLS	28 days	↑	None	None	(Bleeker et al. 2005a)
		SCI (paraplegic)	21 days	↑ (23%)	None	None	(de Groot et al. 2006b)
			11.6 ± 7.9 year	None (preserved)	Usual daily activity (healthy volunteers)	None	(de Groot et al. 2004)
	IMT	Leg casting	7 days	None	Usual daily activity	None	(Sugawara et al. 2004)
	IMT	HDBR	60 days	↑ (13%)	Resistive exercise	Beneficial (partial abolishment)	(Van Duijnhoven et al. 2010a)
	IMT	HDBR	60 days	↑ (13%)	RVE	Beneficial (partial abolishment)	(Van Duijnhoven et al. 2010a)
Microcirculation	Endothelial functions: Forearm plethysmography	HDBR	14 days	↓	Low fat hypoenergetic diet	Beneficial	(Hesse et al. 2005)
	Endothelial functions: skin vaso active substance		60 days	↓	LBNP +aerobic +resistif exercise	Beneficial	(Demiot et al. 2007)

	Markers of endothelium injury: CEC			↑	LBNP+ exercise	Beneficial	
Microcirculation	Endothelial function: skin vasodilation	DI	7 days	↓	None	N/A	(Navasiolava et al. 2010)
Microcirculation	Markers of endothelium injury: EMPs	DI	7 days	↑	None	N/A	(Navasiolava et al. 2010)
Microcirculation	Markers of endothelium injury: EMPs	HDBR	60 days	↑	Chinese herbs	Beneficial	(Yuan et al., 2015)
Microcirculation	Soluble markers of endothelial state: VEGF	DI	7 days	↓	None	N/A	(Navasiolava et al. 2010)
	TPR						
Lower limb resistance vessels	Index of remodeling: peak reactive hyperemic blood flow following 5-min ischemia	HDBR	52 days	↓ 28%			(Bleeker et al. 2005)
upper limb resistance vessels	Marker of remodeling: peak reactive hyperemic blood flow following 10-min ischemia	HDBR	14 days	↓ 31%			(Schoemaker et al 1998)
Skin microcirculation	Maximal cutaneous vasodilator and sweating capacity	HDBR	14 days	impairment	cycling ergometry	beneficial	(Crandall et al. 2003)

HDBR, head-down bed rest; DI, dry immersion; IMT, intima-media thickness; FMD, flow-mediated dilation; SCI, spinal cord-injured; ULLS, unilateral lower limb suspension; LBNP, lower body negative pressure; RVE, resistive vibration exercise; CEC, circulating endothelial cells; EMPs, endothelium-derived microparticles; VEGF, vascular endothelium growth factor

Effets des herbes chinoises TaikongYangXin sur les fonctions vasculaires

au cours d'un alitement anti-orthostatique de 60 jours

Article publié :

"Effect of Chinese herbal medicine on vascular functions during 60-day head-down bed rest"

La médecine traditionnelle chinoise comprend une utilisation très ancienne et empirique d'extraits végétaux pour entretenir une bonne santé et un équilibre dans les fonctions physiologiques. Environs 500 herbes chinoises sont utilisées et beaucoup d'entre elles ont des effets potentiels sur le système cardiovasculaire. Dans la médecine traditionnelle chinoise, l'efficacité ne réside pas dans l'utilisation d'un principe actif bien identifié mais dans la synergie de multiples substances en faibles quantités.

L'objectif de ce travail était d'étudier les effets prophylactiques de la composition du TaikongYangXin, développé par l'agence spatiale chinoise, contre les atteintes vasculaires induites par un alitement de 60 jours.

14 sujets (7 sujets contrôles, 7 sujets prenant quotidiennement du TaikongYangXin) ont participé à cette étude et ont été soumis à un aliment non strict de 60 jours. La macrocirculation a été étudiée par échographie vasculaire au niveau de la carotide et au niveau de l'artère fémorale superficielle. La microcirculation a été étudiée par la technique d'iontophorèse appliquée au niveau cutanée. Biologiquement ont été étudiés les fonctions endothéliales, le bilan lipidique et la sensibilité à l'insuline.

Les 60 jours d'alitement ont induit une diminution de 33 % du diamètre de l'artère fémorale superficielle et de sa compliance alors que l'artère carotide restait inchangée. L'étude par iontophorèse a montré que l'alitement induisait une atteinte de la fonction endothéliale associée à une augmentation des microparticules endothéliales circulantes. La prise quotidienne de TaikongYangXin n'a pas modifié les caractéristiques de la macrocirculation, par contre elle permettait d'améliorer les fonctions endothéliales au niveau de la microcirculation.

TaikongYangXin a donc un effet bénéfique sur la microcirculation, probablement en impliquant la voie du monoxyde d'azote. Plusieurs composés de cette préparation ont potentiellement des effets bénéfiques sur les fonctions endothéliales.

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ORIGINAL ARTICLE

Effect of Chinese herbal medicine on vascular functions during 60-day head-down bed rest

Ming Yuan[1] · Asmaa Alameddine[2] · Mickael Coupé[2] · Nastassia M. Navasiolava[2] · Yongzhi Li[1] · Guillemette Gauquelin-Koch[3] · Yanqiang Bai[1] · Shizhong Jiang[1] · Yumin Wan[1] · Jingyu Wang[1] · Yinghui Li[1] · Marc-Antoine Custaud[2,4]

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Abstract
Purpose Chinese herbal medicine is a promising countermeasure against cardiovascular dysfunction associated with a sedentary lifestyle. We examined the impact of the Chinese herb, Taikong Yangxin, on the micro- and macrovascular dysfunction associated with a 60-day bed rest.
Methods Fourteen healthy men were randomly divided into two groups: those given herbal supplement, and the control group; the two groups underwent a 60-day bed rest. The macrovasculature was assessed by sonography. Skin microvascular functions were assessed with laser Doppler. The plasma level of endothelial microparticles (EMPs), markers of endothelial injury, was determined.
Results Bed rest induced a 33 % decrease in the femoral artery diameter and compliance whereas carotid wall thickness, diameter, and compliance remained unchanged. The early phase of endothelium-dependent vasodilation to ACh was unmodified by bed rest, while the late phase was reduced by 30 % along with a twofold increase in EMPs. In those given Taikong Yangxin, the early phase was amplified by 2.5-fold, and the effects of bed rest on the late phase were prevented.
Conclusion These findings indicate that Taikong Yangxin ameliorates endothelium-dependent vasodilation, likely by improving the NO pathway. The study suggests Taikong Yangxin as a new countermeasure to prevent the changes in microvascular function induced by physical inactivity.

Keywords Physical inactivity · Microcirculation · Endothelium-dependent vasodilation · Endothelial impairment · Conduit vessels

Abbreviations

ACh	Acetylcholine
CHM	Chinese herbal medicine
DBP	Diastolic blood pressure
EMPs	Endothelial microparticles
HDBR	Head-down bed rest
HDL	High-density lipoproteins
HOMA-IR	Homeostasis assessment model-insulin resistance
HR	Heart rate
LDF	Laser doppler flowmetry
LDL	Low-density lipoproteins
NO	Nitric oxide
SBP	Systolic blood pressure
SNP	Sodium nitroprusside
TK	Taikong Yangxin

Introduction

Sedentary lifestyle is an independent risk factor for cardiovascular disease. The associated risks exceed the risks

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✉ Yinghui Li
yinghuidd@vip.sina.com

✉ Marc-Antoine Custaud
mcustaud@gmail.com

[1] State Key Laboratory of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, Beijing, China

[2] UMR CNRS 6214, INSERM 1083, Université d'Angers, UFR Médecine, rue Haute de Reculée, 49045 Angers Cedex, France

[3] Centre National d'Etudes Spatiales, Paris, France

[4] Centre de Recherche Clinique du CHU d'Angers, d'Angers, France

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linked with smoking, elevated blood pressure, hypercholesterolemia, or obesity, while regular exercise is associated with a reduction in adverse vascular events (Thijssen et al. 2010). Experimental models of physical inactivity such as the reduction in locomotion to less than 5000 steps per day (Boyle et al. 2013), short-term and long-term bed rest (Demiot et al. 2007; Hamburg et al. 2007) or dry immersion (Navasiolava et al. 2010) each induce significant alterations to vascular homeostasis including impaired endothelial integrity, compromised microvascular endothelial function (Hesse et al. 2005; Demiot et al. 2007; Hamburg et al. 2007; Navasiolava et al. 2010; Boyle et al. 2013), and arterial remodeling (Thijssen et al. 2010). All of these are prevented or blunted by an increase in exercise (Demiot et al. 2007; Thijssen et al. 2010; Widlansky 2010). However, exercise is not always possible, such as in cases of injury, illness, or other physical limitations. In these cases, inactivity must be addressed by other measures. For example, hypoenergetic, low-fat nutrition was shown to improve endothelial function in volunteers subjected to 2-week head-down bed rest (HDBR) (Hesse et al. 2005).

Derived from a longstanding history and cultural use, traditional Chinese medicine focuses on the integrity of the human body and its close relationship with the environment (Lu et al. 2004). Traditional Chinese medicine is well established and commonly implemented in Chinese society. Chinese herbal medicine (CHM) is one of the most important modalities of traditional Chinese medicine. Approximately, 500 Chinese herbs are currently used, and a number of them are associated with primary cardiovascular indications (Mashour et al. 1998; Tang et al. 2009). The Taikong Yangxin (outer space heart-nourishing) prescription was developed by the China Astronaut Research and Training Center (Astronaut Center of China, ACC) to boost the physical condition of astronauts and improve their adaptability to an extreme environment (Li et al. 2010). We have previously tested the effects of Taikong Yangxin on the cardiovascular response to an orthostatic test (Yuan et al. 2012). We have shown in particular that Taikong Yangxin contributed to prevent the loss of vasoconstriction capacity in orthostatic position at the femoral area level (Yuan et al. 2012).

In the current study, we hypothesized that the physical inactivity during HDBR would induce generalized vascular impairment prevented by Taikong Yangxin. We analyzed vascular remodeling at the macro- and microcirculatory level with specific emphasis on endothelial function and examined whether the CHM Taikong Yangxin prescription could improve the compromised vascular state induced after extended HDBR.

Materials and methods

Subjects

A total of 14 healthy, non-athletic men (age 30 ± 1 year, weight 62 ± 1 kg, height 169 ± 1 cm; mean ± SE) were included in the 60-day HDBR experiment. The subjects had no history of cardiovascular or other major diseases, and they were not taking medication prior to the experiment. The experiment was organized as a double-blind trial. Volunteers were randomly assigned into either the "control" group given a placebo ($n = 7$) or the "herbs" group given the CHM Taikong Yangxin ($n = 7$). "control" and "herbs" groups were studied in parallel to account for seasonal variability in biorhythms and endothelial response. All procedures and risks associated with the experiments were explained, and written consent was obtained from all participants. The experiment was performed in accordance with approved guidelines. The experimental protocol conformed to the Helsinki Declaration and was approved by the ethical committee of the ACC scientific board.

Study design

The earth star international bed rest experiment project (ES-IBREP) protocol was held at the ACC in Beijing, China, in cooperation with the French National Center of Space Studies (CNES, France). Participants arrived 15 days prior to the HDBR for baseline measurements (B-15 to B-1) and left after 24 days of recovery (R+1 to R+24). The −6° HDBR lasted for 60 days. The subjects were permitted a daily 10-min period of standing for hygienic requirements. They were held in the common room and could interact freely. They had also leisure time to read, watch TV, etc. Medical staff supervised the subjects 24 h/day throughout the study. During the entire experiment, coffee, tea, and nicotinic substances were prohibited. Daily caloric intake was 2600–2800 kcal, which consisted of 55–60 % carbohydrate, 27–30 % fat and 13–14 % protein. Water intake was ad libitum. The photoperiod was 16 h light and 8 h dark. Body weight, heart rate, and blood pressure were regularly measured. This 60-day HDBR study was an integrative international study with several protocols performed on different domains. Some results on this integrative study have already been published (Chan et al. 2010; Arbeille et al. 2011; Coupé et al. 2011; Yuan et al. 2012; Shi et al. 2014). This study was registered to the Chinese clinical trial registry with the number ChiCTR-TRC-12002254.

Countermeasure: Chinese herbal medicine

The Taikong Yangxin (TK) prescription is a Chinese herb extract composed of over 10 Chinese herbs. The ingredients include *Panax ginseng* (root), *Astragalus membranaceus* (root), *Ligusticum wallichii* (rhizome), *Schisandra chinensis* (berries), *Ophiopogon japonicus* (root), *Rehmania glutinosa* (root), *Drynaria fortunei* (rhizome), and *Poria cocos* (sclerotium). To prepare the Taikong Yangxin extract, water was added to mashed herbs (10:1 water to herb, in volume) and the mixture was boiled for 1 h; the liquid component was then collected and removed, and fresh water was added to the herbs. The mixture was boiled again for 1 h, and the liquid was once again collected. The liquid components were combined and concentrated to a density of 1 g of herb weight per 1 ml (i.e., drug to extract ratio of 1:1). The Taikong Yangxin extract was administered in the form of a 6 g oral honeyed pill, three times a day. The control group received a placebo composed of starch and dextrin.

Macrovessels: ultrasound measurements

Measurements at the level of the left common carotid artery and superficial femoral artery were performed using an echo-Doppler device (Vivid-7, GE), operated by the same person, prior to (B-6) and at the end (day 52) of the HDBR, at the same time of the day, with participants in supine position. Images of the common carotid artery were performed 3 cm before the bifurcation. Images of the superficial femoral artery were made 3 cm distal to the bifurcation of the femoral artery, and 10 s clips were recorded. Systolic (SBP), diastolic (DBP) blood pressure, and heart rate (HR) were measured before sonography using Dinamap (GE Carescape V100) and were continuously measured during sonography using an infrared finger photoplethysmograph (Cardiopres®, CardioSpace System, CNES). Analysis was performed using an online service (www.televasc.fr) to estimate the intima-media thickness of the carotid artery and the basal diastolic diameter and compliance of both the carotid and femoral arteries. Compliance was calculated using the following equation:

$$\text{Compliance coefficient} = \pi(2D \times \Delta D + \Delta D^2)/4\Delta P,$$

where D is the mean arterial diastolic diameter, ΔD is the mean change in diameter during the heart cycle, and ΔP is the variation in mean pulse pressure.

Evaluation of skin microcirculation

The functional properties of skin microcirculation, at the calf level, were evaluated prior to (B-12) and at the end of HDBR (day 55). We examined basal blood flow, endothelium-dependent and endothelium-independent vasodilation, and maximal vasodilation.

To evaluate the skin perfusion, the technique of laser Doppler flowmetry (LDF; Periflux PF4001, Perimed, Sweden) coupled with iontophoresis was applied as previously reported (Demiot et al. 2007; Navasiolava et al. 2010). We used two specifically designed iontophoretic probes (PF 481-1, Perimed) to allow for local temperature measurement, current application, local heating, and cutaneous blood flow recording. A third probe (PF408; Perimed) was used as a reference 5 cm from the iontophoretic sites. Skin temperature was maintained between 34 and 35 °C. The probes were placed within the same topography in all subjects respective to the distal apex of patella.

Stable baseline data were measured for 5 min before current application was performed.

The iontophoresis of 4 % acetylcholine chloride (ACh; 10 s, 0.1 mA anodal current) and 2 % sodium nitroprusside (20 s, 0.1 mA cathodal current) was applied to assess endothelium-dependent and -independent vasodilation, respectively. The response was estimated over 20 min following stimulation. ACh induces a biphasic vascular response with an early (peak) and late (plateau) phase. Both the peak and plateau vasodilation were estimated for the ACh-induced response. The SNP-induced vascular response was measured at the plateau. At the end of the experiment, the active probes were continuously warmed to 44 °C for 20 min to cause maximal cutaneous vasodilation.

For LDF data analysis, we defined the following parameters: 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 following current application), plateau (the mean for the last minute of recovery after stimulation), and maximal vasodilation (the mean for the last minute of local heating). Response to iontophoresis was expressed as a percentage of maximal vasodilator response to local heating from the same active probe.

Blood studies

Two samples of antecubital venous blood samples were collected, one in the morning before breakfast at baseline (B-11), and the second was taken at the end of HDBR (day 60). Total cholesterol, HDL (high-density lipoprotein) cholesterol, triglycerides, and glucose were assessed by enzymatic calorimetric methods. LDL (low-density lipoprotein) cholesterol was calculated using the Friedewald formula. Insulin was measured using a radioimmunoassay method. Homeostasis

Table 1 Effect of 60-day HDBR without (control) or with Traditional Chinese medicine (herbs) countermeasure on subject characteristics and biological data

Variable	Control		Herbs	
	Before	End	Before	End
Age (years)	30.3 ± 1.2		30.3 ± 2.1	
Body mass (kg)	63.1 ± 1.0	64.5 ± 0.7	60.6 ± 2.0	61.4 ± 2.0
SBP (mmHg)	119 ± 4	115 ± 7	122 ± 3	116 ± 5
DBP (mmHg)	71 ± 4	71 ± 3	71 ± 3	72 ± 4
HR (bpm)	74 ± 4	76 ± 3	72 ± 3	70 ± 4
Carotid intima-media thickness (mm)	0.39 ± 0.03	0.40 ± 0.04	0.38 ± 0.03	0.36 ± 0.03
EMPs (*n*/μl)	45.5 ± 6.6	96.7 ± 22.9*	57.3 ± 14.2	49.7 ± 16.7
Skin temperature (°C)	34.8 ± 0.3	34.3 ± 0.1	35.0 ± 0.1	34.3 ± 0.1*
Fasting glucose (mmol/l)	4.26 ± 0.13	4.40 ± 0.12	4.47 ± 0.21	4.21 ± 0.08
Fasting insulin (μU/ml)	13.2 ± 1.8	14.0 ± 1.6	12.8 ± 1.9	10.8 ± 1.5
HOMA-IR	2.54 ± 0.42	2.73 ± 0.30	2.48 ± 0.29	2.0 ± 0.3
Total plasma cholesterol (mmol/l)	3.96 ± 0.15	3.58 ± 0.14	3.85 ± 0.37	3.14 ± 0.18
HDL cholesterol (mmol/l)	1.58 ± 0.20	1.22 ± 0.11*	1.36 ± 0.13	1.34 ± 0.10
LDL cholesterol (mmol/l)	1.52 ± 0.08	1.38 ± 0.07	1.36 ± 0.15	1.19 ± 0.07
Triglycerides (mmol/l)	1.84 ± 0.19	2.30 ± 0.43	1.62 ± 0.34	1.32 ± 0.16

Values are mean ± SE

* $p \leq 0.05$ vs. before

model assessment-insulin resistance (HOMA-IR) index was calculated as fasting insulin concentration (μU/ml) × fasting glucose concentration (mmol/l)/22.5 (Matthews et al. 1985). Assessment of circulating endothelial microparticles (EMPs) was performed by a BD FACSAria II cell sorter. Briefly, after a centrifugation procedure, platelet-free plasma samples were frozen at −80 °C until experimental analysis. Aliquots (50 μl) of platelet-poor plasma were incubated with 4 μl of anti-CD31-FITC and 4 μl of anti-CD42b-PE (BD Pharmingen™, San Diego, CA, USA) for 20 min, then diluted with 1 ml of PBS in BD TruCOUNT tubes (BD, San Jose, CA, USA) and analyzed. EMPs were defined as $CD31^{+}/CD42b^{-}$ particles. Data were processed using the BD FACSDiva software, and values were reported as numbers per microliter.

Statistical analysis

Data are presented as mean ± SE. The overall effect of bed rest and the effect of the countermeasures were tested with 2-way repeated-measures ANOVA, with day of measurement as the within-subject factor (baseline, end of HDBR) and group as the between-subject factor (control and herbs groups). Statistically significant differences were further analyzed by pairwise comparisons (least significant difference). A p value of ≤ 0.05 was considered significant. Analyses were performed using SPSS 15.0 for Windows.

Results

General data

During the HDBR, body weight, blood pressure and heart rate did not change significantly (Table 1).

Macrovascular measurements

Intima-media thickness of the common carotid artery (Table 1) and carotid artery diameter and compliance (Fig. 1a, b) showed no change after HDBR in both groups. HDBR induced an approximately 33 % decrease in the superficial femoral artery diameter, which was similar across groups (Fig. 1c). After HDBR, we observed a sharp drop in the superficial femoral compliance of approximately 68 % in the control group and 43 % in the Herbs group, without significant difference between groups (Fig. 1d).

Testing of microvascular functions

Basal skin blood flow, maximal vasodilation in response to heating, and endothelium-independent vasodilatory response to SNP were not significantly modified upon HDBR in both groups (Fig. 2a–c). In the control group, the peak response to ACh was not altered by bed rest. However, the plateau vasodilation was significantly reduced by

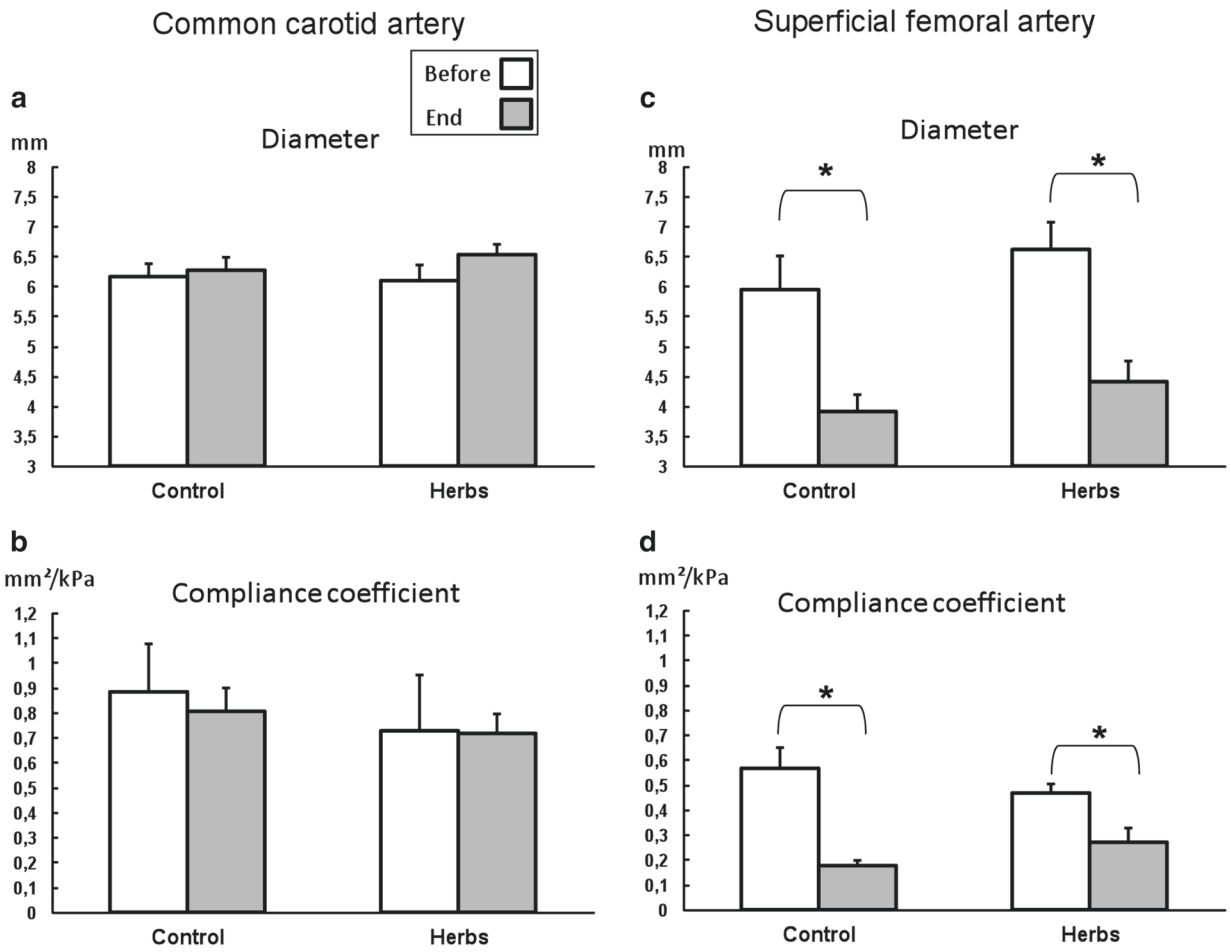


Fig. 1 Resting diameter and compliance coefficient of the common carotid and superficial femoral arteries before and at the end of 60-day HDBR. Values are mean ± SE, $*p \leq 0.05$ vs. before

approximately 30 % in this group. In the Herbs group, the peak response to ACh was elevated approximately 2.5-fold at the end of HDBR. This response was significantly higher compared to the control group ($p = 0.001$). The plateau vasodilation to ACh was not significantly modified in the Herbs group ($p = 0.18$), although the data trended towards an increase (Fig. 2d, e).

Blood studies

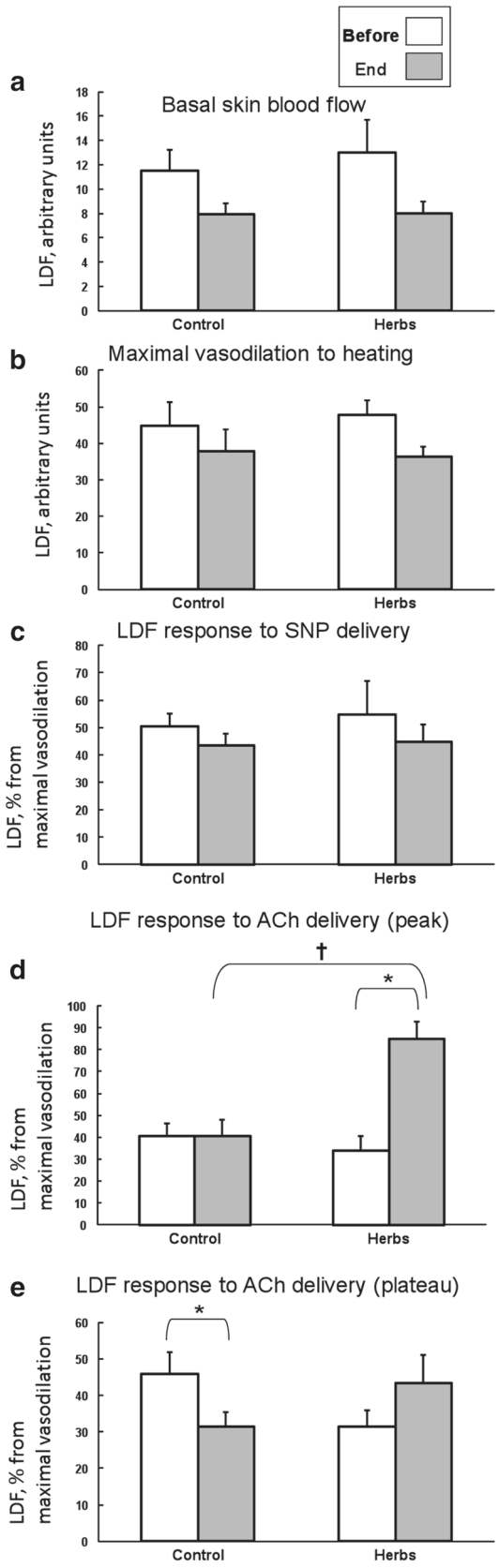
Fasting blood glucose and insulin, estimated insulin resistance, triglycerides, total cholesterol and LDL fraction were not significantly modified by HDBR in both groups. The HDL fraction decreased at the end of HDBR in the control group ($p = 0.014$), whereas no change was observed in the Herbs group. LDL cholesterol and triglyceride levels did not significantly change after HDBR in both groups. We observed a significant increase (twofold) in circulating EMPs at the end of HDBR in the control group ($p = 0.038$). EMPs levels were unchanged in the Herbs group (Table 1).

Discussion

The CHM, Taikong Yangxin prescription, protected endothelial integrity and improved microvascular function after 60 days of HDBR in men. However, at the macrocirculatory level, CHM had no effect on the observed decrease in diameter and impaired elastic properties resulting from bed rest at the femoral artery level.

Effect of HDBR on microcirculatory vessels

In the control group, we observed an attenuation of the plateau phase of endothelium-dependent vasodilation following HDBR at the skin microcirculatory level. This was accompanied by an increase in circulating EMPs. Decreased vascular shear stress due to physical inactivity is an important mechanism to be taken into account. Inactivity-induced inward remodeling preserves shear stress of conduit vessels (Thijssen et al. 2010), but not of resistance vessels. Meanwhile, a chronic decrease in shear stress during physical



◀**Fig. 2** Calf skin blood flow defined by laser Doppler before and at the end of 60-day HDBR: basal (**a**); stimulated by local heating up to 44 °C (**b**), SNP iontophoresis (**c**), and ACh iontophoresis on peak (**d**) and plateau (**e**) phases. Values are mean ± SE, $*p < 0.05$ vs. before, $^{\dagger}p < 0.05$ vs. control group

inactivity is especially harmful for microcirculation, as the physiologic level of shear stress is much greater in arterioles (40–60 dynes/cm^2) than in large arteries (10–30 dynes/cm^2) (Boisseau 2005). Our results on endothelial impairment are in line with previous findings involving experimental physical inactivity (Hesse et al. 2005; Demiot et al. 2007; Navasiolava et al. 2010; Boyle et al. 2013).

Effect of HDBR on conduit arteries

We observed a 33 % decrease in baseline diameter and a drop in the compliance of the superficial femoral artery at the end of the 60-day HDBR. Contrarily, the carotid diameter and compliance remained stable. Our data agree with the results of van Duijnhoven et al. (2010) (The second Berlin bed rest study) who reported a 24 % decrease in diameter of the superficial femoral artery during the 60-day HDBR, but no changes in the carotid artery diameter. Previous data have indicated that physical inactivity with the associated decrease in shear stress is a primary stimulus for the decrease in superficial femoral artery diameter (de Groot et al. 2006; Thijssen et al. 2010). A decrease in transmural pressure, itself depending on variations in hydrostatic pressure, may also help explain vascular remodeling at the superficial femoral artery level. The differential response of the carotid and femoral arteries to HDBR may be explained by the differences in shear stress changes resulting from their relative location with respect to the hydrostatic indifferent point, along with their differing wall structures. HDBR significantly diminishes transmural pressure at the femoral level, but not at the carotid level. Furthermore, the muscular layer plays an active role in inward remodeling. This may impact the response to HDBR as the femoral artery is a muscular artery, while the carotid is an elastic artery with relatively small muscular media. Unlike the findings reported by van Duijnhoven et al. (2010), we did not find an increase in the intima-media thickness of the carotid artery after HDBR.

Effect of Chinese herbal medicine: possible mechanisms of improved endothelium-dependent vasodilation at the microcirculatory skin level

In assessing the effects of CHM, it must be taken into account that the herbal formulas used in traditional Chinese

medicine consist of least three and can contain as many as twenty-five ingredients (Tang et al. 2009). The balance and interaction of these components (synergic, catalytic, diminishing negative side effects of certain components, etc.) are considered more important than the effect of individual ingredients. Many herbs are used to primarily treat cardiovascular pathologies (atherosclerosis, hypertension, cerebral and peripheral vascular diseases, etc.) (Lu et al. 2004). These pathologies are typically associated with nitric oxide insufficiency.

Tang et al. (2009) revealed that commonly used "cardiovascular" Chinese herbs, including ginseng (9 herbs were tested) contain high concentrations of nitrate, generate NO from nitrite, and as such can relax blood vessels via endothelium-dependent and -independent mechanisms. We surmise that some, if not all, Chinese herbs used to treat cardiovascular diseases have a robust NO bioactivity that may act to restore NO homeostasis.

The Taikong Yangxin Prescription includes ginseng, astragalus, ligusticum and schisandra, all of which show vasodilative effects. *Panax ginseng* is an essential base of the Asiatic pharmacopeia, with a well-established reputation in Asia. Its active components, ginsenosides, increase vascular relaxation, as demonstrated in ex vivo studies for cerebral (Chen et al. 1997) and pulmonary (Chen 1996) circulation. This relaxation is eliminated by an inhibitor of NO synthase. Furthermore, a study using cultured endothelial cells showed that ginsenosides enhance the conversion of arginine to citrulline with NO production. Taken together, these data suggest the involvement of the NO pathway in the vasodilative action of ginseng (Chen 1996; Chen et al. 1997). This effect of ginseng may help explain our finding of an improved peak phase response to ACh in the Herbs group, which is considered to be mainly mediated by the NO pathway (Durand et al. 2004). Similarly, *Astragalus membranaceus* ameliorates endothelium-dependent vasodilation, as shown in aortic rings from obese rats (Tang et al. 2010) and aortic rings injured by free fatty acids (Wang and Yu 2011) or homocysteine (Qiu et al. 2010). An endothelial culture study showed that astragaloside can improve the NO–NO synthase pathway dysfunction resulting from oxidative stress (Qiu et al. 2010). Interestingly, one study was performed in humans to test on orthostatic tolerance, 14 days of Radix Astragali extract taken orally (Gao et al. 2008). This extract did not change significantly global hemodynamic responses during a head-up tilt test combined with lower body negative pressure; however, effects on microcirculation could not be tested. *Ligusticum wallichii* and *Schisandra chinensis* are widely used to treat vascular disorders in oriental countries. Their active components induce both endothelium-dependent (i.e., NO) and endothelium-independent vasodilation of aortic rings (Chan et al. 2006; Park et al. 2007, 2009). Thus, we speculate that the components of the Taikong Yangxin prescription might improve endothelium-dependent vasodilation through their effects on the NO pathway.

It must be noted that the effect of some of the components of Taikong Yangxin varies depending on the target vessel. Previous data indicate that ginsenosides cause the relaxation of the precontracted pulmonary and intrapulmonary arteries in the rabbit and also the mesenteric vein in dog, but this same compound potentiated the contractile response of the renal vein in both dogs and rabbits (Chen et al. 1984). Similarly, the active component of *Ligusticum wallichii* induced vasodilation in isolated coronary artery rings artery, but induced vasoconstriction in renal, femoral, and mesenteric arteries (Chiou et al. 1991). Both of these are active components in Taikong Yangxin. In this study, for the same set of subjects, we demonstrated that Taikong Yangxin helped maintain vasoconstriction during orthostatic stress within the superficial femoral artery area following HDBR (Coupé et al. 2011).

Limitations

The absence of exhaustive knowledge of CHM-TK compounds is the main limitation of this study. We did not determine the relative serum concentrations of the individual TK components. A central tenet of CHM is that the benefit supplied by the various herbal formulas results from the combined action of the multitude of active compounds (Lee 2000). TK contains numerous active components, and directly determining the major causal component(s) by simple blood serum concentrations would likely not yield decisive insights.

Based on long-term empirical knowledge, Taikong Yangxin combination was developed and tested in this study. We did not test individually each compound however. It is obviously a weakness of this work if we want to understand specifically the effects of each compound. However, our aim was to test the entire prescription of Taikong Yangxin as a potential countermeasure for long duration space flight.

Another limitation of this work is a relatively small subject number. Most of the studies performed with models of enhanced physical inactivity such as long-term bed rest or dry immersion involve small subject number and should be considered as basis for larger protocols.

Conclusion

This study suggests that the Chinese herbal medicine, Taikong Yangxin, may be a novel countermeasure to prevent changes in microvascular dysfunction induced by physical inactivity. At the macrovascular level, this countermeasure

did not preserve the diameter and the elastic properties. The Taikong Yangxin extract prevented the functional impairment of microcirculatory endothelium induced by 60-day HDBR. Several active components of Taikong Yangxin are known to increase NO–NO synthase pathway-dependent signaling. The resultant increase in NO production within the endothelium may in part explain the observed effects of Taikong Yangxin in this study.

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Conflict of interest None.

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Effets cardiovasculaires du salidroside dans le modèle du rat diabétique Goto-Kakizaki

Article publié :

"The cardiovascular effects of salidroside in the Goto-Kakizaki diabetic rat model"

Nous avons montré précédemment chez l'homme que les extraits végétaux combinés dans le TaikongYangXin avaient un effet bénéfique sur la fonction endothéliale au niveau de la microcirculation dans un contexte de sédentarité poussée.

L'objectif de cette deuxième étude était de tester l'hypothèse que le Salidroside, le principal composé de la plante Rhodiola Rosea, un composé végétal souvent utilisé, avait aussi des effets bénéfique vasculaires dans un modèle de rat spontanément diabétiques, le rat Goto-Kakizaki (GK).

4 groupes ont été étudiés (rats contrôles ou GK ; avec un placébo ou avec un traitement oral quotidien par Salidroside). Nous avons fait une évaluation cardiovasculaire (suivi télémétrique de la pression artérielle, de la fréquence cardiaque, réactivité vasculaire in vitro au niveau de l'aorte et de l'artère

mésentérique de 2ème ordre, expression de la eNOS/phospho-eNOS) et métabolique (taux plasmatiques de fructosamine, test oral de tolérance au glucose).

Les rats GK présentaient une hypertension artérielle une intolérance au glucose marquée, ainsi qu'une atteinte de la vasodilation endothéliale-dépendante et endothéliale-indépendante. Le salidroside n'a eu aucun effet contre l'hypertension ou contre les troubles du métabolisme glucidique chez le rat GK. Le salidroside a par contre montré un effet au niveau de la vasodilation endothéliale-dépendante et endothéliale-indépendante. Cet effet dépendrait de mécanismes d'action au niveau de l'endothélium lui-même et au niveau de la guanylate cyclase soluble des cellules musculaires lisses.

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A. ALAMEDDINE[1], Z. FAJLOUN[2], J. BOURREAU[1], G. GAUQUELIN-KOCH[3], M. YUAN[4], D. GAUGUIER[5], S. DERBRE[6], A. AYER[1], M.A. CUSTAUD[1], N. NAVASIOLAVA[1]

THE CARDIOVASCULAR EFFECTS OF SALIDROSIDE IN THE GOTO-KAKIZAKI DIABETIC RAT MODEL

[1]UMR CNRS 6214 - INSERM 1083, Faculte de Medecine, Universite d'Angers, Angers, France; [2]AZM Center for Research in Biotechnology, Doctoral School of Sciences and Biotechnology, Lebanese University, El Mittein Street, Tripoli, Lebanon; [3]CNES, Paris, France; [4]State Key Lab of Space Medicine Fundamentals and Application, China Astronaut Research and Training Center, Beijing, China; [5]Institute of Cardiometabolism and Nutrition, University Pierre and Marie Curie, Hospital Pitie, Paris, France; [6]EA 921 SONAS, SFR QUASAV, Universite d'Angers, Angers, France

Many factors, including hyperglycemia, hypertension, obesity, dyslipidemia, and a sedentary lifestyle, contribute to a high prevalence of cardiovascular disease. Specific vascular impairment treatments in the context of diabetes and vascular risk need to be improved. Salidroside is the primary active component of *Rhodiola rosea* and has documented antioxidative, cardioprotective, and vasculoprotective properties. The aim of this study was to test the hypothesis that salidroside has protective effects against hyperglycemia, hypertension, and vasodilation impairment in the Goto-Kakizaki (GK) rat model of diabetes. We evaluated cardiovascular parameters (e.g., daytime/nighttime systolic and diastolic blood pressure, heart rate, and activity), metabolic parameters (e.g., body weight, food and water consumption, serum fructosamine level, glucose tolerance), eNOS / phospho-eNOS expression level and *in vitro* vascular reactivity of aorta and second-order mesenteric arteries in Wistar-Kyoto (control) and GK (diabetic) rats treated with salidroside (40 mg/kg) or placebo (water) for 5 weeks. GK rats showed hypertension, marked glucose intolerance, and impaired endothelium-dependent and endothelium-independent vasodilation capacity. Salidroside showed beneficial effects on endothelial and non-endothelial vasodilation and likely acts on the endothelium and smooth muscle cells through the soluble guanylyl cyclase pathway. Despite its vascular effects, salidroside had no effect on blood pressure and heart rate in GK and control rats, it did not improve glucose metabolism or limit hypertension in the GK model of type 2 diabetes.

Key words: *type 2 diabetes, salidroside, telemetry, Rhodiola rosea, vascular function, glucose intolerance, nitric oxide, hypertension, guanylyl cyclase*

INTRODUCTION

Many factors, including hyperglycemia, hypertension, obesity, dyslipidemia, and a sedentary lifestyle, contribute to the high prevalence of cardiovascular disease (CVD). The American Heart Association (AHA) considers diabetes mellitus and metabolic syndrome major risk factors for CVD (1).

Endothelial dysfunction, which is associated with decreased nitric oxide (NO) bioavailability and impaired vasodilation, is one of the earliest stages of cardiovascular events in diabetes. Impaired vasodilation has been noted in animal models of hyperglycemia and diabetes (2, 3), as well as in clinical studies for both type 1 and type 2 diabetes patients (4, 5). It has also been demonstrated that endothelial dysfunction plays an important role in the association of diabetes and hypertension (6).

Goto-Kakizaki (GK) rats are a unique non-obese rat model of polygenic type 2 diabetes that were developed by selective inbreeding of glucose-intolerant Wistar rats (7). GK rats are characterized by disturbed glucose-stimulated insulin release (8, 9) and islet blood flow (10), defective intracellular glucose metabolism (11), insulin resistance (12), and hyperinsulinemia (8, 10). They manifest stable pathological features that resemble human type 2 diabetes, including hyperglycemia, hypertension, and endothelial dysfunction (13). However, GK is a lean diabetes model, whereas most (but not all) patients with type 2 diabetes are overweight.

Salidroside (p-hydroxyphenethyl-β-D-glucoside) is the primary active component of *Rhodiola rosea*. This plant has been used for hundreds of years as a traditional medicine for different therapeutic purposes (14). Salidroside was found to have neuro-, cardio-, and hepato-protective activity (14). Salidroside and *Rodiola rosea* extracts have also been tested in humans to improve mental performance and to combat depression (14).

Salidroside might have beneficial effects on vascular functions. The noted *in vitro* cardiovascular effects of salidroside include cardio-protection (15) and protection of endothelial cells against hypoxia (16).

In *in vitro* studies, salidroside stimulates glucose uptake in skeletal muscle cells by activating phosphorylation of AMP-activated protein kinase (17). These findings suggest the possibility of its potential therapeutic application as an antidiabetic therapy. However, most studies regarding the effects of salidroside on

vascular function and glucose metabolism have been performed *in vitro*. *In vivo* studies are necessary to evaluate the potential therapeutic benefits of salidroside in the context of type 2 diabetes.

The aim of our study was to test the hypothesis that salidroside has protective effects against hyperglycemia, hypertension, and endothelial dysfunction in the rat GK model of diabetes.

MATERIALS AND METHODS

Animal protocols

Male Goto-Kakizaki (GK) rats (aged 12 weeks, n=26) were obtained from the academic laboratory UMRS 972 (Paris). Male Wistar Kyoto (WKy) control rats (aged 12 weeks, n=26) were obtained from Charles River Laboratories. Rats were synchronized to a 12 h light/12 h dark schedule, under controlled environmental conditions at an ambient temperature of 20–24°C with *ad libitum* food (standard chow SDS M20 rat) and water. Food and water consumption was monitored. Daily oral gavage with salidroside (40 mg/kg - WKy Sal, GK Sal) or water (placebo - WKy placebo, GK placebo) was started in 20-week-old rats and continued for 35 days. The selected dose of salidroside was similar to that used by other researchers (18, 19).

For telemetric assessment of heart rate (HR), blood pressure (BP), and spontaneous locomotor activity, 12 WKy rats (6 WKy placebo, 6 WKy Sal) and 11 GK rats (6 GK placebo, 5 GK Sal) were implanted prior to gavage at 17 weeks of age. For *in vitro* vascular reactivity tests, non-implanted WKy rats (7 WKy placebo, 7 WKy Sal) and GK rats (8 GK placebo, 7 GK Sal) were used. A schematic of the protocol used in this study is shown in *Fig. 1*.

All manipulations with animals were performed in accordance with the United States National Institutes of Health guidelines and European Community standards on the Care and Use of Laboratory Animals (Ministere de l'Agriculture, France, Authorization No. 49072). The Ethics Committee for Animal Experimentation of Pays de la Loire approved this study (Protocol No. CEEA. 2010.40).

Salidroside

Salidroside was purchased from Shanghai Tauto Biotech Company, Ltd. (China). The identity of salidroside was verified using nuclear magnetic resonance analyses. The ^{1}H spectrum was recorded in CD_3OD on a Jeol GSX 270 MHz (Jeol Europe, Croissy-sur-Seine, France) spectrometer and corresponded to that of salidroside (20). Its purity was evaluated using high-performance liquid chromatography (HPLC). HPLC analysis was performed on a Waters 2695 apparatus (Waters, Guyancourt, France) consisting of a pumping system, vacuum degasser, and DAD detector, and assisted by the Empower 2 software (Waters). A 10 µL sample (1 mg/mL) was directly injected onto a Lichrospher 100 RP18 column (150 × 4.6 mm; 5 µm; Agilent Technologies) using a gradient acidic water/methanol solvent system. The flow rate was 1 mL/min with UV detection at 254 and 275 nm. Salidroside was 100% pure at both wavelengths.

Cardiovascular parameters

A telemetry system (Data Science International® - DSI, St Paul, MN, USA) was used to monitor BP, HR, and locomotor activity for conscious, freely-moving rats. After 2–3 weeks of acclimatization to laboratory conditions and daily handling, 12 WKy rats and 11 GK rats were surgically fitted with intraperitoneal radiotelemetry transmitters (TA11PA-C40, DSI) according to the recommendations of DSI as previously described (21). Surgery was performed under isoflurane anesthesia. Anesthetized rats received an intramuscular injection of Temgesic® (buprenorphine, 0.1 mg/kg) to provide analgesia during the surgery. A blood pressure catheter was placed in the lower abdominal aorta and secured with surgical glue (3 M Vetbond™, USA). Its placement was verified using a radio receiver. The transmitter was secured in the abdomen by suturing to the muscle wall with a non-absorbable suture. Rats recovered for 14 days and received three subcutaneous injections of antibiotic (streptomycin, 40 mg/kg/day). Post-operative analgesia was provided by 40 mg/kg of pediatric ibuprofen (Advil®) in the drinking water for 3 days post-surgery. Rats were housed individually in standard cages during the first 3–4 days of recovery. After this period, each rat was placed with a non-operated partner. Non-operated partners were animals from the same cages in which the operated rats were housed before surgery. WKy rats were randomly assigned to the WKy placebo (n=6) or WKy Sal (n=6) groups, and GK rats were randomly assigned to the GK placebo (n=6) or GK Sal (n=5) groups.

Cardiovascular parameters and activity were then recorded telemetrically from 19 to 24 weeks of age.

Oral glucose tolerance tests (OGTT)

OGTT were performed 6 days before treatment with salidroside or placebo and on day 28 of treatment. Blood glucose (sampled from *v. caudale laterale*) was measured after an overnight fast before and 30 min, 1 h, and 2 h after a glucose

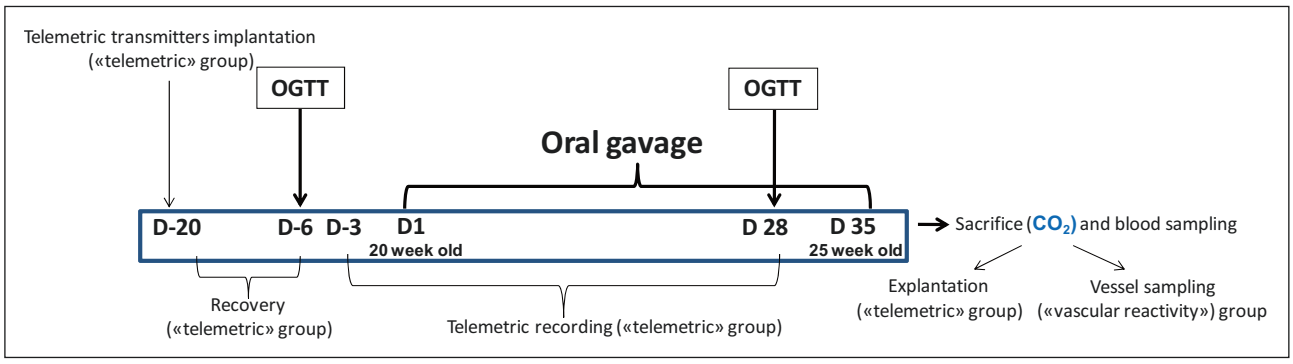


Fig. 1. Diagram of the study protocol. OGTT, oral glucose tolerance test; D -20, D -6, and D -3 refer to 20, 6, and 3 days before starting oral gavage, respectively; D1, D28, and D35 indicate days of gavage.

bolus (2 g/kg diluted in 1 mL of water). Blood tests were performed under isoflurane anesthesia using the Accu-Check Performa system (Roche Diagnostics GmbH, Germany).

Fructosamine assessments

After the 5-week treatment period, all rats were sacrificed by CO_2 inhalation. Blood samples were collected by cardiac puncture in glass tubes, and serum was separated by centrifugation at 4°C for 15 minutes. Serum samples were stored at –79°C. Fructosamine levels were assayed by photometric analysis on a Cobas® 8000 Modular Analyzer System (Roche Diagnostics, Japan). Immediately following blood sampling, rats from the "telemetric" group were explanted, and vessels from rats in the "vascular reactivity" group were dissected and divided into several segments used for vascular reactivity tests and for Western blot analysis.

Vascular reactivity

Two segments of second-order mesenteric arteries and two segments of thoracic aorta (2 mm long) were dissected from each rat (WKy placebo n=7, WKy Sal n=7, GK placebo n=8, GK Sal n=7). Segments were mounted on a wire myograph (DMT, Aarhus, Denmark) in physiological salt solution maintained at 37°C and pH 7.4 as described previously (22). After wall-tension normalization and stabilization for 45 minutes, vessel viability was tested with potassium-rich solution (80 mM). Endothelium

Table 1. Metabolic and cardiovascular parameters in control (WKy) and diabetic (GK) rats treated with salidroside or placebo.

Variable	**WKy placebo**	**WKy Sal**	**GK placebo**	**GK Sal**
Body weight before treatment (week 20), g	436 ±6	437±3	366±8*	359±6
Body weight (week 24) g	463±7	462±6	380±7*	367±7
Mean food consumption for 5 weeks (from 20 to 24 weeks), g/kg/day	48 1	46±1	56±2*	55±3
Mean water consumption for 5 week (from 20 to 24 weeks), mL/kg/day	50±1	50±1	99±13*	95±11
Serum fructosamine for week 25 µmol/L	153±3	152±2	187±9*	199±12
Fasting blood glucose, before treatment mg/dL	100±3	98±3	156±12*	163±13
Fasting blood glucose, week 4 of treatment mg/dL,	113±4	103±3	122±8	141±15
Blood glucose, before treatment T_120, mg/dL	124±3	118±4	301±23*	331±30
Blood glucose, week 4 of treatment T_120, mg/dL	125±3	121±2	278±20*	297±25
SBP day, before treatment mmHg	121±3	115±3	135±3*	134±4
SBP day, mean for 4 week of treatment mmHg (mean for 4 week of treatment)	121±3	117±2	138±4*	139±3
SBP night, before treatment mmHg	126±4	122±3	143±4*	143±4
SBP night, mean for 4 week of treatment mmHg	126±3	123±2	145±4*	146±3
DBP day, before treatment mmHg	85±4	82±2	96±2*	95±2
DBP day, mean for 4 week of treatment mmHg	85±3	83±2	98±2*	99±3
DBP night, before treatment mmHg	89±4	87±2	104±2*	104±2
DBP night, mean for 4 week of treatment mmHg	89±3	87±2	104±2*	105±3
HR day, before treatment bpm	290±4	280±3	272±2*	278±5
HR day, mean for 4 weeks of treatment bpm	294±5	284±4	279±3*	289±5
HR night, before treatment bpm	347±6	337±3	324±7*	339±15
HR night, mean for 4 weeks of treatment bpm	344±5	334±3	323±8*	339±14
Activity day, before treatment counts/min	1.0±0.2	1.0±0.1	0.8±0.05	0.9±0.1
Activity day, mean for 4 weeks of treatment counts/min	0.9±0.1	1.0±0.1	0.9±0.1	1.0±0.1
Activity night, before treatment counts/min	3.1±0.3	3.8±0.3	3.6±0.2	4.2±0.5
Activity night, mean for 4 weeks of treatment counts/min	2.8±0.3	3.4±0.3	2.9±0.1	3.5±0.4
Phospho-eNOS/eNOS aorta	1.45±0.21	1.30±0.27	1.61±0.30	1.59±0.36
Phospho-eNOS/eNOS mesenteric artery	0.94±0.13	0.88±0.08	0.90±0.07	0.94±0.13

Values are expressed as mean ± S.E.; * P<0.05 WKy-placebo vs. GK placebo; No significant difference was observed for WKy placebo vs. WKy Sal; No significant difference was observed for GK placebo vs. GK Sal.

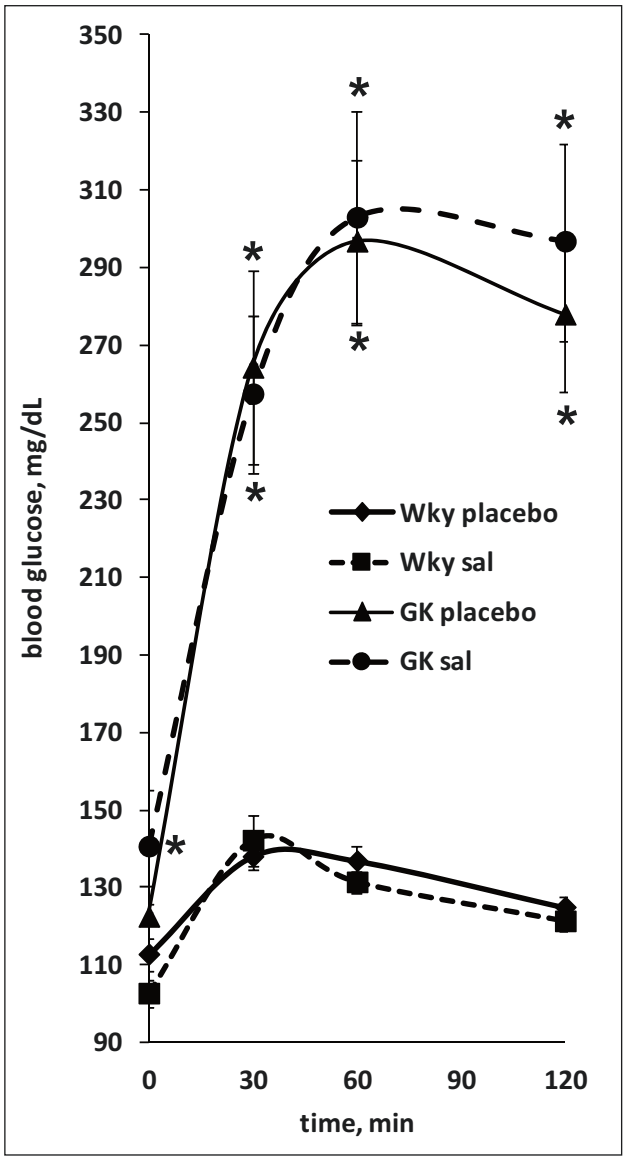


Fig. 2. Evaluation of glucose tolerance on D28 after treatment with salidroside or placebo. * P<0.05 vs. WKy-placebo rats.

integrity was controlled by evaluating acetylcholine-induced relaxation as described previously (22).

Endothelium-independent vasodilatory functions were assessed by CRC to sodium nitroprusside (SNP 10^{-9}–10^{-5} M) after pre-contraction with Phe 10^{-6} M. Between the curves, we applied a wash out and equilibration period of 20 min. The relaxant responses to acetylcholine and SNP were calculated as percentages of the precontraction by 10^{-6} M Phe.

Western blot analysis

Other segments of second-order mesenteric arteries and thoracic aorta were homogenized. Proteins (25 mg total protein from each sample) were separated by SDS-PAGE using a 4% stacking gel, followed by a 10% running gel. Proteins were detected with specific antibodies (eNOS 1:1,000, phospho-eNOS 1:500, and β-actin 1:1,000 in bovine serum albumin in Tris-buffered saline with Tween [T-TBS-BSA] 5%; Transduction Laboratories). Protein expression was visualized using the ECL Plus chemiluminescence kit (Amersham).

Statistical analysis

Data are presented as mean ± standard error (S.E.). For weight dynamics, food and water consumption, glucose tolerance, and telemetric variables, we used two-way analysis of variance (ANOVA), with time as the within-subject factor and with group as the between-subject factor. Statistically significant differences were further analyzed by pairwise comparisons. For studies on isolated vessels, analyses of dose-response curves were performed. Sensitivity (median effective concentration (EC50)) and maximal response were calculated from the respective dose-response equations. Comparison of differences was performed with one-way ANOVA, with a post hoc Bonferroni tests. Differences were considered statistically significant if P≤0.05. Analysis was performed using GraphPad Prism 6.

RESULTS

General characteristics

The body weights of 20-week-old WKy rats were approximately 20% higher compared to those of GK rats of the same age. Weight gains for the 24th weeks of life were higher in WKy rats (approximately +25 g - WKy vs. approximately +10 g - GK) (*Table 1*). However, food consumption per unit of body weight was 15–20% higher in GK rats, and relative water consumption was twice higher in GK rats compared to WKy rats. Serum fructosamine concentrations at 25 weeks of age were approximately 30% higher in GK rats. Fasting glucose in GK rats slightly increased, and fed glucose levels during OGTT were significantly higher in GK rats compared to those in WKy rats (*Table 1*, *Fig. 2*).

Salidroside did not affect body weight, serum fructosamine concentration, or glucose tolerance (*Table 1*, *Fig. 2*).

Blood pressure, heart rate, and activity

Telemetric monitoring of cardiovascular parameters showed a significant increase in systolic and diastolic blood pressure in GK rats (approximately 20 mmHg higher for SPB and 15 mmHg higher for DBP), along with a reduction in heart rate. There was no difference in activity between the different groups (*Table 1*). Salidroside had no significant effect on blood pressure, heart rate, or activity in GK rats and control rats.

Vascular reactivity

In aortic rings from GK placebo rats, maximal vasodilation capacity to acetylcholine (ACh) was impaired. There was also a slight decrease in maximal SNP vasodilation capacity (*Fig. 3*, *Table 2*). Mesenteric artery segments from GK placebo rats showed relaxation was very slightly impaired (small decrease in log EC50 but maximal vasodilation to ACh was unchanged) in response to ACh, with a decrease in maximal vasodilation capacity to SNP (*Fig. 4*, *Table 2*).

Chronic salidroside treatment did not affect WKy rats; however, in GK rats, vasodilatory response to SNP was preserved both in mesenteric arteries and aortic rings. Salidroside treatment improved vasodilation in response to ACh (maximal response) in aortic rings from GK rats.

Western blot analysis

There was no difference in eNOS, phospho-eNOS, and the ratio of phospho-eNOS to eNOS expression level in the

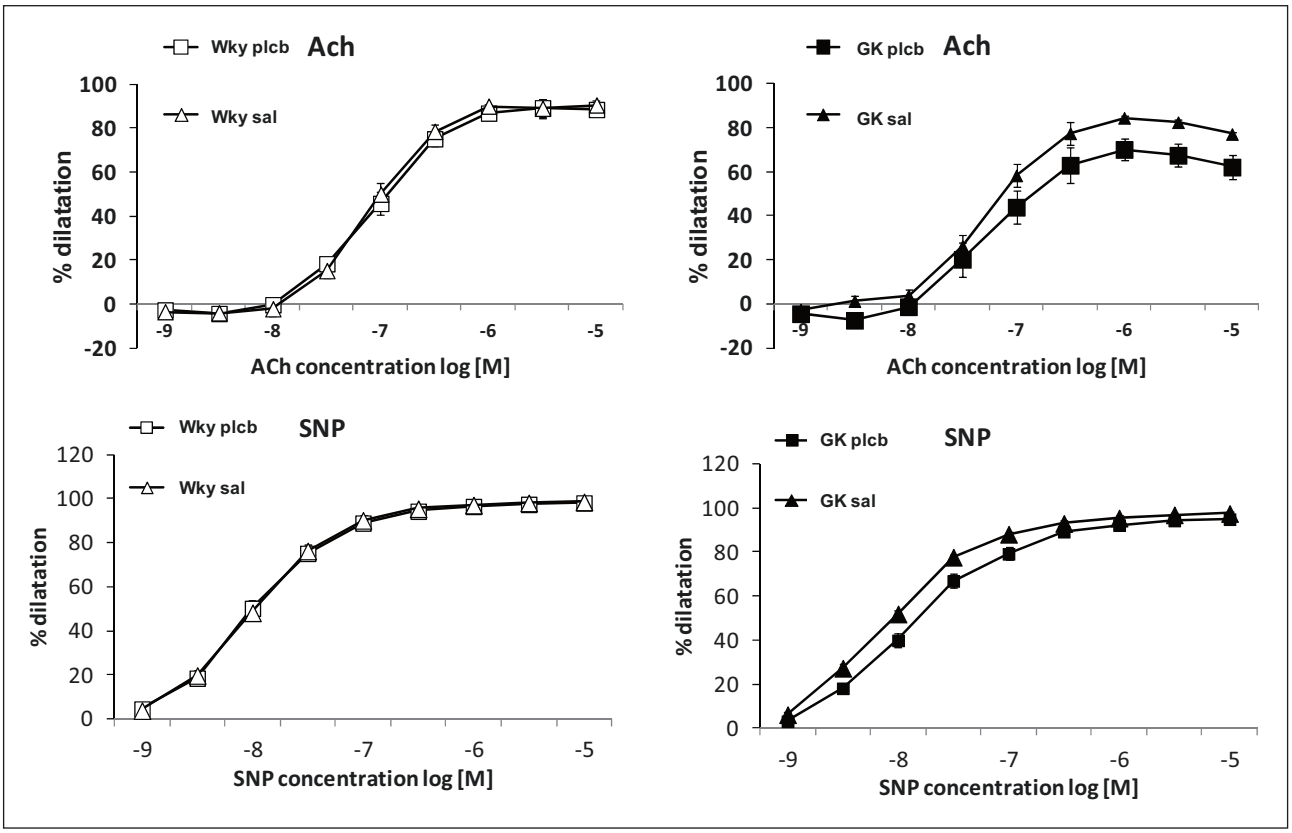


Fig. 3. Concentration-response curves for ACh and SNP in aorta from diabetic and control rats treated with salidroside or placebo.

Table 2. Maximum response and log EC_{50} for various drugs applied to the aorta and mesenteric artery from control (WKy) and diabetic (GK) rats treated with salidroside or placebo.

Parameter	WKy placebo	WKy Sal	GK placebo	GK Sal
Aorta				
ACh log EC50	–7.07±0.054	–7.096±0.04	–7.29±0.092	–7.3±0.048
ACh Max (%)	89.88±2.56	90.64±2.0	67.10±3.32*	81.86±1.905†
SNP log EC50	–8.058±0.048	–8.038±0.044	–7.96±0.048	–8.21±0.06†
SNP Max (%)	97.56±1.02	98.36±0.98	94.07±1.125*	97.14±0.86†
Mesenteric artery				
ACh log EC50	–7.6±0.05	–7.72±0.03	–7.39±0.04*	–7.29±0.023
ACh Max (%)	99.75±2.32	96.2±1.31	96.96±2.2	98.82±1.19
SNP log EC50	–8.15±0.089	–8.208±0.072	–8.74±0.57	–8.35±0.12
SNP Max (%)	96.54±1.90	90.13±1.55	86.87±3.06*	98.78±1.82†

Values are expressed as mean ± S.E.; * P<0.05 vs. WKy-placebo rats; † P<0.05 vs. GK-placebo rats; log EC_{50}, logarithm of the molar concentration of each drug causing 50% of the maximal relaxation; Max, maximum response; ACh, acetylcholine; SNP, sodium nitroprusside.

mesenteric artery and aorta between the different groups (*Fig. 5*, *Table 1*). Chronic salidroside treatment had no significant effect on eNOS expression and activation in GK rats and control rats.

DISCUSSION

Our GK rat model data are consistent with previously published results in this model. In addition to severe glucose intolerance and diabetic status in the absence of obesity, GK rats are hypertensive and have impaired endothelial-dependent and endothelial-independent vasodilation. Chronic treatment with salidroside did not affect glucose tolerance or hypertension but did affect endothelial-dependent and endothelial-independent vasodilation.

Cardiovascular impairment in the Goto-Kakizaki rat model

Goto-Kakizaki rats aged 20 and 25 weeks displayed fasting hyperglycemia and enhanced glucose intolerance similar to previous reports in GK rats (23, 24). Although food consumption of GK rats was higher than WKy rats, body weight and weight

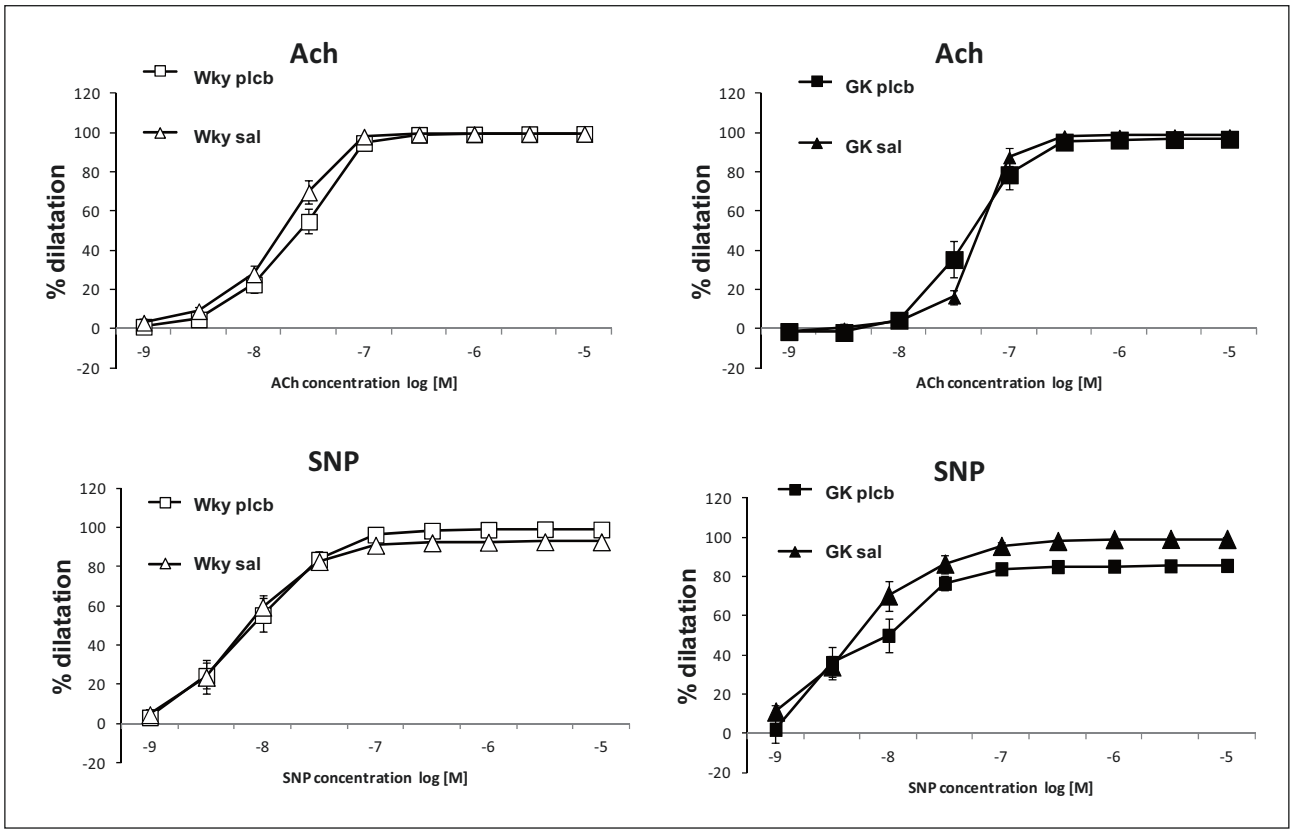


Fig. 4. Concentration-response curves for ACh and SNP in mesenteric arteries from diabetic and control rats treated with salidroside or placebo.

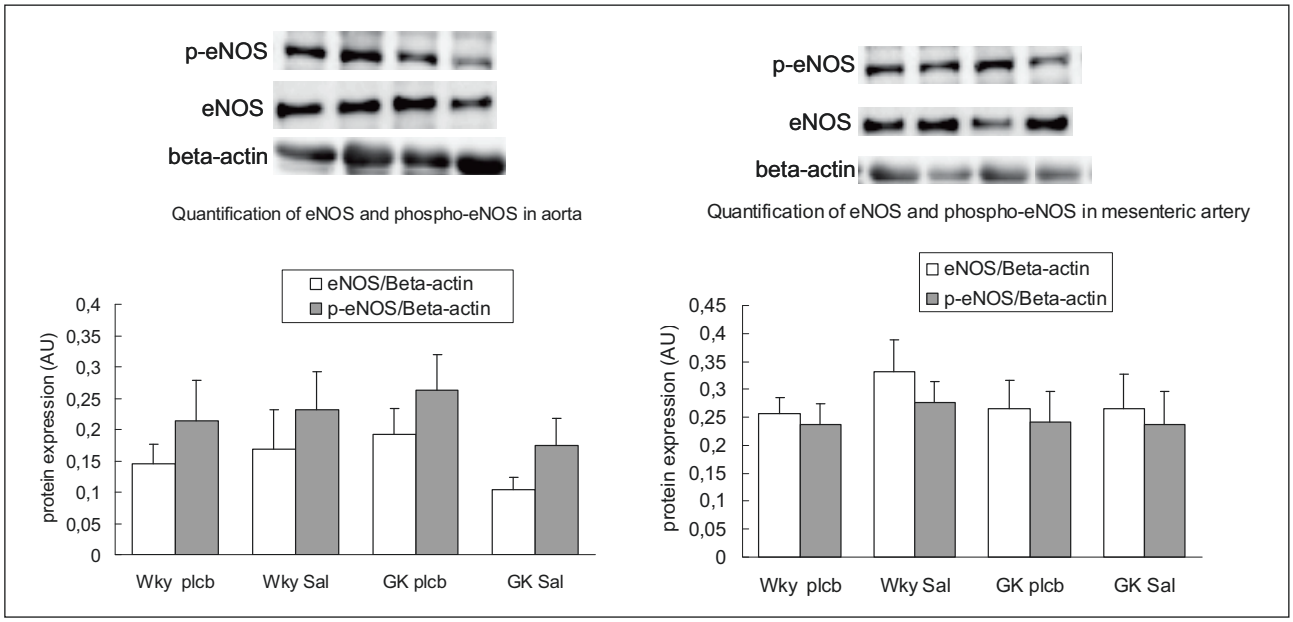


Fig. 5. Western blot for eNOS and phospho-eNOS expression in aorta and mesenteric artery from diabetic and control rats treated with salidroside or placebo. Data is given as a ratio (phospho-) eNOS/β-actin.

gain in WKy rats was higher compared to GK rats. As noted by Landersdorfer *et al*. (25), this might be explained by metabolic impairments specifically related to direct perturbations in insulin metabolism or by higher metabolic rates in GK rats compared to WKy rats. The increased food consumption in GK rats is related to leptin resistance (25).

Telemetric recordings demonstrate that GK rats are hypertensive even when fed a typical diet (0.25% sodium content). GK rats have significantly higher systolic and diastolic blood pressure than WKy rats, which might be due to reduced dilation capacity of vessels in response to endothelial dysfunction (13). We also observed decreased heart rates in GK rats compared

to those in WKy rats. This finding may be related to changes in the autonomic nervous system or baroreflex sensitivity, or to inherited metabolic disorders, particularly those that alter insulin metabolism (26). Reduced heart rates were previously described in streptozotocin-induced diabetic rats, which are also characterized by hyperinsulinemia and hyperglycemia (27).

Studies with isolated vessels have demonstrated that endothelium-dependent dilation is impaired in GK rats. As observed in the current study, endothelium-dependent vasodilation is primarily impaired in large vessels, such as the aorta (28) or superior mesenteric arteries (29). However, it appears preserved in smaller arteries, such as second- or third-order mesenteric arteries (30-32). It has been shown that hyperglycemia promotes deregulation of endothelial NO synthase - eNOS (33), which alters blood vessel dilation *via* the NO pathway. Hyperglycemia induces an increase in NADPH oxidase, which is involved in the reactive oxygen species (ROS) intracellular pathway, thus inducing oxidative stress that likely impairs NO synthesis and bioavailability by endothelial cells (34) in GK rats. We found no difference between WKy and GK rats for eNOS and phospho-eNOS expression in the mesenteric artery and aorta. According to observations of other authors, vascular eNOS expression in GK rats is rather increased (35-36), and so is eNOS mRNA expression (28, 37). However, some researchers report reduced vascular eNOS expression in GK rats (38, 39). The level of vascular eNOS phosphorylation in GK rats may be reduced (39, 40) or unchanged (41). In general, it seems that endothelium-dependent vasodilation impairment in GK rats is not due to decline in eNOS expression (28). Impaired vasodilation capacity could also be related to changes in smooth muscle as suggested by the blunted response observed in response to SNP. Witte *et al*. (23) reported that soluble guanylyl cyclase activity is reduced in aorta from GK rats. Decreased activity of soluble guanylyl cyclase is expected to attenuate the relaxation response to both ACh and SNP. This decreased vasodilation capacity might also contribute to elevated blood pressure in the animal model. Hyperglycemia promotes ROS generation. The increase of ROS in vascular smooth muscle cells of GK rats could induce an oxidation of the prosthetic heme group of soluble guanylyl cyclase and thereby blunt sensitivity to NO (23). An attenuated vasodilation response to SNP in uterine arteries from GK rats has also been reported and is linked with soluble guanylyl cyclase-dependent mechanisms (42).

Vascular effects of salidroside

Diabetes is a very common chronic disease, for which efficient treatment needs to be improved. New ways for diabetes management are constantly looked for using various diabetic models.

Sakr (43) had recently shown that oral gavage of sitagliptin, a dipeptidyl peptidase-4 inhibitor, for one month decreased blood glucose level and insulin resistance and improved cognitive functions in type 2 diabetic Sprague-Dawley rats. Similarly, Kang *et al*. (44) demonstrated that oral administration of xenoestrogens for 5 days protects from STZ-induced apoptosis of pancreatic islets, improves blood glucose and insulin level in type 1 diabetic mice. In the present study we aimed to test salidroside in GK spontaneous diabetes model.

Rhodioloside and salidroside have neuro, cardio-, and hepato-protective activity that prevent or reduce stress-induced impairments. Salidroside has been proposed to be a potential compound for anti-diabetic therapy (17). In our study, salidroside did not affect glucose metabolism or hypertension; however, it affected the vascular vasodilatory capacity at the level of the aorta and mesenteric arteries.

Salidroside has potent anti-oxidant properties. Rhodioloside and salidroside inhibit oxidative stress in rat hepatic stellate cells (45) as well as inhibit intracellular ROS production (46). Salidroside acts directly at the endothelial cell level. *In vitro*, salidroside protects cultured endothelial cells against hydrogen peroxide cytotoxicity (47).

To our knowledge, salidroside and *Rodiola rosea* extracts have not been tested *in vitro* on smooth muscle cell vasodilatory function. We have shown *in vivo* that chronic treatment with salidroside prevents endothelium-independent vasodilation impairment in the context of type 2 diabetes. Salidroside did not change eNOS expression and the ratio of phospho-eNOS to eNOS in GK and control rats, suggesting that salidroside is not able to elicit eNOS activation *in vivo*. As far as we know, *in vivo* effects of salidroside on eNOS regulation were not tested earlier. However *in vitro* studies had shown that salidroside was able to normalize eNOS activation in cultured endothelial cells, when eNOS expression was attenuated by high glucose (48) or homocysteine (49), or enhanced by H_2O_2 (50).

We hypothesize that salidroside could thus mainly act through endothelium-independent mechanisms involving guanylyl cyclase. Soluble guanylyl cyclase oxidation impairs its function by modifying the heme group (51). Increased oxidative stress affects soluble guanylyl cyclase that becomes unresponsive to NO. Salidroside, by reducing oxidative stress, could also have beneficial effects on smooth muscle function and soluble guanylyl cyclase activity.

Surprisingly, salidroside did not reduce hypertension in GK rats. Thus, the mechanisms of hypertension in GK rats involve impaired vasodilatory capacity (13) as well as other impaired pathways (6).

In our study, salidroside had no remarkable effect on glucose tolerance and hypertension. However, in the study of Wang *et al*. (52) *Rhodiola crenulata* root (500 mg/kg/day for 4 weeks by oral gavage) improved glucose tolerance and ameliorated metabolic derangements in Zucker diabetic fatty rats. Furthermore, *Rhodiola rosea* whole extract inhibit carbohydrate-degrading enzymes and angiotensin-converting enzyme as shown by Kwon *et al*. (53), suggesting its potential utility in control of postprandial hyperglycemia and hypertension associated with diabetes. It can be that salidroside alone, being just one of the potentially active compounds isolated from *Rhodiola* species, should be not enough for more prominent and full therapeutically beneficial effects on diabetes and cardiovascular pathological manifestations. We should focus our further studies on whole plant extracts.

In summary, we observed impaired endothelial and non-endothelial vasodilation capacity in GK rats. Salidroside has beneficial effects on endothelial and non-endothelial vasodilation and likely acts on the endothelium itself but also on smooth muscle cells and soluble guanylyl cyclase. Despite its vascular effects, salidroside did not improve glucose metabolism or hypertension in the GK model of type 2 diabetes.

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Conflict of interest: None declared.

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Author's address: Dr. Nastassia Navasiolava, UMR CNRS 6214 - INSERM 1083, Universite d'Angers, UFR Medecine, Rue Haute de Reculee 49045, Angers Cedex, France.
E-mail: nastassia.navasiolava@chu-angers.fr

PARTIE IV

Le role combine du récepteur 2 à l'angiotensine 2 et des oestrogènes chez des souris C57BL6 soumises à un régime hyperlipidique et hypercalorique

Article en cours de préparation :

"The combined role of Angiotensin II type II Receptor and estrogen in type II diabetes in C57Bl6 mice."

Dans les travaux précédents nous avons étudié les dysfonctions cardiovasculaires induites par l'inactivité physique et la microgravité. Dans cette étude nous nous sommes intéressés à la fonction métabolique et aux conséquences d'un régime hyperlipidique hypercalorique chez la souris. Il s'agit d'un modèle de diabète de type II induit par une alimentation inadaptée. Nous avons réalisé cette étude pour bien comprendre les mécanismes qui entrent en jeux dans les dysfonctions vasculaires, afin de clarifier l'implication du récepteur AT2R et des œstrogènes sur les complications vasculaires chez les souris C57BL6 diabétique par un régime riche en graisse. Le récepteur AT2R est une nouvelle cible potentielle pour des médicaments protecteurs du système cardiovasculaire. Le rôle de ce récepteur en situation normale et pathologique doit être étudié.

Le diabète est accompagné de nombreux facteurs de risques comme l'hypertension, l'athérosclérose, due à des infiltrations de cellules immunitaires pro-inflammatoires, une dyslipidémie marquée avec une balance HDL-cholestérol (HDL)/LDL-cholestérol (LDL) très en faveur des LDL ce qui induit un fort stress oxydant. Le tout conduit à

une dysfonction endothéliale et l'installation progressive d'une hypertension artérielle.

Le récepteur II à l'angiotensine II est récemment démontré comme protecteur au niveau cardiovasculaire. Sa stimulation augmente la vasodilatation et diminue la pression artérielle, inhibe la croissance et la prolifération cellulaire et inhibe la stimulation des facteurs pro-inflammatoires. Est il aussi protecteur en situation pathologique telle que le diabète ?

D'autre part, beaucoup d'études ont confirmé que les femmes en pré-ménopause sont plus protégées des maladies cardiovasculaires que les hommes, ce qui démontre l'effet protecteur de l'œstrogène

Pour étudier la fonction vasculaire au niveau de la micro et de la macrocirculation nous avons utilisé la méthode des vaisseaux isolés (aorte thoracique) et du rein isolé et perfusé. 2 groupes de souris C57Bl6 et $AT2R^{-/-}$ sont divisés chacun en 4 groupes : contrôle, ovariéctomisés, diabétiques et diabétiques/ ovariéctomisés.

Au niveau du rein, la vasodilatation induite par l'ACh est augmentée dans le groupe diabétique + ovariectomisé $AT2R^{-/-}$ comparée au groupe de souris C57B6. Au niveau de l'aorte, la vasodilatation à l'Ach est augmentée chez les souris diabétiques $AT2R^{-/-}$ et ovariectomiées $AT2R^{-/-}$ par rapport aux souris C57B6. Au niveau de l'aorte, la vasoconstriction à la phényléphrine est significativement diminuée dans les groupes de souris $AT2R^{-/-}$ en cas de diabète, d'ovariectomie et de diabète + ovariectomie.

La présence du récepteur AT2R semble donc diminuer les capacités de vasodilatation au niveau de la vascularisation rénale et au niveau de l'aorte en cas de diabète et d'ovariectomie. Les mécanismes impliqués dans la diminution de cette capacité vasodilatatrice en présente d'AT2R en cas de diabète ne sont pas encore bien connus. Nous pouvons évoquer en cas de diabète une diminution de la libération de molécules vasodilatatrices ou la production de ROS impliquant AT2R.

Title: The combined role of Angiotensin II type II Receptor and estrogen in type II diabetes in C57Bl6 mice.

Authors: Asmaa Alameddine[1], Walid Warda[1,2], Jennifer Bourreau[1], Guillemette Gauquelin-Koch[3], Daniel Henrion[1], Marc-Antoine Custaud[1].

Affiliations:

[1]UMR CNRS 6214 – INSERM1083, Faculté de médecine d'Angers, Université d'Angers, France

[2]Azm Center for Research in Biotechnology, Doctoral School of Sciences and Biotechnology, Lebanese University, El Mittein Street, Tripoli, Lebanon

[3]CNES, Paris, France

Corresponding author:

Asmaa ALAMEDDINE

UMR CNRS6214 – INSERM1083, Université d'Angers, UFR Médecine, Rue Haute de Reculée 49045, Angers Cedex, France

Telephone: +33652244498

Fax:

E-mail: asmaalameddine@hotmail.com

Abstract

Renin-angiotensine system contributes towards the initiation and progression of metabolic syndrome and diabetes through activation of AT1 and AT2 receptors. AT1R counteracts the action of AT2R and participates in vascular injury. Also, vascular dysfunction is gender dependent, and the protective role of estrogens in female is clear. An interaction between estrogens and AT2R is recently demonstrated. The aim of this study was to clarify the combined role of estrogen and AT2R in the progression of diabetes and of vascular dysfunction in C57BL6 mice submitted to high fat diet (DT2). C57BL6 and $AT2R^{-/-}$ female mice were fed with high fat diet for 4 months. These 2 groups are divided into two subgroups: ovariectomised (OVX) and sham operated mice. We evaluated vascular and renal function by using wire myography and isolated and perfused kidney methods.

In kidney, Ach mediated vasodilation is higher in DT2 + OVX $AT2R^{-/-}$ compared to C57B6 mice. In aorta, Ach vasodilation is increased in DT2 $AT2R^{-/-}$ & OVX $AT2R^{-/-}$ compared to C57B6 mice. In aorta, vasoconstriction to Phe is significantely decreased in $AT2R^{-/-}$ in case of DT2, OVX and DT2+OVX.

The presence of AT2R seems to decreases the vasodilation capacity in ovariectomized and diabetic mice at the kidney level and at the aorta level. The mechanism of the implication of AT2R in the decrease of dilation in diabetes is still unknown, but it could be explained by the decrease in production of vasodilatory molecules or release in ROS in $AT2R^{+/+}$ mice in the context of diabetes.

Key words: Type 2 diabetes, angiotensin II type II receptor, estrogen, vascular function, renal reactivity.

Introduction:

Diabetes mellitus is a metabolic disorder syndrome, due to a combination of environmental and hereditary factors, leading to abnormal blood glucose level. Insulin resistance typically precedes the onset of type 2 diabetes and is commonly accompanied by other cardiovascular risk factors: dyslipidemia, hypertension, and prothrombotic factors (Hopkins et al., 1996). The adverse influence of diabetes extends to all components of the cardiovascular system: the microvasculature, the larger arteries, and the heart, as well as the kidneys (AHA 1999).

The rennin-angiotensin system is one of the most important regulator systems in organism. It plays a key role in cardiovascular disease physiopathology by controlling blood fluid volume, electrolyte balance and blood pressure (Ardaillou, 1999) . Angiotensin II is the major effector peptide in the system, its action is mediated by two receptor subtypes AT1 and AT2 (Berk, 2003) (Ardaillou, 1999). AngII acts via l'activation of AT1 by short-term effects (effects on vascular smooth muscle, sympathetic activation, tubular reabsorption of NA+, aldosterone secretion, renal vasoconstriction), and by long-term effects (cardiomyocyte hypertrophy, proliferation of fibroblasts, collagen synthesis, increased wall thickness, proliferation of smooth muscle cells). AT2R counteracts these effects of AT1R by inhibiting cell growth and proliferation and promoting cell differentiation (Meffert et al., 1996), inhibiting pro-inflammatory events, lowering blood pressure (Masaki et al., 1998).

Epidemiologic studies demonstrated that premenopausal women are more protected against heart disease than men, and that vascular and cardiac cells expressing estrogen and these receptors (ER α et β) have protective properties against atherosclerosis and neointimal heperplasia and accelerates re-endothelialization after cell damage (Lenfant et al., 2011). Recent studies demonstrate that the potential beneficial effect of estrogen such as glucose intolerance and vasodilatation is brought about interaction of estrogen with other hormones and receptors such as insulin and recently with AT2R (Armando et al., 2002; Louet et al., 2004).

The mode of action of estrogen on the cardiovascular system includes important and complex regulatory influences on the renin-angiotensin system (RAS) (Schunkert et al., 1997). Although estrogen stimulates the synthesis of the renin substrate angiotensinogen (Clauser et al., 1989) and downregulates AT1 receptors in vascular smooth muscle cells (Kakar et al., 1992), pituitary gland (Seltzer et al., 1992), and adrenal cortex (Roesch et al., 2000) .

The present study was designed to understand the involvement of estrogen and AT2R in the onset of diabetes and their role in vascular and kidney functions in diabetic mice.

Materials and Methods:

Animal protocols

Female Wilde-type (WT) and AT2R$^{-/-}$ aged 3 months are fed by a high fat diet (Fat calories 60%) for 4 months to induce type 2 diabetes (n=9). These two groups are divided into two subgroups: ovariectomised and sham operated mice. Body weight and glycaemia (glucometer AccuChek Go®, Roche) were measured continually. After 4 months mouse is weighted and anesthetized with isoflurane (5%) and received an intramuscular injection of Temgesic ®(Buprénorphine 0.2 mg/ml). The right kidney is isolated and perfused in a isolated kidney system as described below and the thoracic aorta and segments of mesenteric arteries were gently dissected and placed in ice cold physiological salt solution (PPS) as described below.

The protocol was approved by the Committee on the Ethics of Animal Experiments of "Pays de la Loire" (permit # CEEA.2013.118).

The isolated perfused mice Kidney

Mice were anesthetized with 5% isoflurane, and injected with temgesic. To isolate and perfuse kidney an incision in the abdomen is effected, than intestines are retracted with cotton swab and wet compress, exposing the right kidney. Abdominal artery is separated from the vein, a wire is passed below the abdominal artery, and held with a clamp to prevent loss of blood during catheterization, but it is not ligated to avoid ischemia, than when renal artery a wire is passed below it. With micro scissors, we make a small incision in the abdominal artery, just before the renal artery. A catheter connected to a syringe containing heparinized krebs is inserted into the renal artery and ligated with the wire. Than Kidney is perfused with small boluses until it completely bleaches. The abdominal aorta is ligated and cut and kidney is isolated and perfused with a constant flow of krebs (NaCl 120, KCl 5, CaCl2 2.5, MgSO4·7H2O 1.2, KH2PO4 1.2, NaHCO3 25, et glucose 11 mM;37^{0}C, PH=7.4). The flow is regulated until obtaining a pressure between 100 mmHg and 120 mmHg and kidney is stabilized for 15 minutes. The reactivity of kidney and endothelial function in renal micro vessels are tested by CRC to Acetylcholine after pre-contraction with phenylephrine.

Vascular reactivity

2 segments of thoracic aorta (without perivascular adipose tissue) were dissected from each mouse and mounted on a wire myograph and bathed in a physiological salt solution PSS at 37^0 C, PH=7.4 as described previously. After wall-tension normalization and stabilization for 45 minutes, vessel viability was tested using potassium rich solution (KCL, 80 mmol/L). Endothelium dependant vasodilation was tested in precontracted vessels (Phe 10-6 M), by addition of acetylcholine (10-6M). Muscle function was tested by a cumulative concentration-response curve to Phe (10-9 to 10-5 mol/L). Endothelium-dependant and independent vasodilation was tested by a cumulative concentration-response curve to Ach (10-9 to 10-5 mol/L) and to SNP (10-9 to 10-5 mol/L) respectively. A washout and equilibration of 20 min is applied between the curves.

Ovariectomy

The female mice were ovariectomized to study the role of estrogens and their mechanism of action in protecting vascular and kidney function due to diabetes.

The mice were anesthetized with isoflurane and the analgesic is administered under aseptic conditions and underwent ovariectomy surgery (OVX group) or sham surgery (SHAM group). The surgical procedure was performed by a ventral abdominal midline small incision. Ovaries were bilaterally clamped and removed by this incision. Uterine horns were tied and the uterus was left intact. Then, the abdominal wall was sutured. After surgery, mice were maintained under good conditions to recover. In the sham procedure, animals were anesthetized and the abdominal wall was opened in a similar manner to that used for the OVX mice; the ovaries were exteriorized to create similar stress, but they were not removed.

Statistical Analysis

Data are presented as mean ± standard error (SEM). For body weight, fasting blood glucosevariation and studies on isolated vessels and renal reactivity, we used two-way analysis of interaction (ANOVA). Statistically significant differences were further analyzed by pairwise comparisons. For studies on isolated vessels and renal reactivity analyses of dose-response curves were performed. Analysis was performed using GraphPad Prism 6.

Results

General characteristics

The body weights (g) of WT and AT2R$^{-/-}$ mice fed with high fat diet is significantly higher compared to those of control mice, but the weight of WT ovariectomized mice fed with high fat diet is significantly higher compared to the sham operated mice fed with high fat diet, this difference is absent in AT2R$^{-/-}$ mice (Figure 1). Fasting blood glucose (mg/dl) of WT and AT2R$^{-/-}$ mice fed with high fat diet increased significantly compared to those of control mice (Figure 2).

Renal reactivity

In WT Black6 group Ach mediated vasodilation was significantly higher in control group compared to DT2/OVX group, this difference was absent in AT2R$^{-/-}$ group, demonstrate a combined role of AT2R and estrogen (Figure 3 & 4).

Comparing EC50 of Ach dose-response curves of Black 6 mice and AT2R$^{-/-}$ mice indicate that there is no significant difference between groups. However, comparing the Emax indicate that in this point the Ach-mediated vasodilation is higher in DT2/OVX AT2R$^{-/-}$ mice than that of Black6 mice (Table 1).

Vascular reactivity

The phe mediated vasoconstriction didn't change in the different group of Black6 and AT2R$^{-/-}$ mice (Figure 5)

Vasoconstriction to Phe was significantly higher in Black6 mice compared to AT2R$^{-/-}$ mice in all groups except in control groups were difference is not significant. In addition, Phe-mediated contraction in AT2R$^{-/-}$ mice was significantly higher in control group compared to ovariectomized and diabetic groups. These results demonstrate that the AT2R is implicated in the increase of vasoconstriction in diabetic and ovariectomised group but not in control group (Figure 6).

Acetylcholine mediated vasodilation didn't change in the different group of Black6 and AT2R$^{-/-}$ mice (Figure 7). However, the Ach-mediated vasodilation increase in AT2R$^{-/-}$ DT2 and ovx mice compared to DT2 and ovx Black6 mice. This difference is absent between control and DT2/OVX of Black6 and AT2R$^{-/-}$ mice (Figure 8).

Discussion

In kidney, Ach mediated vasodilation is higher in DT2 & OVX AT2R$^{-/-}$ compared to C57B6 mice. In aorta, Ach vasodilation is increased in DT2 AT2R$^{-/-}$ & OVX AT2R$^{-/-}$ compared to C57B6 mice. In aorta, vasoconstriction to Phe is significantely decreased in AT2R$^{-/-}$ in case of DT2, OVX and DT2+OVX.

Metabolic parameter

Estrogen is a female hormone produced in a higher quantity in many sites, such as skin and mesenchymal cells, osteoblasts, different region in the brain, and ovaries, which are the primary source of estrogen. This hormone is known to decrease female weight gain. In our study, the body weight gain of WT mice fed with HFD increases in ovariectomized mice, but this gain is less important in the presence of estrogen. This difference is absent in AT2R$^{-/-}$ female mice, suggesting the combined role of AT2R and estrogen on weight gain. Other studies reported that HFD elicits profound reduction in estrogen level in female AT2R$^{-/-}$ that could alter insulin function than increase body weight of mice. On the other hand, it was also demonstrated that AT2R$^{-/-}$ decrease the level of estrogen production is adipose tissue (Samuel et al., 2013).

However, some studies reported that AT2R$^{-/-}$ male mice in response to HFD had a lower rate of weight gain compared to WT mice, and that there is an inverse relationship between AT2R and male estrogen production which is negligible compared to the females (Yvan-Charvet et al., 2005), this could be explained by the role of AT2R on adipocyte differentiation and lipogenesis and the absence of estrogen and its interaction with AT2R.

Renal reactivity

We found that the Ach-mediated vasodilation decreases in black6 OVX, DT2 and DT2/OVX groups, indicating the implication of estrogen in the endothelium dependent vasodilation to Ach and the implication of diabetes in the alteration of endothelial function.

Indeed, estrogen is usually thought to be renoprotective (Yanes et al., 2008), its action is mediated by two receptors ER α and ER β which are present in endothelial cells and vascular smooth muscle cells and other tissues. The activation of Estrogen receptors leads to the release of nitric oxide (NO), via endothelial NO synthase (eNOS) than activate the endothelium dependent vasodilation. Some studies demonstrate that the female protective factor is lost in the presence of diabetes (Breyer et al., 1996) after a decrease in plasma estradiol as found in female rats with streptozotocin-induced type 1 DM (Wells et al., 2005), which can explain in part the decrease of the Ach-mediated

vasodilation in DT2 groups, In addition, the diabetes itself is a major factor that alter the endothelial function by oxidative stress and ROS production.

However, the effect of estrogen on Ach-mediated vasodilation is absent in $AT2R^{-/-}$ mice demonstrating a combined role of AT2R and estrogen. Recent studies demonstrated that renal AT2R expression in female mice is higher than male mice (Armando et al., 2002) highlighting the role of the reproductive hormone, the estrogen. It has been shown that many advantages such as vasodilation in female is brought about by interaction of estrogen with other receptors such as AT2R (Louet et al., 2004, Armando et al., 2002). Also, it was demonstrated that estrogen leads to decrease the AT1-to-AT2R ratio, and upregulates AT2R expression in the kidneys, heart and reproductive organs (Armando et al., 2002).

The presence of diabetes decrease the Ach-mediated vasodilation compared to non-diabetic Black6 and AT2RKO mice.

In the other hand, Sourris et al demonstrate that $AT2R^{-/-}$ mice exhibited significantly higher renal superoxide production in freshly minced kidney cortex as compared with those from WT animals that it's known to alter the endothelium dependent vasodilation; however, elevated renal superoxide production in the $AT2R^{-/-}$ remained unaffected by diabetes (Sourris et al., 2010). However in our study Ach mediated vasodilation is higher in $AT2R^{-/-}$ mice compared to Black6 mice in all groups, that could be mediated by other molecules such as prostacyclin, bradykinin with inflammatory actions. Indeed, AT2R has an anti-inflammatory response so its absence could lead to a release of inflammatory molecules. Indeed, it was demonstrated that $AT2R^{-/-}$ mice fed with a high fat diet had higher levels of renal TNF alpha (vasodilator factor) and lower IL-10 (prevent vasodilation) compared to wild type mice on the same diet.

Effect of AT2R on vasoreactivity of aortas in diabetic mice

AT2R is recognized as beneficial to cardiovascular system. It's postulated to act in a counter-regulatory manner to those conferred by AT1R (anti-growth, apoptotic and vasodilator action in rat isolated resistance arteries and aortas (Widdop et al., 2003; You et al., 2005a) via activation of a vasodilatory cascade involving bradykinin (BK), nitric oxide (NO), and guanosine cyclic 3',5'-monophosphate (cGMP)), this beneficial action is changed in some context and AT2R may exerts a hypertrophic and antiangiogenic influence on cardiovascular tissues (Lévy, 2004). In the context of diabetes, Koitka et al (Koïtka et al., 2010) suggest that AT2R may have deleterious effects in the development and progression of atherosclerosis.

In the other hand, other studies on hypertensive rats demonstrate a decrease in AT2R expression on endothelial and smooth muscle of mesenteric arteries, although AT_2R stimulation induced a vasoconstriction in these SHR resistance arteries, this effect involved Endothelin-1 and thromboxane A2 (You et al., 2005b). In our study, the phe-mediated vasoconstriction didn't change with diabetes or in the absence of estrogen but it's the absence of AT2R that decrease the Phe mediated vasoconstriction and increase the Ach-mediated vasodilation.

Igarashi et al., (2007) demonstrate that the expression of AT1R was enhanced in diabetic fatty rat aortae, which could contribute to cell proliferation and inhibition of the insulin signaling pathway through AT1 receptors in both diabetic and non-diabetic VSMC which contribute to increase vascular constriction. However, in AT2RKO mice vasoconstriction decrease compared to Black6 mice, that could be explained by the early phase of inflammation induced by the absence of AT2R.

In our study, the presence of AT2R decreases the Ach mediated-vasodilation in ovariectomized and diabetic mice at the kidney level, which it could be explained by generation of reactive oxygen species (ROS) (Retailleau et al., 2010). Using of ROS blockers restores the AT2R mediated-endothelium dependent vasodilation which indicate that this reduction of AT2R mediated dilation is due to oxidative stress (Retailleau et al., 2010).

In conclusion, the presence of AT2R seems to decreases the vasodilation capacity in ovariectomized and diabetic mice at the kidney level and at the aorta level. The mechanism of the implication of AT2R in the decrease of dilation in diabetes is still unknown, but it could be explained by the decrease in production of vasodilatory molecules or release in ROS in $AT2R^{+/+}$ mice in the context of diabetes.

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***Table 1.*Log EC50 and maximum response for Acethylcholine dose applied to the renal vessels from different groups.**

	Control		**OVX**		**DT2**		**DT2/OVX**	
	C57Bl6	AT2RKO	C57Bl6	AT2RKO	C57Bl6	AT2RKO	C57Bl6	AT2RKO
Log EC50	-7.78 ±0.61	-8.34 ± 0.17	-7.58 ± 0.25	-8.03 ± 0.17	-7.46 ± 0.36	-7.63 ± 0.22	-7.53 ± 0.20	-7.59 ± 0.26
Emax	82.17 ± 14.23	73.42 ± 2.41	55.61 ± 4.80	78.92 ± 4.34	51.49 ± 6.48	71.28 ± 5.46	58.88 ± 3.21	82.6 (*) ± 4.68

Values are expressed as mean ± SE; *p 0.05 vs. C57Bl6 DT2/OVX mice; log EC50, logarithm of the molar concentration of each drug causing 50% of the maximal relaxation; EMax, maximum response;

***Figure 1*: The variation of body weight (g) in WT and AT2R$^{-/-}$ mice during 15 weeks of feeding with normal or high fat diet. Mean ± sem is presented.**

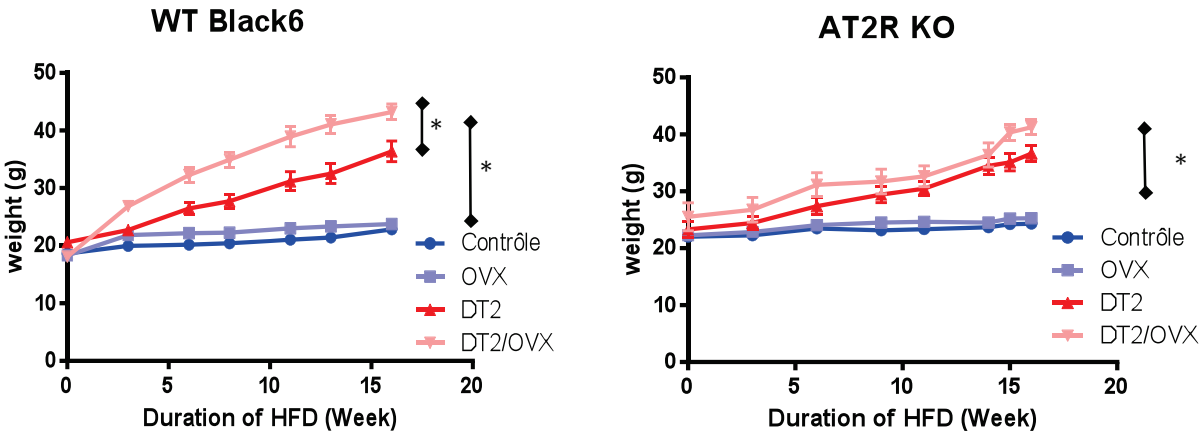


***Figure 2*: The variation of fasting blood glucose (mg/dl) in WT and AT2R$^{-/-}$ mice during 15 weeks of feeding with normal or high fat diet. Mean ± sem is presented. * p<0.05**

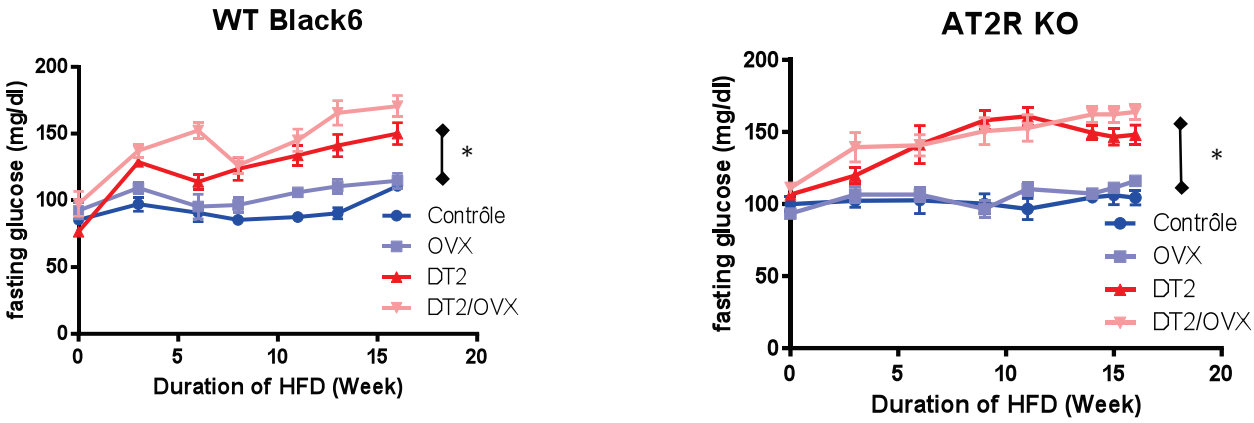


***Figure 3*: Dose-response curves to Acetylcholine in Black6 and AT2RKO mice renal vessels. Mean ± sem is presented (n=9 per group) * p<0.05 control vs. other groups**

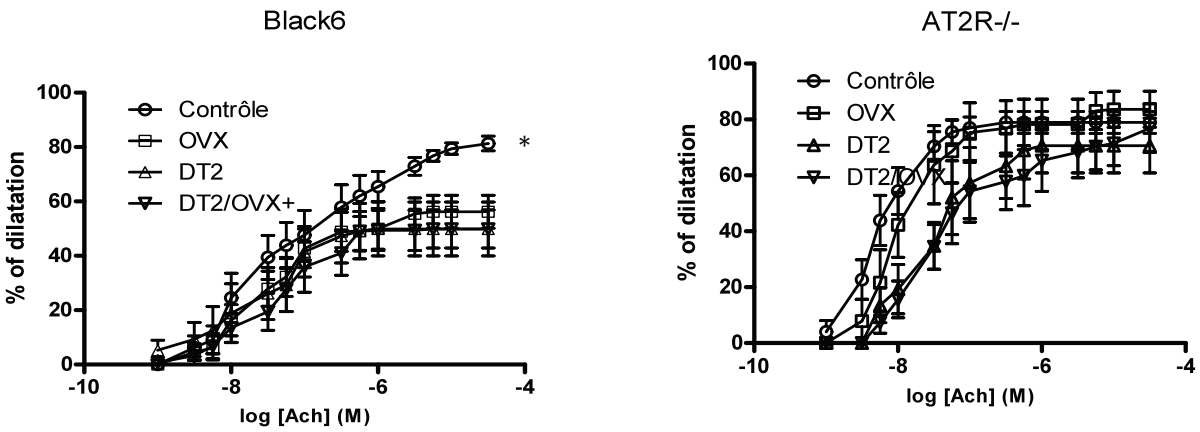


Figure 4: **Acetylcholine -mediated vasodilation performed in renal vessels of isolated kidney from the different groups of WT Black 6 compared to AT2KO mice (control, ovarectomized (OVX), diabetic (DT2), diabetic and ovariectomized (DT2/OVX)). Mean ± sem is presented (n=9 per group)**

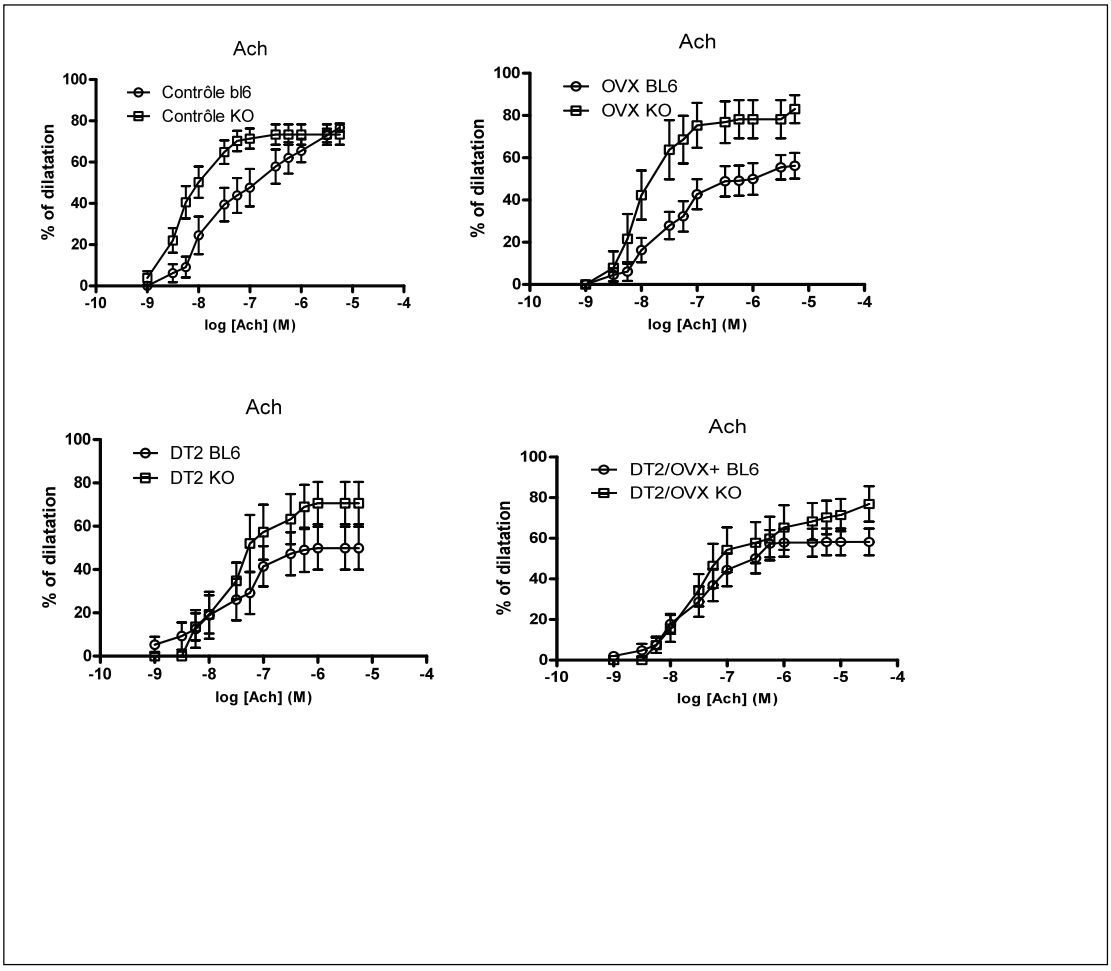


***Figure 5*: Dose-response curve to phenylephrine performed in aortas of the different groups of Black6 (A) and AT2R KO mice (B). Mean ± sem is presented (n=9 per group)**

*** p<0.05 control vs. DT2 and OVX group**

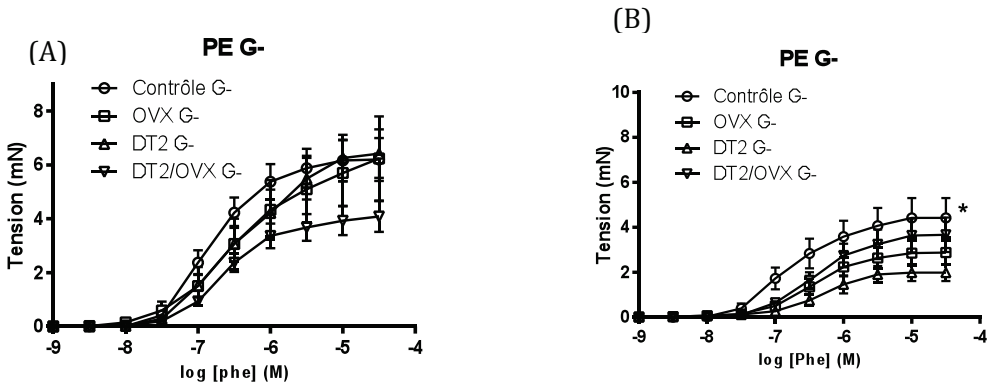


Figure 6: **Phenylephrine-mediated contraction performed in aortas isolated from the different groups of WT Black 6 compared to AT2KO mice (control, ovarectomized (OVX), diabetic (DT2), diabetic and ovariectomized (DT2/OVX)). Mean ± sem is presented (n=9 per group)**

*** p<0.05 control vs. DT2 and OVX group**

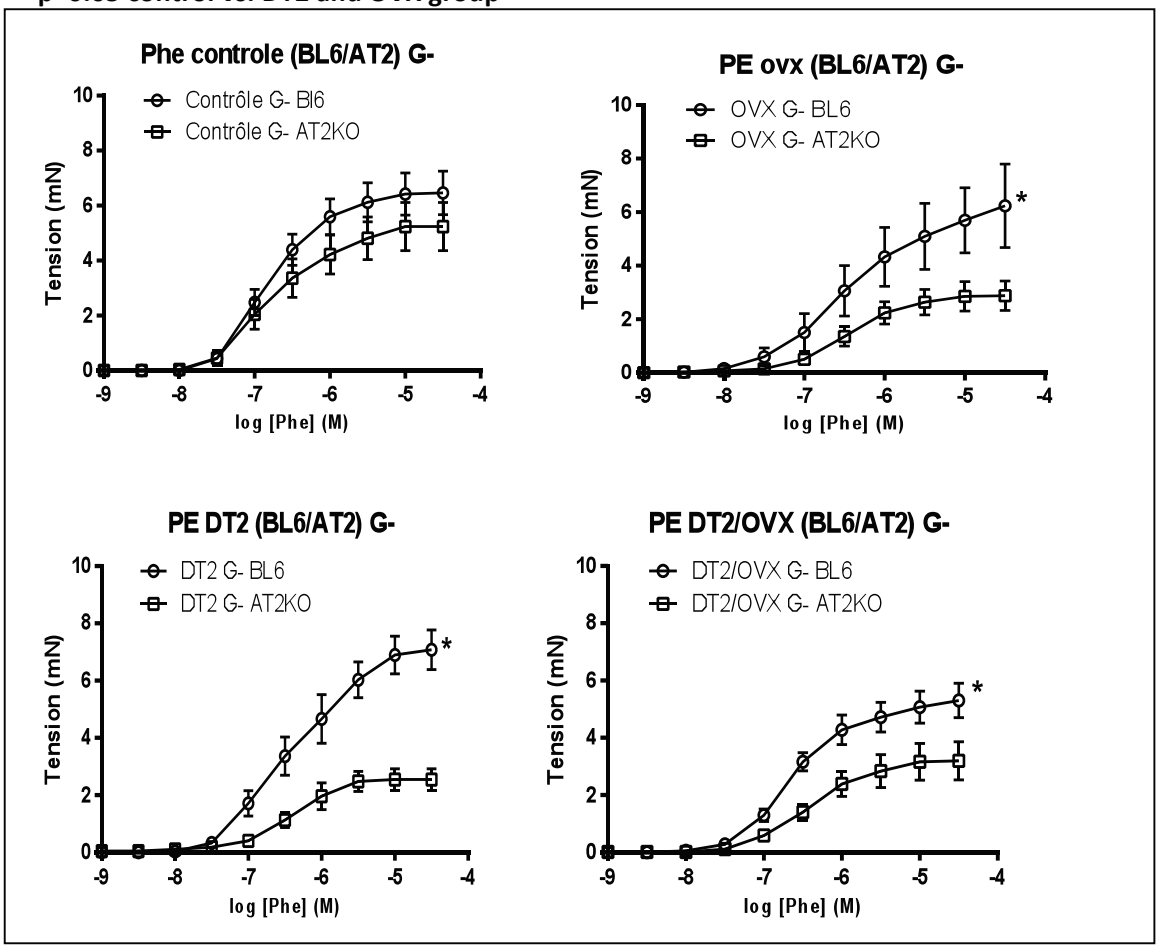


Figure 7: **Dose-response curve to acethylcholine performed in aortas of the different groups of Black6 (A) and AT2R KO mice (B). Mean ± sem is presented (n=9 per group)**

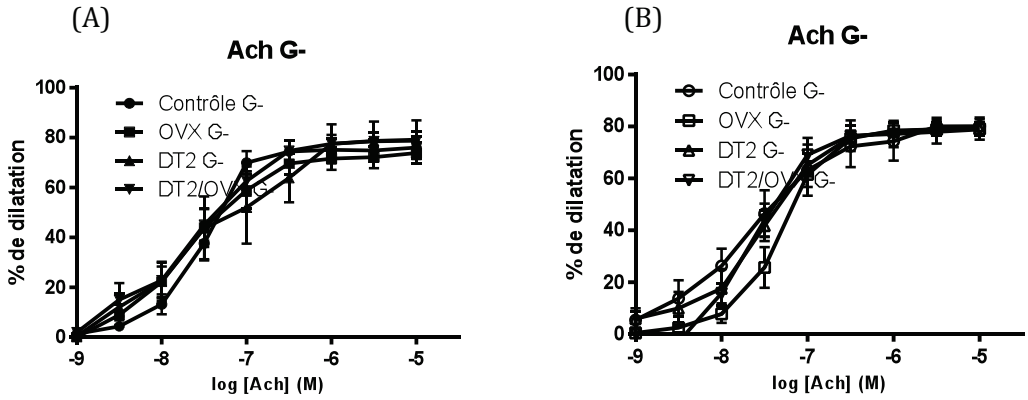
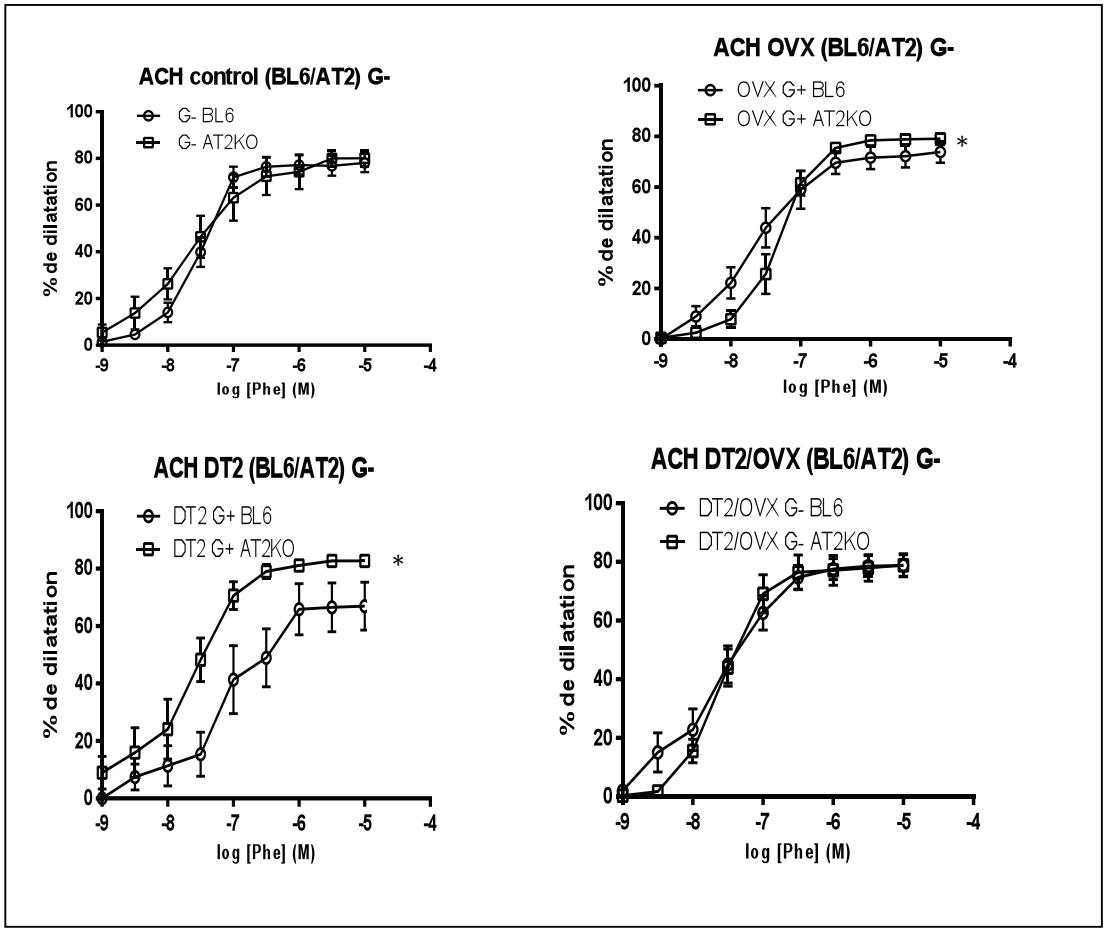


Figure 8: **Acethylcholine-mediated contraction performed in aortas isolated from the different groups of WT Black 6 compared to AT2KO mice (control, ovarectomized (OVX), diabetic (DT2), diabetic and ovariectomized (DT2/OVX)). Mean ± sem is presented (n=9 per group)**

*** p<0.05 C57bl6 vs. correspondent AT2R-/- group**



DISCUSSION GENERALE

Les astronautes en microgravité sont exposés à une inactivité physique très poussée, d'où l'importance de clarifier dans ce contexte toutes les dysfonctions spécifiquement liées à l'inactivité physique.

La sédentarité est considérée comme un facteur majeur du risque vasculaire et est donc responsable d'athérosclérose et de maladies cardiovasculaires. De nombreuses études ont permis d'établir le lien entre l'inactivité physique et des évènements vasculaires précoce qui en résultent concernant le remodelage vasculaire et la fonction vasculaire.

Microgravité, inactivité physique et dysfonctions vasculaires

Les effets de la sédentarité et de la microgravité sur les fonctions vasculaires englobent les artères de conductances, de résistance (macrocirculation) et la microcirculation. Nous n'avons pas considéré ici l'étude des veines et de leur remodelage et nous nous sommes restreint à l'étude du versant artériel. Il existe cependant des modifications de la structure et de la fonctionnalité des veines liée aux modifications de la pression hydrostatique et des muscles squelettiques adjacents (Belin de Chantemèle et al., 2004).

Au niveau des artères de conductances, l'alitement induit une diminution du diamètre des artères fémorales alors que le diamètre carotidien n'était pas modifié (Bleeker et al., 2005). Nous avons observé un résultat comparable après 60 jours d'alitement (Yuan et al., 2015).

Des modifications fonctionnelles importantes sont aussi à noter. En fait, la fonction vasculaire est en grande partie régulée par la couche endothéliale. Inaba et al, (Inaba et al., 2010) ont démontré qu'une diminution de 1% de la dilatation flux dépendante au niveau de l'artère brachiale est associée avec une augmentation de 8 % du risque d'atteinte des maladies cardiovasculaires. D'autre part, Nosova et al,

(Nosova et al., 2014) ont démontré que 5 jours d'inactivité chez des sujets jeunes et en bonne santé, conduisent à une diminution de 2 % de la dilatation dépendante du flux dans les artères fémorales et brachiale. Ce dysfonctionnement vasculaire lié à l'inactivité physique peut être expliqué par le stress oxydatif local et l'augmentation de la sécrétion locale des espèces réactives de l'oxygène qui sont parmi les facteurs les plus importants participants dans la diminution de l'effet vasodilatateur de NO et l'altération de la fonction vasculaire. La diminution chronique de flux au niveau de ces artères de conductance du fait de l'inactivité physique est surement un facteur physique prédominant initiant ce remodelage vasculaire.

Au niveau des artères de résistance il est difficile d'étudier chez l'homme leur structure, mais c'est plutôt leur fonction qui est explorée. Plusieurs études ont eu recours à l'analyse de la variation du pic de l'hyperémie réactive du flux sanguin. C'est une façon de mesurer in vivo la capacité vasodilatatrice globale des artérioles d'un territoire. Cette dernière est diminuée chez les sujets inactifs par rapport aux sujets contrôles (Shinagawa et al., 1966; Thijssen et al., 2006). 13 jours d'alitement conduisent à une altération de la dilatation endothélium dépendante (Hesse et al., 2005), et par suite une augmentation des résistances vasculaires (Bleeker et al., 2005; Christ et al., 2001; Hopman et al., 2002). Il a été démontré que cette altération était prévenue à la suite d'une restriction énergétique, indiquant que cette altération était en partie due à un excès d'apport calorique (Hesse et al., 2005).

La voie du NO est probablement impliquée dans cette atteinte vasculaire, mais la voie du NO seule n'explique pas tout. Nous avons montré que c'est la dilatation tardive à l'Ach qui était diminuée par l'alitement, et cette dilatation implique plutôt la voie des prostacyclines (Yuan et al., 2015). Des changements dans les voies impliquant les agents vasoconstricteurs (l'endotheline (ET-1) et l'angiotensine II (Ang II)) ont été remarqués (Maeda et al., 2001; Wang et al., 2007). Pour bien comprendre les mécanismes d'atteinte des fonctions endothéliales, il faut s'intéresser au métabolisme. En effet une dyslipidémie, une intolérance au glucose avec augmentation des taux circulants de glucose induisent une atteinte des fonctions endothéliales. L'inactivité physique induit rapidement une modification des fonctions métaboliques. Il a été démontré que 3 jours d'alitement conduisent à une

réduction de la sensibilité des muscles à l'insuline (Smorawiński et al., 2000). Dans notre étude d'alitement prolongée de 60 jours, de façon surprenante nous n'avons pas mis en évidence d'insulino-résistance et de dyslipidémie significative hormis une diminution du HDL cholestérol (Yuan et al., 2015). Cette expérience a eu lien à l'agence spatiale chinoise, les habitudes alimentaires pourraient expliquer cette différence avec les autres études conduites en occident.

Voie impliquées dans les altérations vasculaires liées aux dysfonctions métaboliques (insulino-résistance)

Notre étude sur le modèle de souris diabétique induit par l'alimentation nous a permis d'approfondir les liens entre les atteintes métaboliques et les dysfonctions cardiovasculaires. Nous avons montré que le récepteur AT2R, alors qui est plutôt vasculoprotecteur en situation normale, devient un facteur limitant de la vasodilatation en cas de diabète. Le récepteur II à l'angiotensine II (AT2R) est de plus en plus étudié au niveau vasculaire à cause de son rôle vasodilatateur protecteur qui oppose le rôle d'AT1R. Mais il peut aussi prendre un rôle tout différent dans le vieillissement (Pinaud et al., 2007) et l'hypertension artérielle (You et al., 2005) et le diabète. En fait, il a été montré que la dilatation AT2R-dépendante des artères mésentériques de rats ZDF (modèle de diabète de type 2) était réduite par la production de ROS et de thromboxane-A_2 issu de COX-2 (Retailleau et al., 2010). Pour en faire une cible thérapeutique, il devient très important de connaître son mode de fonctionnement dans les différentes pathologies. Ainsi sa stimulation en cas de diabète avéré n'est peut être pas forcément bénéfique pour le patient.(Tarhouni et al., 2013; Tostes et al., 2003). Nous avons aussi étudié le rôle des estrogènes dans la dysfonction vasculaire induite par le diabète. Les œstrogènes et particulièrement le 17 β-œstradiol (E2) en plus de leurs rôles dans le développement sexuel féminin, jouent un rôle vasculoprotecteur essentiel au niveau vasculaire. Les œstrogènes agissent par l'intermédiaire de leurs récepteurs α et β (ER-α et ER-β). Ces récepteurs sont exprimés au niveau membranaire et nucléaire des CMLs et des cellules endothéliales (Tarhouni et al., 2013; Tostes et al., 2003). Au niveau de l'endothélium, l'activation des récepteurs aux œstrogènes entraîne

d'une part une surexpression de la eNOS et une diminution d'expression de certaines sous-unités de la NADPH-oxydase, d'autre part la libération de médiateurs relaxants sur la CML dont le NO, la PGI2, et l'EDHF (Tarhouni et al., 2013). Une étude récente a montré qu'en absence des œstrogènes la dysfonction endothéliale est caractérisée par une baisse de production de l'oxyde nitrique NO et une augmentation de la production de prostacycline (Tarhouni et al., 2014) par activation de l'expression de COX2 (enzyme cyclo-oxygénase implique dans la synthèse de prostacycline) (Vessières et al., 2013).

Quelles seront les contre-mesures adéquates pour diminuer le plus possible les effets de la microgravité et de l'inactivité physique ?

Le développement de contre-mesures est primordial pour prévenir l'effet des facteurs de risques liés à la microgravité et à l'inactivité physique, en particulier pour la population qui ne peut pas réaliser des efforts physiques.

Jusqu'à présent les principales contremesures validées sont basées sur des exercices physiques (aérobiques ou anaérobiques) ou d'autres simulations physiques comme les vibrations. Dans le contexte de la microgravité l'idée de ces contremesures est de reproduire les effets de la gravité sur le corps humain. Comme les effets de la gravité sur notre organisme sont ubiquitaires, le développement de ces contremesures est difficile. La contremesure idéale serait de reproduire une gravité réelle dans le vaisseau spatial à l'aide d'une centrifugation à grand axe, mais elle serait très couteuse en énergie.

Il est nécessaire de compléter ces contremesures physiques par d'autres moyens préventifs basés sur des compléments alimentaires ou des médicaments ciblant des fonctions particulières.

Le Taikong Yangxin est une prescription dans la médecine traditionnelle chinoise, issue de composés végétaux, utilisée par les astronautes et pratique par son petit volume, sa légèreté, l'absence d'effets indésirables. Ils permettent une amélioration de la régulation des fonctions cardiovasculaires et préservent la capacité à effectuer de l'exercice physique (Yu et al., 2008). Dans notre étude, nous avons démontré l'effet de Taikong Yangxin sur l'amélioration de la fonction endothéliale de la

microcirculation de sujets soumis à un alitement prolongé. Le principe d'efficacité de la médecine traditionnelle chinoise est basé sur l'interaction de nombreux composants administrés à faibles doses. Il est néanmoins intéressant d'étudier les principaux composants de cet extrait complexe pour mieux comprendre les mécanismes d'action du Taikong Yanxin.

Le salidroside est un composé présent dans les extraits végétaux de la médecine traditionnelle chinoise. Cette molécule est extraite des rhizomes de *Rhodiola rosea*. Elle a des effets pharmacologiques divers, y compris anti-vieillissement, anti-cancéreux, anti-inflammatoires, hépato-protectifs et anti-oxydants (Yu et al., 2008). Les effets cardiovasculaires du salidroside démontrés in vitro incluent la cardioprotection (Yanes et al., 2008), la protection des cellules endothéliales contre l'hypoxie (Zhang et al., 2009) et la stimulation de la vasodilatation d'aortes précontractées (Zhang et al., 2009). In vivo, il a été montré que le salidroside prévient l'œdème cérébral ischémique (Yanes et al., 2008) et qu'il a des effets protecteurs sur l'ischémie myocardique aigue et améliore l'hemorhéologie dans un modèle de stase sanguine aigue (Yanes et al., 2008). Le salidroside est une molécule protectrice qui pourrait être étudiée aussi comme une contre-mesure en cas de microgravité puisqu'elle agit sur divers fonctions physiologiques cardiovasculaires, métaboliques et psychiques. Nous avons montré dans notre modèle animal de rats GK diabétiques que le salidroside avait des effets vasculaires bénéfiques au niveau de l'endothélium et au niveau des cellules musculaires lisses. Par contre il n'avait pas d'effet bénéfique sur la régulation glucidique de ces rats diabétiques.

Le Taikong Yangxin et le salidroside seraient aujourd'hui des prescriptions possibles utilisables chez l'homme avec des effets vasculo-protecteurs non ciblés. Le Resvératrol est un polyphénol naturel extrait du vin avec des effets vasculo-protecteurs reconnus (Andriantsitohaina et al., 2012). Chez le rat, dans le modèle de microgravité par décharge du train postérieur, il a été montré que le Resvératrol pouvait être une contremesure efficace contre les atteintes musculaires, métaboliques et osseuses liées à la microgravité (Momken et al., 2011). Le Resvératrol serait ainsi une autre contremesure pertinente utilisable chez l'homme.

La recherche de contre-mesures ciblées ne sera-t-elle pas mieux ?

L'étude détaillée des voies de signalisation affectées en situation de microgravité et d'inactivité physique pourrait permettre de développer de nouvelles contremesures pharmacologiques ciblées. L'altération des voies de signalisation de l'insuline est une conséquence de la microgravité et de l'inactivité physique et pourrait devenir une cible privilégiée.

Le récepteur à l'angiotensine II de type 2 est vasculo-protecteur en situation physiologique. Le C21 est un stéroïde agoniste du récepteur 2 à l'angiotensine II largement étudié aujourd'hui pour ses effets sur le système cardiovasculaire (mais elle n'est pas encore administrée à l'homme. Des expériences sur des animaux ont démontré l'effet ciblé du C21. Le traitement avec ce composant réduit l'expression d'AT1R au niveau rénal et agit comme hypotenseur (Ali et al., 2015). Il est en plus considéré comme un anti-inflammatoire en atténuant l'expression du TNF-alpha, d'IL-6 et d'IL-10 (Matavelli et al., 2015; Menk et al., 2015). Au niveau cérébral, il a été démontré que le traitement avec le C21 atténue la démence vasculaire ischémique (Iwanami et al., 2015). Des essais préliminaires sont effectués sur le rein isolé et perfusé de souris C57BL6 contrôles. Ces essais ont montré un effet vasodilatateur du C21. Nous avons cependant montré qu'en situation pathologique, notamment en cas de diabète avéré que le récepteur AT2R perdait sont rôle vasculo-protecteur et même devenait délétaire. Avant de proposer le C21 comme une contre-mesure potentielle, il faudrait vérifier que l'inactivité physique poussée et la microgravité n'entrainent pas une modification de l'activité de ce récepteur le rendant délétère. Un modèle animal de sédentarité / microgravité serait particulièrement utile pour réponde à cette question.

Les modèles animaux de microgravité et d'inactivité physique

L'accès à l'espace est très limité, de même la mise en œuvre des modèles au sol par alitement, immersion sont difficile. Induire une inactivité physique poussée chez des sujets sains reste une intervention contraignante pour ces sujets. Des modèles animaux de microgravité et de sédentarité sont donc très utiles.

Plusieurs modèles animaux ont été créés pour étudier plusieures conditions liées à l'environnement spatial (Morey et al., 1979). Morey-Holton et Globus ont développé

un modèle animal simulant les effets de la microgravité (Morey-Holton and Globus, 2002) par décharge du train postérieur. Il est aujourd'hui largement utilisé.

Les modèles de décharge du train postérieur chez le rat et la souris permettrent de reproduire les transferts liquidiens induits par la microgravité, l'effet confinement et sons sensés aussi reproduire l'inactivité physique. Ils sont utilisés pour l'étude des atteintes du système immunitaire (Lescale et al., 2015; Sonnenfeld, 2003, 2005), osseux (Jia et al., 2014; Morey-Holton and Globus, 1998; Niu et al., 2015; Tou et al., 2008), musculaire (Bigbee et al., 2006; Haddad et al., 2003), nerveux (Trinel et al., 2013), cardiovasculaire (Stabley et al., 2013; Tsvirkun et al., 2012).

Ce modèle reste cependant controversé comme modèle de microgravité. Il a été montré qu'il n'induisait pas de sédentarité mais plutôt une hyperactivité physique après une semaine (Tsvirkun et al., 2012).

Pour les études dans le domaine cardiovasculaire, il est nécessaire de développer un nouveau modèle animal reproduisant les effets de la microgravité au sol. L'effet inactivité physique se doit d'être présent. L'effet transfert liquidien paraît moins important chez les petits quadrupèdes. Il doit induire une diminution de la capacité à l'exercice physique, une atteinte endothéliale et une diminution de la tolérance au glucose.

Il a été proposé un modèle de rat génétiquement inactifs qui a été créé par des croisements consanguins répétés des individus sélectionnés à chaque génération selon leur capacité à effectuer un exercice sur un tapis roulant, à la suite 2 catégories de souris sont obtenus les souris LCR (Low capacity runners) et HRC (Hight capacity runners) (Wisløff et al., 2005). Mais il s'agit de souches très spécifiques.

Dans le modèle de rat GK génétiquement diabétique nous avons démontré que ce modèle présentait un diabète avéré, une atteinte de la vasodilatation endothéliale dépendante et indépendante au niveau de la macro- et de la microcirculation. Cependant les rats GK présentent une hypertension artérielle significative. Le modèle de souris nourris durant 4 mois par un régime riche en graisse présente une altération de la fonction endothéliale au niveau de la microcirculation du rein, par

contre cette altération est absente dans les aortes cela peut être due à ce que le diabète n'est pas encore bien établi.

Pour compléter nos études sur les modèles animaux d'inactivité physique et de microgravité nous pouvons proposer un nouveau modèle combinant activité physique spontanée / inactivité physique & régime normal / régime hypercalorique-hyperlipidique. Le groupe témoin sera celui avec une alimentation normale et l'accès à une roue d'activité et le groupe inactif sera celui avec une alimentation hyperlipidique-hypercalorique et la roue d'activité bloquée. Nous allons devoir dans un premier temps montré que dans ce modèle nous avons bien une atteinte des fonctions endothéliales, une intolérance au glucose et une diminution de la capacité à l'exercice physique.

CONCLUSION

Les dysfonctions vasculaires liées à la microgravité sont en grandes parties dues à l'inactivité physique induite par la microgravité. Dans les différentes études que nous avons effectuées nous avons étudié les altérations vasculaires et métaboliques induits par des facteurs environnementaux tels que l'alimentation inadaptée et l'inactivité physique. Nous avons mis en évidence un dysfonctionnement vasculaire et endothélial au niveau des artères de résistances chez les sujets alités, dans le modèle du rat GK diabétiques et chez les souris nourries par un régime riche en graisse.

Nous avons aussi étudiée les effets potentiels de quelques contremesures. Chez les sujets inactifs par un alitement anti-orthostatique de 60 jours, le Taikong Yangxin améliore la fonction endothéliale en améliorant la voie NO. Chez les rats GK spontanément diabétiques, le salidroside améliore la vasodilatation endothélium dépendante et indépendante.

Le récepteur AT2R joue un rôle important dans l'homéostasie vasculaire. Si en situation normale il est plutôt vasculo-protecteur, en situation pathologique, comme lors d'un diabète avéré, il serait plutôt délétère et limiterai les capacités vasodilatatrices.

Le composant C21, un agoniste sélectif de ce récepteur AT2R porte des espoirs comme nouvelle thérapeutique vasculo-protectrice. A terme ce serait potentiellement une contremesure possible pour prévenir la dysfonction endothéliale. Mais attention en situation pathologique comme lors d'un diabète il pourrait devenir néfaste. Pour continuer à tester de nouvelles contremesures chez l'animal il faut développer un nouveau modèle animal de sédentarité/microgravité. Nous proposons un modèle combinant une restriction de l'activité physique et un régime hypercalorique qu'il faut encore valider.

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Thèse de Doctorat

Asmaa ALAMEDDINE

Dysfonction vasculaire et conditions environnementales dans des modèles expérimentaux chez l'homme et l'animal

Vascular dysfunction and environmental conditions in humans and animal experimental models

Résumé

La gravité est un facteur environnemental majeur. C'est cette force qui a façonné la vie et le fonctionnement de notre organisme est intimement lié à la gravité. Pour rester en bonne santé, nous devons bénéficier de l'influence quotidienne de la gravité et d'un apport alimentaire adapté à notre activité physique.
L'objectif de ce travail de thèse est d'étudier le remodelage vasculaire et la dysfonction endothéliale dans des modèles de sédentarité et de troubles métaboliques ainsi que d'explorer des moyens de contremesures.
Un alitement de 60 jours chez des sujets sains masculins induit un remodelage de la macrocirculation au niveau de l'artère fémorale ainsi qu'une dysfonction endothéliale au niveau de la microcirculation. La prise quotidienne d'extraits végétaux complexes issus de la médecine traditionnelle chinoise (Taikong Yangxin) a permis de prévenir l'atteinte endothéliale.
Dans un modèle de rat diabétique avec atteinte vasculaire (rats GK), nous avons testé le salidroside, important composé issus du Taikong Yangxin. Bien qu'il n'ait pas d'effets sur le diabète, ce composé a montré un effet bénéfique sur la vasodilatation endothéliale -dépendante et -indépendante.
Dans une dernière partie nous avons étudié chez la souris l'implication du récepteur de type 2 à l'angiotensine et des récepteurs aux estrogènes dans les dysfonctions cardiovasculaires induite par une alimentation hypercalorique.
L'inactivité physique induit un remodelage morphologique et fonctionnel au niveau de l'arbre vasculaire ce qui en fait un facteur de risque majeur et indépendant des maladies cardio-vasculaires. Des extraits végétaux simples ou complexes ont des effets bénéfiques sur les fonctions endothéliales. Le récepteur de type 2 à l'angiotensine et ses interactions possibles avec le récepteur aux œstrogènes pourrait être une cible pharmacologique comme contremesure des atteintes vasculaires liées à l'environnement.

Mots clés

Inactivité physique, microgravité, diabète, rats GK, dysfonction vasculaire, herbes chinoises, salidroside, récepteur à l'angiotensine de type 2

Abstract

Gravity is a major environmental factor. This force that shaped the life and the functioning of our body is closely related to gravity. To remain healthy, we should benefit from the daily influence of gravity and a food intake adapted to our physical activity.
The objective of this thesis is to study vascular remodeling and endothelial dysfunction in sedentary models and metabolic disorders and to explore ways of countermeasures.
60 days of head down bed rest in healthy male induce a macrocirculation remodeling at the femoral artery and an endothelial dysfunction at the microcirculation level. Daily intake of complex plant extracts from traditional Chinese medicine (Taikong Yangxin) helped to prevent endothelial dysfunction.
In a diabetic rat model with vascular dysfunction (GK rats), we tested the salidroside, an important compound from the Taikong Yangxin. Although it has no effect on diabetes, this compound showed a beneficial effect on endothelial -dependent and -independent vasodilation.
In the last part of our work, we studied the involvement of type 2 angiotensin receptor and estrogen receptor in cardiovascular dysfunction induced by a high calorie diet in mice.
Physical inactivity induces morphological and functional remodeling in the vascular tree, making it a major risk factor independent of cardiovascular diseases. Simple or complex plant extracts have beneficial effects on endothelial function. Angiotensin type 2 receptor and its interaction with the estrogen receptor could be a pharmacological target as a countermeasure against vascular damage related to the environment.

Key Words

Physical inactivity, microgravity, diabetes, GK rats, vascular dysfunction, Chinese herbs, salidroside, angiotensin type 2 receptor

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