



Régulations cardiovasculaires au repos et à l'exercice chez l'Homme : nouvelles perspectives de la variabilité de fréquence cardiaque et de la sensibilité du baroréflexe en boucle ouverte

Thimothée Fontolliet

► To cite this version:

Thimothée Fontolliet. Régulations cardiovasculaires au repos et à l'exercice chez l'Homme : nouvelles perspectives de la variabilité de fréquence cardiaque et de la sensibilité du baroréflexe en boucle ouverte. Médecine humaine et pathologie. Université de Lyon, 2017. Français. NNT : 2017LYSES007 . tel-02079088

HAL Id: tel-02079088

<https://theses.hal.science/tel-02079088>

Submitted on 25 Mar 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



N° d'ordre NNT : 2017LYSES007

THESE de DOCTORAT DE L'UNIVERSITE DE LYON
opérée au sein de
L'Université Jean Monnet

Ecole Doctorale 488
Science, Ingénierie, Santé

Spécialité de doctorat : Physiologie neuro-cardiovasculaire
Discipline : Biologie Médecine Santé

Soutenue publiquement le 12 juin 2017, par :

Timothée Fontolliet

***Régulations cardiovasculaires au repos et à
l'exercice chez l'Homme:
nouvelles perspectives de la variabilité de
fréquence cardiaque et de la sensibilité du
baroréflexe en boucle ouverte***

Devant le jury composé de :

Philippe Arbeille, Professeur, Université François Rabelais, Tours
Jacques Regnard, Professeur, Université de Franche-Comté, Besançon
Raffaello Furlan, Professeur, Humanita University, Milan
Frédéric Roche, Professeur, Université Jean Monnet, Saint-Etienne
Vincent Pichot, Docteur, Université Jean Monnet, Saint-Etienne
Jean-Claude Barthélémy ; Professeur, Université Jean Monnet, St-E.

Guido Ferretti, Professeur, Université de Genève, Genève

Rapporteur
Rapporteur
Examineur
Examineur
Examineur
Co-directeur
de thèse
Co-directeur
de thèse

À mon père

Remerciements

Je tiens, avant tout, à remercier chaleureusement celui qui m'a accepté comme assistant doctorant, puis suivi de près quatre années durant. Celui qui, de par ses conseils, son écoute, sa disponibilité et sa confiance m'a permis de réaliser cette thèse dans les meilleures conditions. De par son attitude empathique, son oreille attentive, son soutien permanent, très justement balancé entre encadrement et indépendance, le Pr Guido Ferretti a su parfaitement m'accompagner dans le monde complexe de la physiologie cardiovasculaire et me guider dans la tâche complexe qu'est la thèse de doctorat. Sa connaissance et son pragmatisme m'ont éclairé à moult reprises. Il est devenu, au fil des ans, plus qu'un chef de labo et un directeur de thèse. Je tiens, par ces quelques lignes, à le remercier chaleureusement et à lui réitérer toute l'amitié et la reconnaissance que je lui porte.

De même, je tiens à remercier chaleureusement mon deuxième co-directeur de thèse, le Pr Jean-Claude Barthélémy. Bien que plus éloigné géographiquement, étant basé à Saint-Etienne, il a toujours répondu présent lorsque je m'adressais à lui, que ce soit pour des questions scientifiques, administratives ou personnelles. Je lui en suis très reconnaissant.

Mes remerciements vont également à :

Aurélien Bringard, collègue et partenaire de recherche avec qui j'ai partagé quatre années de discussions, réflexions et autres questionnements. Ce fut toujours pour moi un plaisir d'apprendre et de me perfectionner grâce à ses nombreuses connaissances et autres conseils avisés. Il a été un excellent collègue de bureau, toujours prêt à écouter mes questionnements de thésard.

Mes autres collègues du laboratoire, Chistian Moia et Tiziano Binzoni, qui m'ont apporté toutes leurs connaissances respectives et leur sympathie durant ces années de collaboration. .

Vincent Pichot, grand spécialiste du traitement de signal, qui, en me permettant d'utiliser le programme *BRSanalysis* et en me conseillant toujours de manière attentive, a apporté une aide précieuse à mon travail. .

Le Pr Philippe Arbeille de l'Université François Rabelais à Tours, ainsi que le Pr Jacques Regnard, de l'Université de Franche-Comté à Besançon, qui ont aimablement accepté de juger mon travail en étant membres du jury et

rapporteurs de cette thèse de doctorat, de même que le Pr Raffaello Furlan, de l'Humanita University de Milan, le Pr Frédéric Roche et le Dr Vincent Pichot, de l'Université Jean Monnet à Saint-Etienne, tous membres du jury, qui ont aimablement accepté de juger ce travail.. Je leur en suis très reconnaissant.

Et j'aimerais également mentionner :

L'ensemble des collaborateurs que j'ai eu le plaisir de rencontrer et qui ont participé, de près ou de loin, à l'élaboration des articles présentés dans cette thèse. Qu'ils trouvent ici toute ma gratitude.

Ma mère, qui a pris le temps de relire ce travail et de le corriger pour en améliorer la forme. Elle fut, comme cela a toujours été le cas, d'une aide précieuse et d'un soutien permanent. Merci.

Julie, qui m'a soutenu durant cette période chargée qu'est la rédaction d'une thèse de doctorat. Sa présence à mes côtés est un inépuisable moteur qui m'aide à avancer chaque jour. Merci d'être là.

ABSTRACT

Autonomic nervous system (ANS) and cardiovascular regulation are closely linked. For decades, researches have tried to understand how heart rate variability (HRV) and baroreflexes can be used as significant markers of the autonomic nervous control, and sometimes of its impairments. The general aim of this thesis is to gain further insights into the role of ANS in modulating cardiac and vascular functions. The project consisted of four studies.

In the first study, we analysed the effects of gravitational acceleration on cardiovascular autonomic control. This special condition showed interesting results for cardiorespiratory variables. Our results did not agree with the notion of sympathetic up-regulation in hypergravity. We speculated that only the sympathetic branch of the ANS might have been active during elevated gravitational acceleration exposure. Furthermore, the vascular response occurred in a condition of massive baroreceptor unloading.

Our second work targeted the effect of lung denervation on HRV in bilateral lung transplant recipients. As the graft is no longer connected to the ANS, this is an excellent experimental model for the study of cardiovascular regulation without modulation of heart activity by parasympathetic and/or sympathetic lung afferents. The hypothesis was that the modulation of the high frequency component of HRV by the breathing frequency is mediated by the ANS. This hypothesis would be supported by the results if the high frequency component of HRV is suppressed in bilateral lung transplant recipients. Lung denervation implied strong HRV reduction, all indices being decreased, indicating that neural modulation from lung afferents contributes largely to HRV. Baroreflex sensitivity was reduced. The higher low-versus-high frequency ratio implied that the total

power drop was mostly due to the high frequency component, indicating that neural modulation from lung afferents largely contributes to the high frequency component of HRV. The changes in blood pressure variability were smaller than those in HRV, suggesting that the effects of lung denervation were specific to HRV modulation. This finding confirms that blood pressure variability and HRV are under different control mechanisms.

The third article concerns the effects of autonomic blockades on cardiovascular modulation, at rest and during exercise. We hypothesized that HRV should decrease with vagal or sympathetic blockades, and disappear during simultaneous blockade of both ANS branches. The results suggest that the parasympathetic outflow to the heart is the main determinant of HRV, while the role of the sympathetic branch is less important. Indeed, sympathetic blockades failed in changing HRV indices at rest, indicating that a selective blockade of cardiac β -adrenergic receptors has no effects on spontaneous heart rate oscillations. These effects are specific to HRV, as the effects observed on blood pressure variability are indirectly related to the action of the administered drugs. The changes in baroreflex sensitivity were consistent with the changes in arterial blood pressure variability, suggesting that baroreflexes may modulate the LF power of arterial blood pressure.

The last article focused on a closed-loop approach to analyse the dynamics of displacement of the baroreflex operating point upon exercise onset. The patterns of the dynamics of baroreflex resetting upon exercise onset was characterised by two distinct phases, independently of the posture. We speculate that withdrawal of vagal action on the heart, possibly as a consequence of central command, may explain the change in baroreflex sensitivity upon exercise start,

whereas the sympathetic branch of the autonomic nervous system may represent the distal efferent component of the neural loop controlling the baroreflex resetting process. In conclusion, the change in baroreflex sensitivity and the resetting process are distinct phenomena, under different control systems.

RÉSUMÉ

Le système nerveux autonome (SNA) contribue de façon importante aux régulations des fonctions cardiovasculaires. Pendant des décennies, les chercheurs ont essayé de comprendre comment la variabilité de la fréquence cardiaque (VFC) et le gain du baroréflexe cardiaque pouvaient être utilisés comme marqueurs significatifs du contrôle neurovégétatif cardiaque, et parfois de son altération. L'objectif général de cette thèse est de mieux comprendre le rôle du SNA dans la modulation et les adaptations des fonctions cardiaques et vasculaires. Le projet comprenait quatre études.

Dans la première étude, nous avons analysé les effets de l'accélération gravitationnelle graduées sur la régulation neurovégétative de la fréquence cardiaque et de la vasomotricité artériolaire. Dans ces expositions expérimentales des variables cardiovasculaires et respiratoires ont été modifiées de façon spécifique. Nos résultats ne sont pas compatibles avec la mise en jeu d'une régulation sympathique au niveau cardiaque en situation d'hypergravité brève. Nous avons supposé que seule la branche sympathique du SNA était active durant une exposition à une accélération de gravité élevée. La réponse adaptative de la vasomotricité artérielle vasculaire est observée en condition de grande décharge des barorécepteurs.

Notre deuxième travail eu pour objet l'effet de la dénervation pulmonaire sur la VFC, et a donc été conduit chez des patients ayant subi une greffe pulmonaire complète. Le greffon n'étant plus relié au SNA, il s'agit d'un excellent modèle expérimental pour l'étude de la régulation cardiovasculaire en l'absence de modulation de l'activité cardiaque par des afférences nerveuses pulmonaires parasympathiques et/ou sympathiques. Puisque la VFC dans les hautes

fréquences est reconnue comme largement déterminée par le profil ventilatoire, on s'attend à ce que la composante à haute fréquence de la VFC soit absente chez les sujets transplantés bi-pulmonaires. Les résultats montrent que cette dénervation pulmonaire implique une forte réduction de la VFC totale et dans les deux bandes de fréquence étudiées, hautes et basses. Cela indique donc qu'une large contribution de la modulation nerveuse de la VFC répond aux afférences pulmonaires. La sensibilité du baroréflexe est réduite. Le rapport plus élevé entre les basses et les hautes fréquences traduit une réduction de puissance totale principalement due à la diminution de la composante haute fréquence. Ces résultats montrent que les afférences pulmonaires contribuent largement à la modulation neurovégétative de la composante à hautes fréquences de la VFC. La variabilité de la pression artérielle est beaucoup moins modifiée que celle de la VFC par la transplantation bipulmonaire, ce qui met en évidence que les afférences pulmonaires contribuent spécifiquement à la modulation de la VFC. Cette observation est un argument fort pour reconnaître des voies de régulation différentes pour les variabilités de fréquence cardiaque d'une part et de pression artérielle d'autre part.

Le troisième article traite des effets sur la modulation cardiovasculaire de blocages pharmacologiques du SNA sur les régulations de fréquence cardiaque et de vasomotricité périphérique, au repos et pendant l'exercice. Nous avons émis l'hypothèse que la VFC devrait diminuer suite à un blocage vagal ou sympathique, et disparaître pendant le blocage simultané des deux branches. Les résultats suggèrent que l'afférence parasympathique au cœur est le principal déterminant de la VFC, le rôle de la branche sympathique étant, quant à lui, moins important. En effet, les blocages sympathiques ne modifient pas les

indices de HRV au repos, ce qui indique qu'un blocage sélectif des récepteurs β -adrénergiques cardiaques n'a aucun effet sur les oscillations spontanées du rythme cardiaque. Ces effets sont spécifiques à la VFC car les effets observés sur la variabilité de la pression artérielle sont indirectement liés à l'action des substances actives administrées. Les changements de la sensibilité du baroréflexe étaient consistants avec les variations de la variabilité de la pression artérielle, suggérant que les baroréflexes pourraient moduler la composante LF du spectre de variabilité de la tension artérielle.

Avec le dernier article, nous nous sommes concentrés sur l'analyse de la dynamique de déplacement du point d'opération du baroréflexe en début d'effort. Les schémas de la dynamique du rétablissement du baroréflexe à l'apparition de l'exercice ont été caractérisés par deux phases distinctes, indépendamment de la posture. Nous supposons que le retrait de l'action vagale sur le cœur, peut être dû à une commande centrale, pourrait expliquer le changement de la sensibilité du baroréflexe au début de l'exercice, alors que la branche sympathique du SNA pourrait représenter la composante efférente de la boucle nerveuse de contrôle du processus de réinitialisation du baroréflexe. En conclusion, le changement de la sensibilité du baroréflexe et le processus de réinitialisation sont deux phénomènes distincts, sous l'influence de systèmes de contrôle différents.

Table des matières

Titre	
Dédicaces	II
Remerciements	IV
Abstract	VI
Résumé	IX
Table des matières	XII
Liste des publications	1
Liste des abréviations	2
1. Introduction	4

Première partie

2. Fondamentaux physiologiques	10
2.1. Système cardiovasculaire	11
2.1.1. Structure contractile cardiaque	14
2.1.2. Structure conductrice cardiaque	14
2.2. Système nerveux autonome	17
2.2.1. Système nerveux parasympathique	18
2.2.2. Système nerveux sympathique	19
2.2.3. Influence du SNA sur le cœur	21
2.3. Pression artérielle	23
2.3.1. Régulation de la pression artérielle	24
2.3.2. Baroréflexe	26
2.3.2.1. Fonctionnement du baroréflexe	26
2.3.2.2. Méthodes d'investigations du baroréflexe	27
3. Phénomènes physiologiques mesurés	30
3.1. Variabilité de fréquence cardiaque	31

3.1.1.	Analyses temporelles de la variabilité de fréquence cardiaque	33
3.1.1.1.	Interprétation, avantages et inconvénients	35
3.1.2.	Analyses fréquentielles de la variabilité de fréquence cardiaque	36
3.1.2.1.	Transformée rapide de Fourier	36
3.1.2.1.1.	Avantages et inconvénients	38
3.1.2.2.	Modèle autorégressif	39
3.1.2.2.1.	Avantages et inconvénients	40
3.1.3.	Composants spectraux	40
3.2.3.1.	Interprétation des composants spectraux	44
3.1.4.	Transformée d'ondelette	47
3.2.	Sensibilité du baroréflexe	48
3.2.1.	Importance clinique de la sensibilité du baroréflexe	49
3.2.2.	Mesure de la sensibilité du baroréflexe	50
3.2.2.1.	Technique spectrale croisée	50
3.2.2.2.	Méthode séquentielle	50

Seconde partie

4.	Méthodologie commune	54
4.1.	Acquisition des données	55
4.1.1.	Logiciel <i>BRSanalysis</i>	56
4.1.1.1.	Traitement des données sur <i>BRSanalysis</i>	57
4.2.	Analyses statistiques	61
5.	Travaux de recherche	63
5.1.	Article I : Effects of gravitational acceleration on cardiovascular autonomic control in resting humans	64
5.1.1.	Introduction	65
5.1.2.	Manuscrit	67
5.1.3.	Discussion	81
5.2.	Article II : Heart rate variability and baroreflex sensitivity in bilateral lung transplant recipients	83
5.2.1.	Introduction	84

5.2.2. Manuscrit	85
5.2.3. Conclusion	112
5.3. Article III : Effects of autonomic blockades on human cardiovascular control, at rest and during exercise	114
5.3.1. Introduction	115
5.3.2. Manuscrit	117
5.3.3. Conclusion	140
5.4. Article IV : Dynamics of the RR-interval versus blood pressure relationship at exercise onset humans	143
5.4.1. Introduction	144
5.4.2. Manuscrit	146
5.4.3. Conclusion	159
 6. Conclusions et perspectives	 160
6.1. Conclusions générales	161
6.2. Perspectives	163
 7. Références bibliographiques	 165

Liste des publications

T. Fontolliet, V. Pichot, G. Antonutto, J. Bonjour, C. Capelli, E. Tam, J.C. Barthélémy, G. Ferretti. *Effects of gravitational acceleration on cardiovascular autonomic control in resting humans*, Eur J App Physiol. 2015 Jul;115(7):1417-27.

Bringard, A. Adami, N. Fagoni, T. Fontolliet, F. Lador, C. Moia, E. Tam, G. Ferretti. *Dynamics of the RR-interval versus blood pressure relationship at exercise onset in humans*. Eur J App Physiol. 2017, In press.

T. Fontolliet, P. Gianella, V. Pichot, J-C. Barthélémy, P.M. Soccà, G. Ferretti, F. Lador. *Heart rate variability and baroreflex sensitivity in bilateral lung transplant recipients*. Am J Respir Crit Care Med. 2017, Submitted.

T. Fontolliet, V. Pichot, A. Bringard, N. Fagoni, A. Adami, E. Tam, R. Furlan, J-C. Barthélémy, G. Ferretti. *Effects of autonomic blockades on human cardiovascular control at rest and during exercise*. Am J Physiol Reg Integr Comp Physiol. 2017, Submitted.

Liste des abréviations

<i>A</i>	Atropine
<i>ABP</i>	Arterial blood pressure
<i>ACTH</i>	Adrénocorticotrophine
<i>ADH</i>	Hormone antidiurétique
<i>a_g</i>	Accélération de gravitation
<i>ANOVA</i>	Analyse de variance
<i>ANS</i>	Autonomic nervous system
<i>BLT</i>	Bilateral lung transplantation
<i>BLTR</i>	Bilateral lung transplant recipient
<i>BP</i>	Blood pressure
<i>BRS</i>	Baroreflex sensitivity / Sensibilité du baroréflexe
<i>C</i>	Control
<i>CB</i>	Cadenced breathing
<i>CO</i>	Cardiac output
<i>CVLM</i>	Partie caudale de la moelle allongée ventrolatérale
<i>DAP</i>	Diastolic arterial pressure
<i>DB</i>	Double blocage
<i>ECG</i>	Électrocardiogramme
<i>FB</i>	Free breathing
<i>FFT</i>	Fast Fourier transform / Transformée de Fourier rapide
<i>GABA</i>	Acide γ-aminobutyrique
<i>G_z</i>	Accélération verticale
<i>HR</i>	Heart rate
<i>HF</i>	High frequency / Haute fréquence
<i>HFnu</i>	Normalized high frequency / Haute fréquence normalisée
<i>HG</i>	Handgrip
<i>HRV</i>	Heart rate variability
<i>LF</i>	Low frequency / Basse fréquence
<i>LFnu</i>	Normalized low frequency / Basse fréquence normalisée
<i>LF/HF</i>	High and low frequency ratio / Ratio de haute fréquence sur basse fréquence
<i>M</i>	Metoprolol
<i>MAP</i>	Mean arterial pressure
<i>MAPm</i>	Minimum of mean arterial pressure upon exercise onset
<i>MSNA</i>	Muscle sympathetic nerve activity / Activité nerveuse sympathique du muscle
<i>P_{TOT}</i>	Total power / Puissance totale
<i>TPR / R_p</i>	Total peripheral resistance / Résistance périphérique totale
<i>RR / RR_i</i>	R-R interval / Intervalle R-R

<i>RVLM</i>	Partie rostrale de la moelle allongée ventrolatérale
<i>RSV</i>	Résistances vasculaires systémiques
<i>SAP</i>	Systolic arterial pressure
<i>SAP_m</i>	Minimum of systolic arterial pressure upon exercise onset
<i>SD</i>	Standard deviation / Déviation standard
<i>SV</i>	Stroke volume
<i>ULF</i>	Ultra low frequency / Ultra basse fréquence
<i>VES</i>	Volume d'éjection systolique
<i>VFC</i>	Variabilité de fréquence cardiaque
<i>VLF</i>	Very low frequency / Très basse fréquence
<i>VPA</i>	Variabilité spontanée de la pression artérielle

1. Introduction

L'homme s'intéresse depuis plusieurs siècles aux pulsations cardiaques, exprimées comme telles pour la première fois dans les écrits du scientifique grec Herophilos en 300 av. JC (Billman, 2011). Au cours de l'histoire de la médecine et de la physiologie, les chercheurs comprirent que la fréquence de ces pulsations cardiaques variaient dans le temps, et Hales fut le premier, en 1733, à décrire la variation de l'intervalle de chaque battement durant le cycle respiratoire. La technologie et les découvertes s'accéléchèrent pour atteindre, de nos jours, des niveaux de compréhension importants au regard du chemin parcouru. Il est généralement admis depuis longtemps que même dans un état stationnaire, la fréquence des pulsations cardiaques varie spontanément autour d'une valeur moyenne. L'étude de la variabilité spontanée de la fréquence cardiaque (VFC) est un important sujet de recherche dans le domaine de la physiologie cardiovasculaire depuis 35 ans au moins.

Même si de nombreuses avancées ont permis de mieux comprendre les enjeux médicaux de la VFC et de la régulation de la pression artérielle, restent certaines zones d'ombre, sujets de nombreux débats scientifiques, autour des mécanismes de régulation de ces phénomènes. De même, selon les conditions physiologiques et environnementales rencontrées, le rôle que joue le système nerveux autonome (SNA) au sein de ces mécanismes reste encore controversé. Cette thèse de doctorat, sans avoir la prétention de répondre à l'ensemble des questionnements du vaste sujet qu'est la régulation des mécanismes cardiovasculaires centraux et périphériques, essaye d'apporter un éclairage supplémentaire, et ce par l'analyse de ces phénomènes dans diverses conditions particulières.

L'ensemble des recherches de cette thèse ont été réalisées au sein du laboratoire de physiologie intégrative des départements APSI / NEUFO de la Faculté de Médecine de l'Université de Genève. Cette unité de recherche est dirigée par le professeur Guido Ferretti, co-directeur de cette thèse. Dépendant de l'école doctorale « Science, Ingénierie, Santé » de l'Université Jean Monnet, faisant partie du réseau de l'Université de Lyon, cette thèse a été réalisée en étroite collaboration avec le laboratoire de recherche SNA-Epis dirigé par le Dr. Jean-Claude Barthélémy, deuxième co-directeur de ce doctorat. De plus, certains travaux présentés dans ce travail sont le fruit de collaborations avec le Karolinska Institutet de Stockholm et le service de pneumologie des hôpitaux universitaires de Genève.

Les quatre travaux de recherche formant le corps de cette thèse et introduits dans le prochain paragraphe sont complétés par un rappel des fondamentaux physiologiques traités ainsi que par une description des phénomènes physiologiques mesurés et de leurs méthodes d'analyse respectives.

Bien que les travaux étayant ce travail de thèse puissent, au premier abord, sembler traiter de sujets relativement différents, ils regroupent un ensemble pertinent de conditions de recherche, permettant une étude de large spectre des problématiques physiologiques et fondamentales des phénomènes liés à la VFC et à la variabilité spontanée de la pression artérielle (VPA), ainsi que de leur régulation par les branches sympathique et vagale du système nerveux autonome. Dans un premier temps, nous avons cherché à expliquer les modifications de la régulation cardiovasculaire enregistrées dans une condition bien particulière, celle de l'hypergravité. Cette dernière, simulée par une

centrifugeuse humaine, modifie les paramètres cardiovasculaires par une importante accumulation de volume sanguin dans les régions déclives du corps. Les veines, réservoir sanguin, accumulent d'importantes quantités de sang, diminuant le retour veineux, le volume d'éjection systolique et, de ce fait, le débit cardiaque (Bonjour *et al.*, 2010). Ce contexte physiologique particulier est un terrain d'étude intéressant et inexploré quant à ses effets sur la VFC, la VPA et la sensibilité des baroréflexes artériels (BRS). Il est le thème du premier article de cette thèse.

Le second article traite d'une tout autre condition, en analysant les résultats obtenus sur un protocole de recherche réalisé avec l'aimable accord de transplantés pulmonaires. Cette opération, impliquant une condition de dénervation pulmonaire, a permis de quantifier l'importance des afférences nerveuses pulmonaires sur la VFC et le BRS.

Le troisième article est, quant à lui, une étude des effets d'administrations de substances pharmacologiques stoppant les influx sympathiques et vagues de manière alternée ou simultanée, et ce au repos et durant l'exercice. Le blocage d'une partie ou de la totalité des efférences du SNA menant au cœur permet d'analyser les effets d'une branche du système neurovégétatif sur le système cardiovasculaire, indépendamment de l'autre, et d'en extraire ainsi des informations importantes sur leur influence sur le spectre de puissance de la VFC, de la VPA et du BRS.

Les trois premiers articles ont analysé des données au repos ou à l'exercice lors d'état stationnaire. Ils ne nous offrent donc aucun éclairage sur les mécanismes impliqués lors de phases de transition. Ainsi, la dernière contribution de ce travail de thèse est un article consacré à l'étude spécifique du baroréflexe

lors de la phase de transition entre repos et exercice. L'étude de cette phase de transition, impliquant des méthodes d'analyse différentes, cherche à mettre en lumière les mécanismes complexes de la régulation du baroréflexe et de sa sensibilité en début d'exercice. Nous nous sommes donc concentrés sur l'analyse de la dynamique de déplacement du point d'opération du baroréflexe. Ce travail complète ainsi notre spectre de recherche autour des phénomènes de régulation de la fréquence cardiaque et de la pression artérielle par le système nerveux autonome.

Première partie

2. Fondamentaux physiologiques

2.1. Système cardiovasculaire

Afin de comprendre les actions du SNA sur le système cardiovasculaire, il est important d'en connaître la structure. Le système cardiovasculaire est composé de plusieurs éléments : le cœur, officiant comme pompe permettant d'alimenter le réseau cardiovasculaire, lui-même composé par les artères et les artérioles permettant la distribution du sang du cœur vers les organes, et par les veines et veinules, formant le réseau ramenant le sang vers le cœur. Entre ces éléments, le réseau capillaire permet un maximum d'échange entre la circulation sanguine et les tissus environnants. L'organisation structurelle du système cardiovasculaire est représentée dans la Figure 1. La structure du cœur est représentée dans la Figure 2.

Le cœur, agissant comme une pompe alimentant les réseaux vasculaires, est constitué de quatre chambres (deux oreillettes et deux ventricules) séparées en deux entités fonctionnelles indépendantes sur le plan hémodynamique, chacune composée d'une oreillette et d'un ventricule placé en série. De chaque côté, oreillette et ventricule communiquent à travers un orifice contenant deux valves unidirectionnelles, dont l'ouverture ou la fermeture dépendent de la pression prédominantes à chaque instant dans les deux chambres. La contraction du ventricule gauche, à travers la valve semi-lunaire aortique, alimente le réseau systémique, qui transporte le sang artériel, presque complètement oxygéné au niveau de la mer, aux tissus périphériques, où les échanges de gaz et de nutriments ont lieu entre capillaires et tissus. Après son passage dans le lit capillaire, le sang, moins richement oxygéné, est ramené par les veines au cœur droit, où le ventricule droit le pompe dans la circulation

pulmonaire. Les échanges gazeux pulmonaires ont lieu dans les capillaires pulmonaires, où le sang artériel se constitue avant d'être véhiculé à nouveau au cœur gauche par les veines pulmonaires.

Un tel système fonctionne dans la mesure où une pression de remplissage positive existe et évite le collapse des vaisseaux. La pression de remplissage du système cardiovasculaire humain est de l'ordre de 7 mmHg. La pression permettant au ventricule gauche de pomper le sang dans la circulation

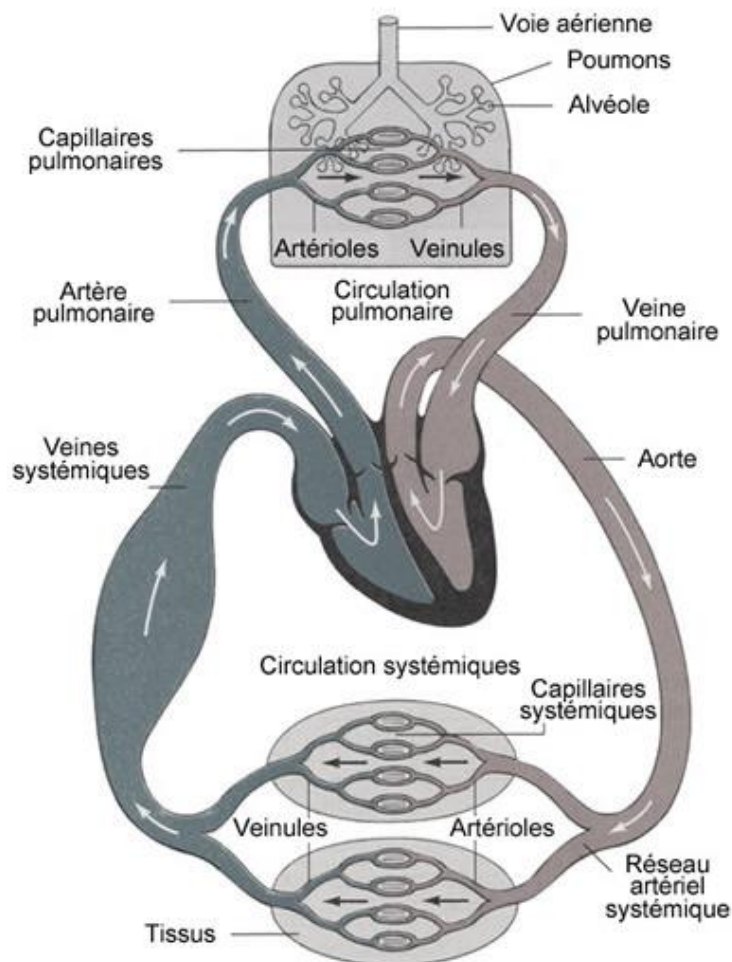


Figure 1 : Illustration du système cardiovasculaire segmenté en circulation systémique et pulmonaire.

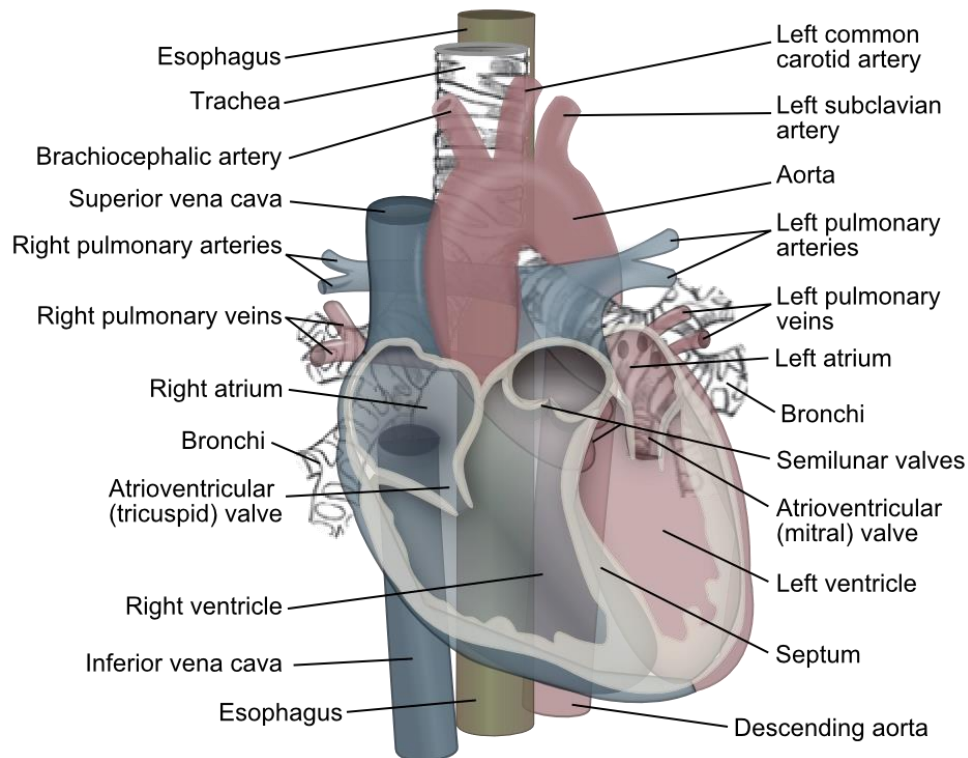


Figure 2 : Le cœur vu en coupe frontale avec ses différentes artères et veines permettant la circulation et la distribution du sang dans l'ensemble du corps. Les vaisseaux bleus représente le passage du sang désoxygéné, les vaisseaux rouge celui du sang oxygéné.

systemique est toutefois bien plus haute que la pression de remplissage. De norme, elle atteint au repos 120 mmHg durant la contraction cardiaque (systole), et 80 mmHg durant la phase de relâchement (diastole). Une telle pression est nécessaire pour surmonter les résistances vasculaires périphériques, localisées surtout au niveau des artéioles, où a lieu le contrôle de la distribution du débit sanguin aux différentes circulations régionales.

Afin de faire fonctionner ce cycle en permanence, en fonction des diverses situations physiologiques que le corps humain peut rencontrer, le cœur et les artéioles reçoivent des influx nerveux. Ces influx, médiés par le SNA, modulent la fréquence et la force des contractions cardiaques, ainsi que le calibre des artéioles.

2.1.1. Structure contractile cardiaque

Le muscle cardiaque, appelé myocarde, permet la contraction des ventricules. Il s'agit d'un muscle strié involontaire, composé de cardiomyocytes. Ces cellules contractiles spécialisées sont reliées par des disques intercalaires constitués de deux éléments importants, les desmosomes et les « gap junctions » qui, grâce à leur structure tubulaire protéique, permettent une circulation facilitée des potentiels d'action électriques indispensables à la polarisation et à la dépolarisation de l'ensemble du tissu cardiaque. Les desmosomes sont, eux, des filaments jouant le rôle de liens mécaniques entre chaque entité cellulaire.

2.1.2. Structure conductrice cardiaque

Des cellules particulières non contractiles diffusent efficacement le potentiel d'action dans l'ensemble du tissu myocardique. Ce potentiel d'action est généré par le mouvement d'ions (K^+ , Na^+ , Ca^{++}) à travers des canaux ioniques transmembranaires. Ce déplacement d'ions, par définition chargé électriquement, implique un changement des charges de part et d'autre des membranes cellulaires, créant ainsi une dépolarisation qui, atteignant un seuil limite, dépolarise la cellule selon une loi du « tout ou rien » : la dépolarisation est complète et toujours à la même puissance ou ne l'est pas du tout. Cette dépolarisation de l'ensemble des cellules contractiles du cœur est synchrone.

Action potential of cardiac muscles

Grigoriy Ikonnikov and Eric Wong

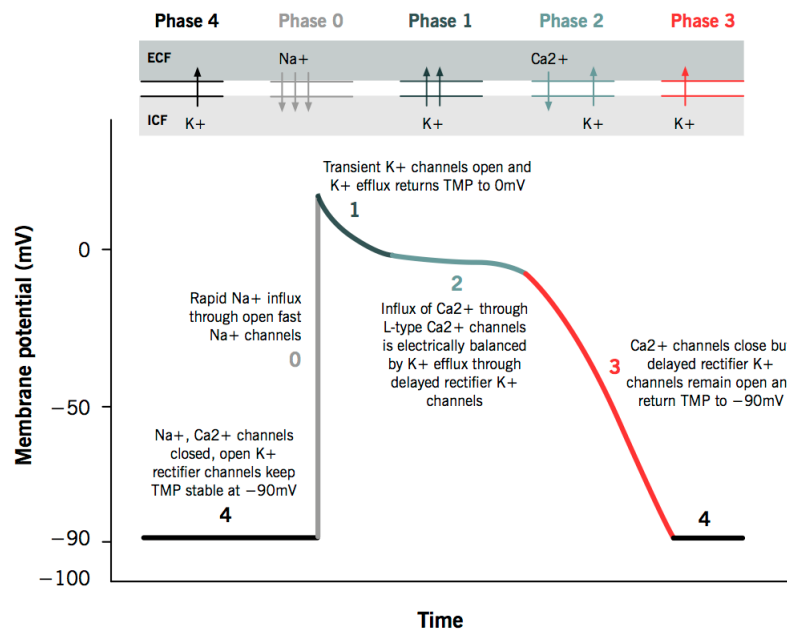


Figure 3 : Graphique décrivant le potentiel membranaire en fonction du temps ainsi que les déplacements ioniques à travers cette membrane cellulaire.

Le potentiel d'action s'initie au sein du nœud sinusal, sur la face postérieure de l'oreillette droite. Les cellules le constituant ont la particularité d'être autorythmiques et génèrent un potentiel d'action spontané sans stimulation externe. La régulation inhibitrice permanente du système nerveux parasympathique fixe sa fréquence de dépolarisation à environ 70 battements par minute chez l'homme. Ce rythme de dépolarisation est influencé par les neurotransmetteurs dont nous discutons dans le chapitre « Système nerveux autonome ». L'acétylcholine, au travers de l'activation de récepteurs muscariniques spécifiques, réduit la concentration d'adénosine monophosphate cyclique (AMPc), ce qui allonge la dépolarisation diastolique, diminuant ainsi le rythme cardiaque. L'adrénaline et la noradrénaline agissent sur des récepteurs bêta1–adrénergiques et provoquent, au contraire, une accumulation d'AMPc

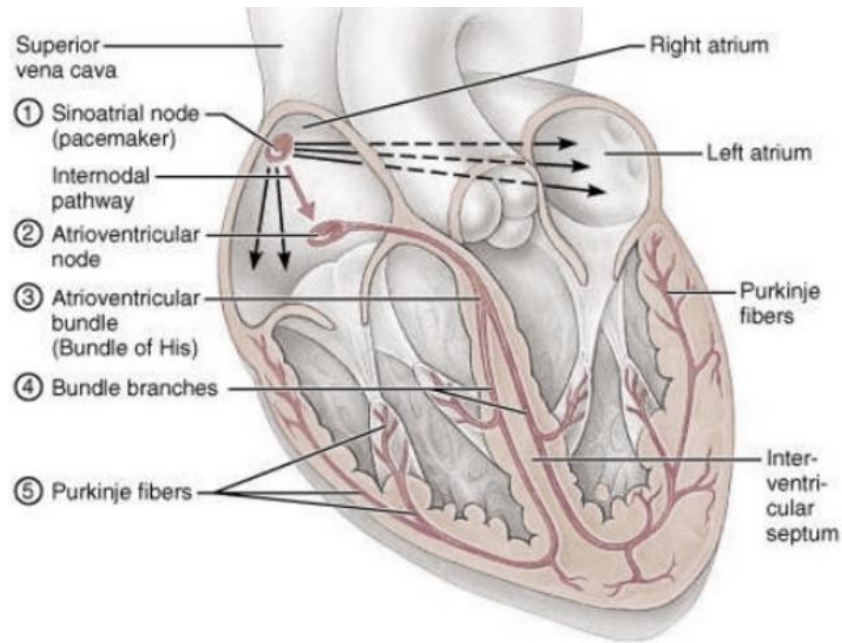


Figure 4 : Schéma descriptif de l'ensemble des tissus conducteurs du cœur.

ayant pour effet d'augmenter la fréquence des cycles de polarisation et de dépolarisation du myocarde. Par la suite, le potentiel d'action est transmis aux ventricles par le nœud atrioventriculaire et par le faisceau de Hiss, ce qui implique un délai de 40 millisecondes pour remplir efficacement les ventricles avant la contraction de leurs cellules myocardiques via les fibres de Purkinje.

2.2. Système nerveux autonome

Les différents systèmes du corps humain et les organes en faisant partie sont influencés par le SNA. Ce système, aussi appelé système nerveux végétatif, est donc lié à la plupart des organes du corps humain. Sa fonction est de réguler et de moduler leurs activités. Cette action sur les organes s'effectue automatiquement et majoritairement de manière inconsciente.

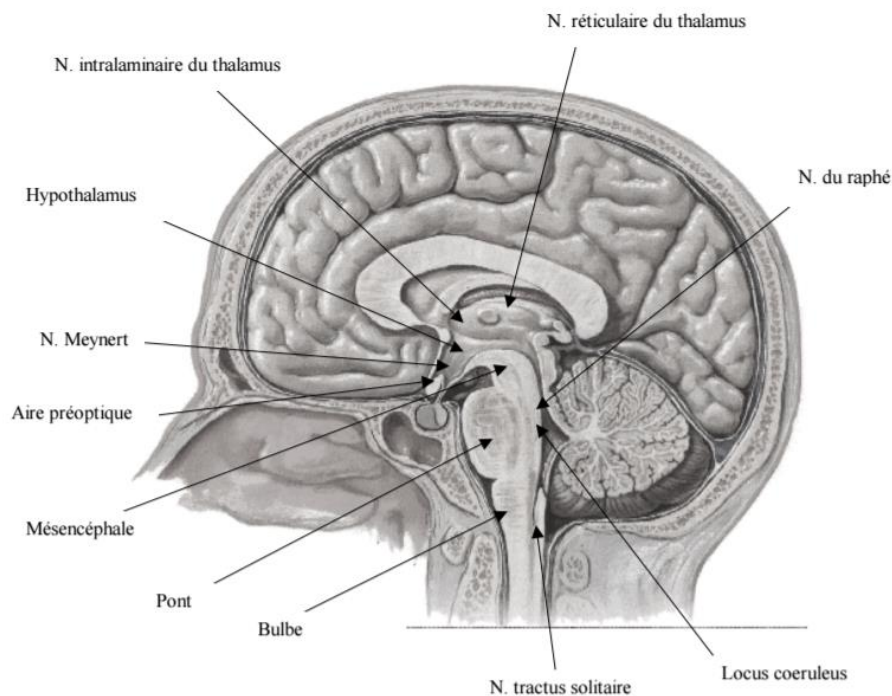


Figure 5 : Schéma neuro-anatomique localisant, entre autre, les zones de contrôle du système nerveux autonome.

Le SNA est divisé en deux différents sous-systèmes travaillant en parallèle, dans des directions souvent opposées, la plupart du temps sur des organes cibles similaires. La première de ces deux branches, appelée parasympathique, possède des nerfs efférents, partant, d'une part, du tronc cérébral et, d'autre part, de la partie sacrale de la colonne vertébrale (S2-4). L'autre branche, appelée système nerveux sympathique, possède un réseau neuronal prenant naissance dans les parties thoracique et lombalgique (L2-3) de

la colonne vertébrale. Un résumé de l'organisation structurelle du SNA est reporté dans la Figure 6.

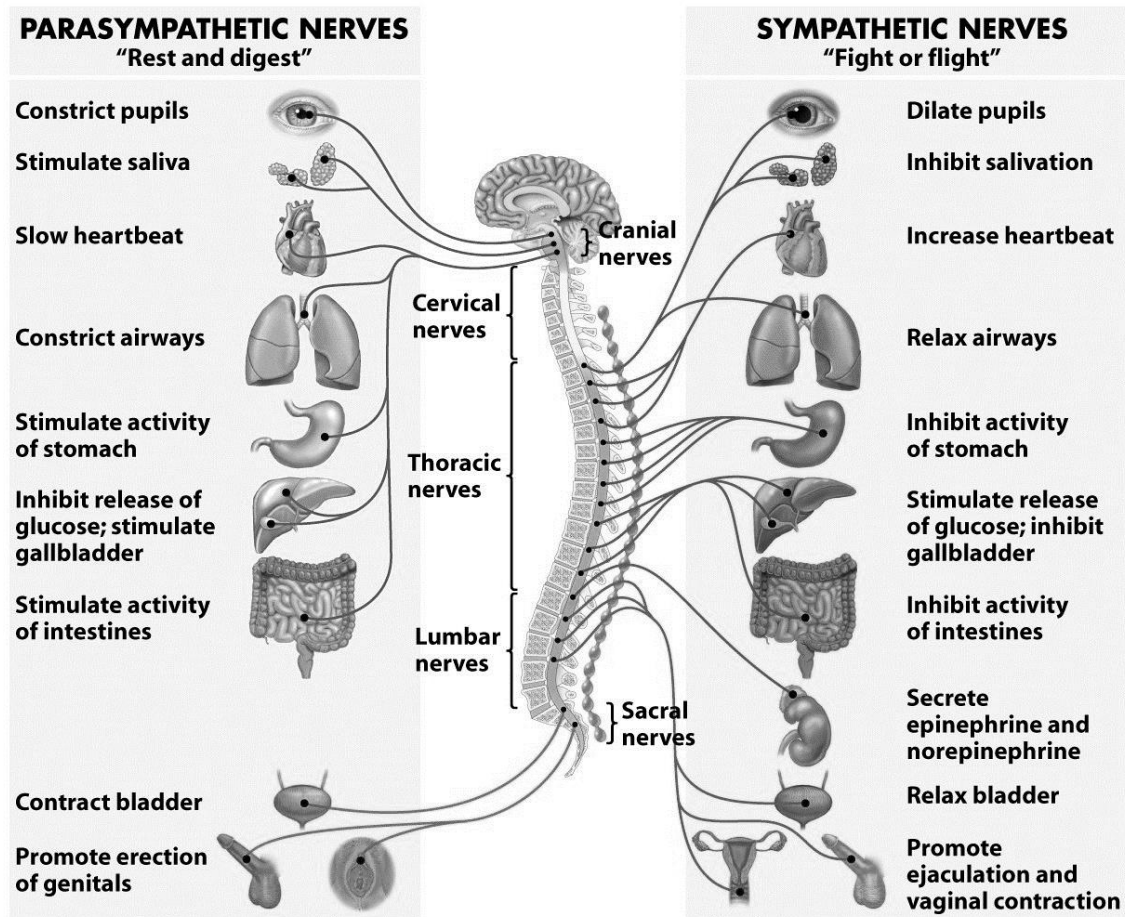


Figure 6 : Système nerveux autonome et ses deux branches distinctes : le système nerveux sympathique et le système nerveux parasympathique. Description des origines nerveuses au sein du système nerveux central de chacun des systèmes.

2.2.1. Système nerveux parasympathique

Le système nerveux parasympathique est responsable de la partie « repos et digestion » de nos activités. Il se manifeste principalement durant les situations neutres, et son rôle principal est de réduire la consommation d'énergie, en gérant les activités basales telles que la digestion, la miction, la motilité et la défécation. Il aide par ailleurs à stocker les réserves corporelles. La structure de la branche parasympathique est constituée de deux neurones. Un long premier neurone pré-

ganglionnaire part du tronc cérébral, formant les nerfs facial (VII), oculomoteur (III) glossopharyngien (IX) et vague (X), ainsi que de la partie sacrale de la moelle épinière. Le deuxième neurone, post- ganglionnaire, très court, est soit très proche de l'organe cible, soit carrément intégré à celui-là.

À hauteur du ganglion, le neurone pré-ganglionnaire libère un neurotransmetteur, l'acétylcholine, véritable messenger chimique qui agit sur les récepteurs nicotiniques du neurone post-ganglionnaire. En réaction, ce dernier libère à nouveau de l'acétylcholine, qui stimule cette fois les récepteurs muscariniques de l'organe cible, qui adaptera son activité si nécessaire. Au repos, dans des conditions normales, lorsque les deux branches du SNA sont actives, l'activité parasympathique est de règle prédominante (Mendelowitz, 1999), ce qui est principalement dû à l'activité du nerf vague.

2.2.2. Système nerveux sympathique

C'est le système nerveux sympathique qui, lors de situations d'urgence ou de danger, prépare le corps aux réponses dites « fight-or-flight ». Ainsi, il est responsable de tous les mécanismes qui augmentent la consommation d'énergie et prépare le corps à l'action par l'augmentation de la pression artérielle, la tachycardie, la vasoconstriction périphérique, etc. Ce système fonctionne lui aussi selon un modèle à deux neurones. Un premier neurone pré-ganglionnaire court, situé dans la partie thoraco-lombaire de la moelle épinière, libère aussi de l'acétylcholine dans le ganglion para- ou pré-vertébral, qui active les récepteurs nicotiniques situés sur le second neurone post-ganglionnaire. Ce dernier libère à son tour de la noradrénaline, qui active les récepteurs adrénergiques des tissus cibles périphériques qui peuvent être de deux classes différentes : les récepteurs

alpha (α) et les récepteurs bêta (β). Les premiers sont de deux types : α_1 et α_2 . Les α_1 sont situés sur la membrane des cellules musculaires lisses du système vasculaire. Leur excitation a un effet vasoconstricteur participant à l'augmentation de la pression artérielle. Bien que les récepteurs α_2 soient présents sur les membranes des cellules musculaires lisses du système vasculaire, ils ne jouent qu'un rôle mineur dans le contrôle des résistances vasculaires périphériques. Les récepteurs bêta sont aussi de deux types : les β_1 et les β_2 . Les β_1 , que l'on retrouve sur les fibres myocardiques, participent aux réponses sympathiques chronotropes positives et inotropes positives décrites dans le chapitre suivant, tandis que les β_2 sont localisés dans les fibres musculaires lisses de certaines artéioles et ont, contrairement aux récepteurs alpha, un effet vasodilatateur lorsqu'ils sont stimulés.

Les organes qui réagissent à la noradrénaline peuvent présenter l'une ou l'autre classe sur leur membrane. La liaison de la noradrénaline aux récepteurs α a un effet exciteur. La liaison aux récepteurs β a un effet variable selon le type de récepteur cible, comme cité plus haut. Par exemple, la liaison de la noradrénaline aux récepteurs β_1 du muscle cardiaque stimule, par différents effets, l'activité cardiaque. Les cellules chromaffines de la médullosurrénale et les neurones post-ganglionnaires des glandes sudoripares s'activent aussi de manière particulière. Les cellules chromaffines sont stimulées par les neurones pré-ganglionnaires et sécrètent de la norépinephrine directement dans le sang. Pour les glandes sudoripares, à l'exception des paumes de la main et de la surface plantaire du pied, les neurones pré-ganglionnaires libèrent de l'acétylcholine, qui active cette fois, en bout de chaîne, des récepteurs muscariniques.

2.2.3. Influence du SNA sur le cœur

Le SNA influence le cœur de quatre manières différentes :

Le chronotropisme définit l'influence sur la fréquence cardiaque. En effet, la fréquence d'émission des potentiels d'action par le nœud sinusal peut être augmentée via une activation sympathique des récepteurs β_1 . On parle alors de chronotropisme positif. Si la fréquence est diminuée via l'activation vagale des récepteurs muscariniques, il s'agit alors de chronotropisme négatif.

L'inotropisme définit le potentiel contractile des cellules musculaires myocardiques. Ces dernières peuvent, en réponse à un potentiel d'action, se contracter de manière plus ou moins importante. Le SNA peut agir sur cette contractilité de manière positive via la branche sympathique, ou de manière négative, par influence parasympathique. Cependant, l'influence parasympathique sur le cœur se fait majoritairement sur le tissu conducteur cardiaque, via les récepteurs muscariniques (M_2) présents au niveau des nœuds sinusal et atrioventriculaire.

Le dromotropisme détermine la capacité de conduction de l'influx nerveux par les cellules myocardiques du nœud sinusal jusqu'aux cellules ventriculaires via le réseau nodal. Un stimulus a un effet dromotrope positif lorsqu'il augmente cette vitesse de conduction du potentiel d'action, via le système nerveux sympathique. Le stimulus est dit négatif lorsqu'il la diminue par influence parasympathique.

Le bathmotropisme renvoie à la notion d'excitabilité d'une cellule cardiaque. On distingue les effets bathmotropes positifs qui, par la branche sympathique, facilitent la stimulation des cellules myocardiques, et les effets

bathmotropes négatifs, qui diminuent l'excitabilité de ces cellules par influence parasympathique.

2.3. Pression artérielle

La pression artérielle se définit comme la force appliquée par le sang par unité de surface des parois vasculaires. Comme on l'a vu dans le paragraphe précédent, le cycle cardiaque possédant une phase d'éjection systolique et une autre de remplissage diastolique, la pression exercée par le flux sanguin sur les parois des artères varie de manière importante. Elle est maximale lors de l'éjection du sang par la contraction du myocarde. On parle alors de pression artérielle systolique. Elle devient minimale à la fin de la phase de remplissage du cœur. Il s'agit de la pression artérielle diastolique. La pression artérielle moyenne correspond à la moyenne intégrale des variations de pression instantanée durant un cycle cardiaque. Cette pression dépend, d'une part, du débit cardiaque (Q_c), lui-même dépendant de la fréquence cardiaque (FC) et du volume d'éjection systolique (VES) selon la formule suivante :

$$Q_c = FC \cdot VES$$

La pression dépend d'autre part des résistances vasculaires systémiques (RSV) correspondant à la résistance à l'écoulement du sang dans les vaisseaux artériels. Le tout s'exprime par la formule :

$$\Delta P = Q_c \cdot RSV = FC \cdot VES \cdot RSV$$

2.3.1. Régulation de la pression artérielle

Les régulations nerveuses de la pression artérielle agissent rapidement, permettant de moduler à court terme une pression adéquate en changeant le diamètre des vaisseaux sanguins, ce qui modifiera la RSV, et distribuer le sang dans les différents organes en fonction des besoins. Durant un exercice physique, par exemple, les muscles squelettiques augmentent leur activité, donc leur besoin en oxygène. Pour y répondre, le corps dérive une part du sang vers les tissus musculaires. Le centre nerveux régissant les mécanismes de vasodilatation et de vasoconstriction se trouve au sein du bulbe rachidien, proche du centre cardiaque. C'est le centre vasomoteur. Il envoie continuellement des influx nerveux aux muscles lisses composant la paroi des vaisseaux artériels via des afférences nerveuses sympathiques. Par conséquent, les artères, et spécifiquement les artéioles, sont toujours sous l'influence d'un tonus sympathique que l'on appelle tonus vasomoteur.

Le centre vasomoteur modifie son activité en recevant des influx sensitifs provenant de différentes structures. Parmi ses influx, ceux qui proviennent de barorécepteurs sont d'une grande pertinence pour cette thèse. Les barorécepteurs sont des récepteurs sensoriels sensibles au changement de la tension exercée sur les parois artérielles. Ils sont principalement localisés dans les sinus carotidiens et le sinus de l'aorte. Lors de l'augmentation de la pression artérielle, ces récepteurs sont étirés et envoient, via les nerfs crâniens IX (vague), et X (glossopharyngien), un influx nerveux au noyau du tractus solitaire, qui participe à l'intégration des réponses cardiovasculaires. L'influx nerveux libère dans cette structure du glutamate, un neurotransmetteur qui va relayer l'information via un réseau polysynaptique vers différents centres médullaires. La

Figure 7 résume l'ensemble des mécanismes nerveux de régulation de la pression artérielle.

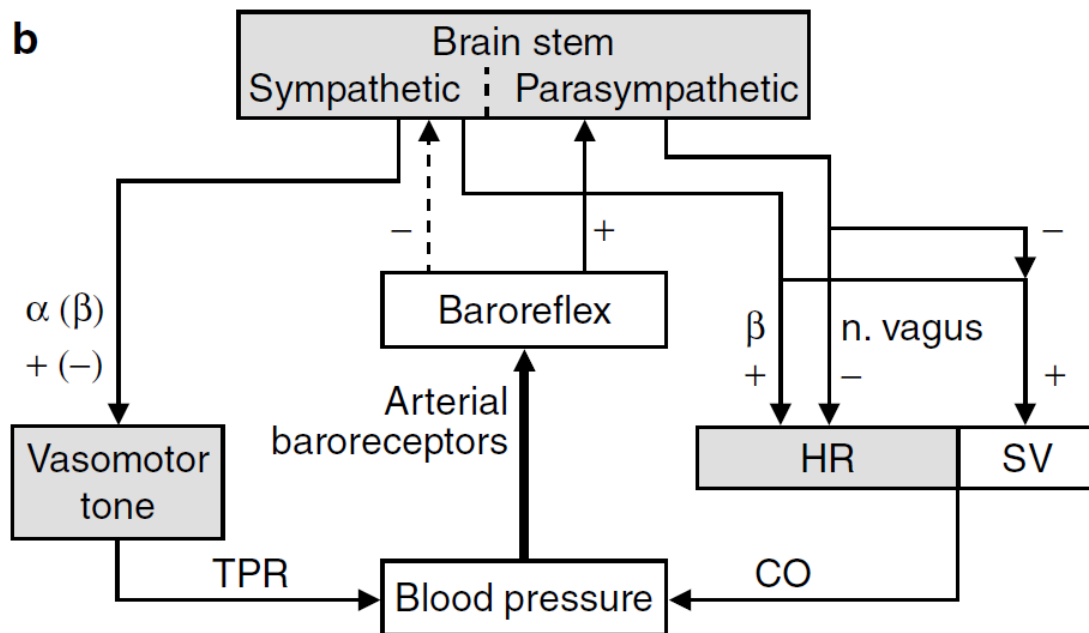


Figure 7 : Schéma élaboré des mécanismes de contrôle de FC, PA et des différents mécanismes de rétroaction du baroréflexe, illustrant les différentes actions vagales, α - et β -sympathiques indépendantes. BP = pression artérielle ; CO = débit cardiaque ; HR = fréquence.

Parmi les mécanismes de régulation à long terme, une place particulièrement importante appartient au système rénine-angiotensine-aldostérone. Activée par la rénine, libérée par les reins lors d'épisodes de volémie basse, l'angiotensine II agit comme vasoconstricteur. L'angiotensine II stimule aussi la sécrétion d'hormone antidiurétique et d'aldostérone, qui augmente la pression artérielle. L'hormone antidiurétique (vasopressine ou ADH) est, elle, sécrétée par la neurohypophyse et permet une vasoconstriction conséquente lors de chute importante de la pression artérielle. L'angiotensine II déclenche finalement la sensation de soif, amenant à une consommation d'eau augmentant le volume sanguin, donc la pression artérielle. L'ensemble du système rénine-

angiotensine-aldostérone permet ainsi de garder une homéostasie hydrosodée constante, primordiale pour maintenir une pression artérielle stable.

Le facteur natriurétique auriculaire, libéré par les oreillettes lors de leur étirement dû à l'augmentation de la pression artérielle, stimule l'excrétion du sodium et de l'eau, diminuant ainsi le volume sanguin. Il provoque aussi une vasodilatation généralisée.

2.3.2. Baroréflexe

2.3.2.1. Fonctionnement du baroréflexe

Les médiations sympathiques et parasympathiques du baroréflexe sont, par la suite, régulées via des cheminements physiologiques différents. La réponse vagale est envoyée à travers un simple arc réflexe en passant par le noyau ambigu et le noyau dorsal du nerf vague. La composante cardiaque du baroréflexe est largement modulée dans ces aires régulatrices. La réponse sympathique est plus complexe avec d'importants points de contrôle dans le noyau du tractus solitaire ainsi que dans les parties rostrale (RVLM) et caudale (CVLM) du bulbe rachidien, ou moelle allongée, ventrolatérale. Le noyau moteur dorsal, à travers un interneurone inhibiteur, envoie l'information nerveuse au CVLM et au RVLM. L'activation de fibres sensibles au GABA dans le CVLM inhibe les neurones sympatho-excitatoires du RVLM, ce qui résulte en une diminution de la pression artérielle et du tonus sympathique (Guyenet *et al.* 2010 ; Madden et Sved, 2003). Ces efférences ont donc deux destinations distinctes. L'une centrale, en direction du cœur et l'autre périphérique, vers le système vasculaire.

Le système baroréflexe décrit ci-dessus correspond à ce que l'on appelle le baroréflexe artériel, ou à haute pression, répondant aux rapides variations de pression de la partie artérielle de la circulation. Cependant, il existe aussi un baroréflexe à basse pression qui perçoit les changements de distension du système veineux, jouant un rôle important de capacitance. Ce baroréflexe à basse pression module donc principalement la volémie, qui influence aussi directement la pression artérielle (Daugirdas, 2001). Un changement de volume sanguin important activant aussi secondairement le baroréflexe à haute pression, suite à la modification de la pression artérielle par l'augmentation du retour veineux qui influence le débit cardiaque, il n'est pas aisé, en pratique, de dissocier les deux baroréflexes.

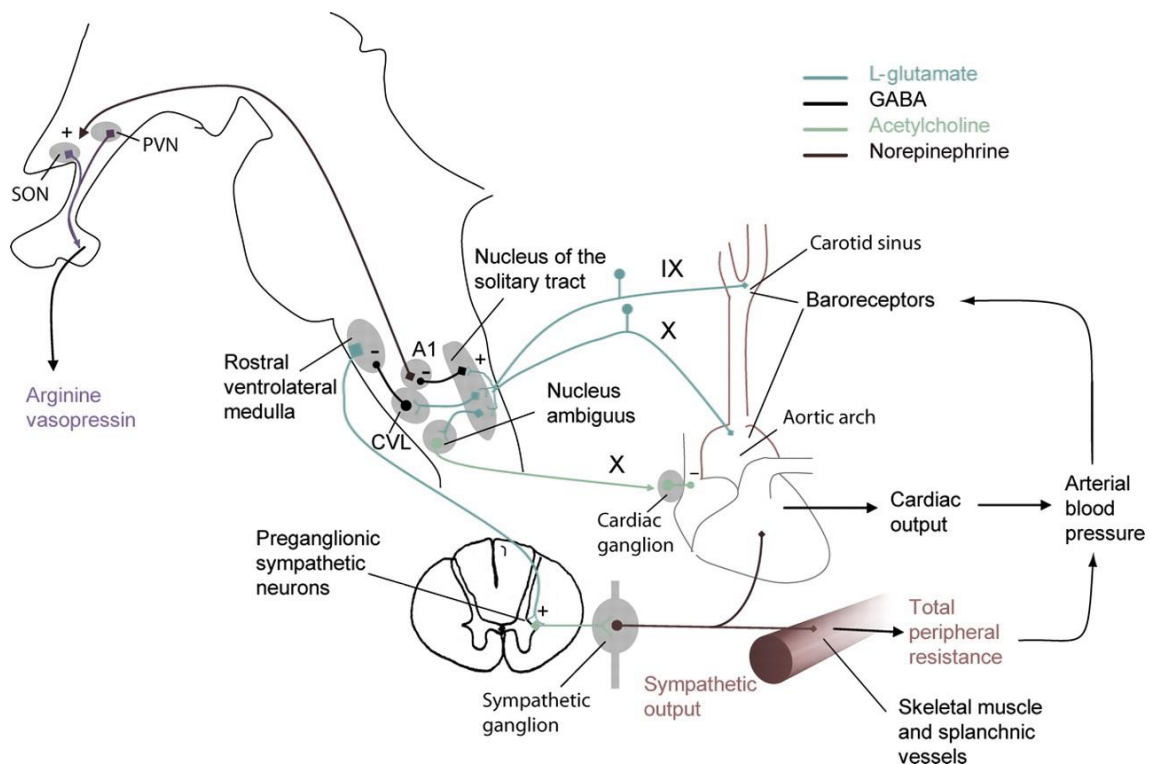


Figure 8 : Schéma représentant l'ensemble du système de régulation du baroréflexe par les voies efférentes sympathiques et parasympathiques et des neurotransmetteurs impliqués dans la transmission de l'information.

2.3.2.2. Méthodes d'investigation du baroréflexe chez l'homme

Les différentes analyses du baroréflexe reposent sur deux méthodes d'investigation distinctes. L'approche « en boucle ouverte » implique soit la modification de la pression de distension des carotides par des procédures d'aspiration ou de pression au niveau du cou, soit l'administration de substances hypotensives, par exemple le nitroprussiate de sodium, et hypertensives, telle que la phényléphrine, à des doses croissantes. Les réponses de la pression artérielle et de la fréquence cardiaque sont, par la suite, enregistrées et analysées (Eckberg et Sleight, 1992). L'étude en boucle ouverte des baroréflexes a conduit à la construction de la courbe classique du baroréflexe artériel, que Kent *et al.* (1972) ont traitée avec un modèle logistique

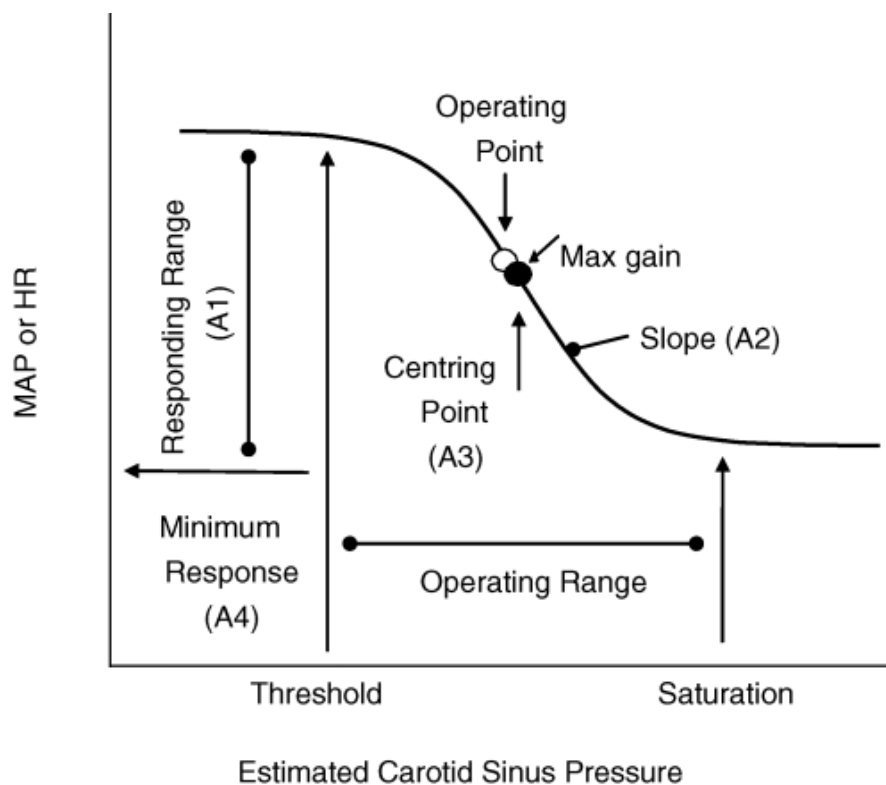


Figure 9 : Modèle de la courbe classique du baroréflexe basé sur les travaux de Kent *et al.* (1972) décrivant les réponses de la fréquence cardiaque (HR) et de la pression artérielle moyenne (MAP) dérivées de l'application, sur les carotides, d'une pression extérieure. Le « responding range » (A1) représente le changement maximum de la variable dépendante. Le « operating range » est la différence entre la pression du sinus carotidien au seuil et la saturation du réflexe. Le gain maximal est calculé comme étant la valeur du gain au point central. Il est utilisé comme index de la réponse du baroréflexe au niveau carotidien. La pente de la fonction du baroréflexe carotidien représente le coefficient du gain (A2) (Raven *et al.*, 2006).

Ce modèle a identifié plusieurs paramètres décrivant la réponse baroréflexe. Le dit « operating point » est défini comme étant, sur la courbe de baroréflexe, le point où la pression artérielle et la fréquence cardiaque sont prédominantes avant l'introduction d'un stimulus aigu. Les études en boucle ouverte démontrent que ce point peut varier selon les stimuli envoyés par le système nerveux central et périphérique (Raven *et al.*, 2002 ; Raven, 2008 ; Fadel and Raven, 2012 ; Mitchell, 2013). Le gain maximal du baroréflexe représente quant à lui la valeur du gain au point médian de la pression, autrement dit le point central de la courbe du baroréflexe (Raven *et al.*, 2006).

Par ailleurs, les méthodes « en boucle fermée » s'appuient sur un suivi continu, battement par battement, des changements de la pression artérielle et de la fréquence cardiaque. Elles n'impliquent donc pas de modification de la pression par stimuli externes. De cette manière, la boucle de rétroaction restant fermée, donc dans des conditions physiologiques, l'influence des changements de fréquence cardiaque sur la pression artérielle peuvent être mesurés. Ayant utilisé ce type de méthode dans nos travaux de recherche, nous développerons de manière plus précise ces méthodes en boucle ouverte dans un chapitre ultérieur.

3. Phénomènes physiologiques mesurés

3.1. Variabilité de la fréquence cardiaque

L'étude de la variabilité spontanée de la fréquence cardiaque, ou de l'intervalle RR, est devenue un outil important de l'étude de l'activité du SNA, de même qu'un standard clinique reconnu. Afin de comprendre cette méthode, il est important de revenir sur les activités électriques du cycle cardiaque. Ces dernières se mesurent grâce à un électrocardiogramme (ECG), qui se divise en plusieurs segments nommés communément P, Q, R, S et T, représentés dans la Figure 10. L'onde P correspond à la dépolarisation des oreillettes, le complexe QRS à la dépolarisation ventriculaire et l'onde T à la repolarisation du cœur en fin de cycle. Le pic R, facilement repérable dans des enregistrements effectués parallèlement à l'axe électrique du cœur, est donc utilisé afin de mesurer

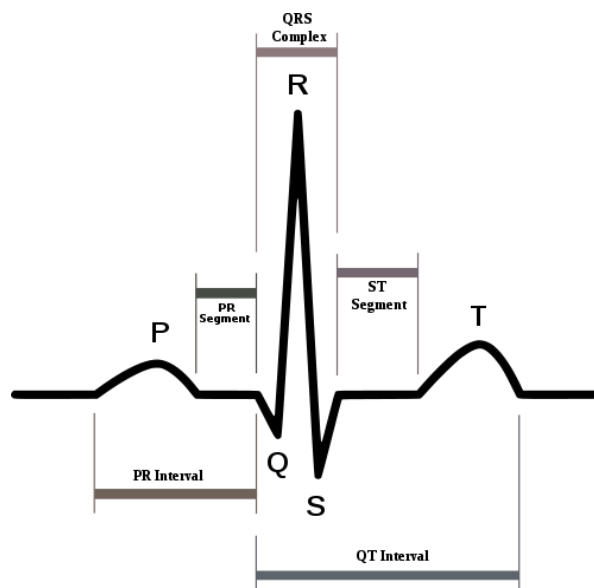


Figure 10 : Déflexions p, q, r, s et t sur le tracé de l'ECG.

précisément l'intervalle entre deux pics consécutifs, donc la durée d'un cycle cardiaque. Il a vite été évident que même au repos, en condition stable, la durée

d'un cycle cardiaque subit des variations spontanées. On discerne rapidement les différences de variabilité, comme le montre la Figure 11, avec le tracé d'une jeune personne en bonne santé (A), durant un exercice physique (B), et celui d'une personne âgée avec insuffisance cardiaque (C).

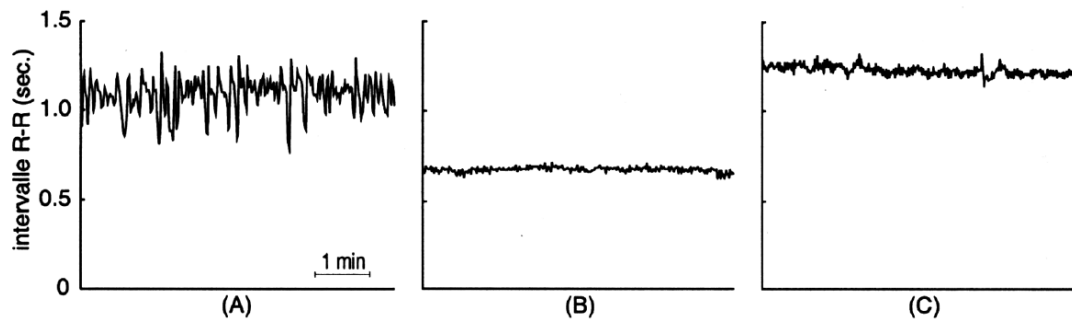


Figure 11 : Exemple de mesures de l'intervalle RR durant 5 minutes, dans trois cas différents, avec une variabilité élevée (A) et faible (B et C)

Ces variations spontanées peuvent être analysées dans les domaines du temps et de la fréquence. Toutefois, afin d'obtenir une définition des événements suffisamment fine, il est important que le signal ECG ait une fréquence d'échantillonnage supérieure à 250Hz (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Il est important de nettoyer le tracé afin d'obtenir un signal propre, sans battement ectopique ou manquant. Cette étape est réalisée en utilisant des filtres éliminant les faux battements ainsi que des algorithmes d'interpolation qui permettent de moyenniser un battement devant être remplacé en prenant en compte les battements précédents et suivants. Une fois ces manipulations réalisées, deux méthodes distinctes peuvent être utilisées. Elles sont décrites dans les paragraphes suivants.

3.1.1. Analyse temporelle de la variabilité de fréquence cardiaque

La plus ancienne, mais aussi la plus commune des méthodes d'évaluation en milieu clinique, consiste, en déterminant chaque complexe QRS et donc chaque intervalle RR normal (aussi nommé « normal to normal » (NN), à connaître la FC à un instant t (Task force, 1996 ; Malik, 2004 ; Lahiri *et al.*, 2008). Cette méthode temporelle ne permet pas de différencier de manière spécifique l'activité des branches sympathique et parasympathique du SNA, et il est recommandé de l'utiliser sur de longues périodes d'au moins 18 heures (Buijs et Swaab ; 2013). Différents indices dérivés de la mesure directe de l'intervalle RR peuvent être calculés sur la base de ces mesures. Le premier, nommé SDNN, consiste à calculer la déviation standard de l'ensemble des intervalles RR sur l'enregistrement. Il donne une information globale de la variabilité. La durée de l'enregistrement fera donc varier l'indice, et des sessions d'enregistrement de durées inégales ne doivent, de ce fait, pas être comparées (Task force, 1996 ; Crawford *et al.*, 1999 ; Kleiger *et al.*, 2005).

De ce paramètre, deux variantes créées en divisant l'ensemble de l'enregistrement en périodes de cinq minutes sont possibles. La première, le SDNN index, mesure la moyenne des déviations standards des intervalles RR de l'ensemble des périodes de cinq minutes. Le SDANN index calcule, sur l'ensemble de l'enregistrement, la déviation standard de la moyenne des intervalles RR pour chaque période de cinq minutes. Il renseigne plutôt la variabilité à long terme. D'autres variables peuvent être dérivées des différences entre les intervalles RR. Ainsi, NN50 représente le nombre d'intervalles RR

successifs variant de plus de 50 ms. SDSD est la déviation standard de la différence entre les intervalles RR successifs. rMSSD est la moyenne quadratique (i.e. la racine carrée de la moyenne arithmétique des carrés de ces nombres) des intervalles RR successifs, et exprime, entre autre, la variabilité à court terme des hautes fréquences décrites dans le chapitre suivant, et modulées principalement par la respiration. Le pNN50 est le nombre d'intervalles RR successifs variant de plus de 50 ms divisé par le nombre total d'intervalles. Il reflète aussi la variabilité des hautes fréquences, rapportées à la modulation parasympathique du cœur par le SNA (Task force, 1996 ; Kleiger *et al.*, 2005). De l'ensemble des paramètres temporels, aucun n'est considéré comme représentant principalement les modulations sympathiques du cœur.

Un autre type d'analyse temporelle, dit géométrique, consiste à convertir les séries d'intervalles RR en formes géométriques, telles que l'histogramme, le HRV triangular index, ou encore la méthode basée sur les graphiques de Lorenz ou de Poincaré (Kleiger *et al.*, 1987 ; Malik, 2004). Même si elles permettent une appréciation visuelle intéressante, ces méthodes restent cependant peu répandues dans la recherche fondamentale. L'ensemble des paramètres calculés par analyse temporelle sont résumés dans le Tableau I.

Tableau I

Variable	Unité	Description
SDNN	Ms	Ecart-type de tous les intervalles RR sur la période d'enregistrement
SDNN index	Ms	Moyenne des écarts-types des intervalles RR de chaque période successive de 5 minutes durant l'enregistrement.

SDANN index	Ms	Ecart-type de la moyenne des intervalles RR de chaque période successive de 5 minutes durant l'enregistrement.
NN50	-	Nombre d'intervalles RR successif normaux supérieurs à 50 ms sur l'ensemble de l'enregistrement.
SDSD	Ms	Ecart-type de la différence entre les intervalles RR successifs sur l'ensemble de l'enregistrement.
rMSSD	Ms	Racine carrée de la moyenne des intervalles RR successifs élevés au carré.
pNN50	%	Pourcentage d'intervalles RR successifs normaux supérieurs à 50 ms, sur l'ensemble de l'enregistrement.

3.1.1.1. Interprétation, avantages et limites

Les méthodes temporelles sont fréquemment utilisées dans les études cliniques. Les données recueillies montrent qu'une réduction de la variabilité de la fréquence cardiaque est généralement associée à une augmentation des risques de mortalité liés à différentes cardiopathies telles que l'insuffisance cardiaque (Ponikowski *et al.*, 1997 ; Sandercock and Brodie, 2006 ; Tuininga *et al.*, 1994), l'infarctus du myocarde (Pipilis *et al.*, 1991 ; Singh *et al.*, 1996 ; Zuanetti *et al.*, 1996), ou encore le syndrome de mort subite (Kudaiberdieva *et al.*, 2007).

Ces méthodes temporelles ont l'avantage d'être relativement simples et apportent, dans un contexte clinique, des informations et des pronostics sur les patients, qui s'avèrent utiles pour le personnel médical. Cependant, ces méthodes sont sensibles aux biais induits par des signaux non stationnaires et ne permettent pas de fournir des informations sur les différentes fréquences du signal de la variabilité de l'intervalle RR. Car comme tout signal, celui de la

variabilité cardiaque est fait de fréquences dont l'analyse apporte de nombreuses informations physiologiques. C'est pour cela que les méthodes fréquentielles, discutées dans le chapitre suivant, se révèlent être de puissants outils d'analyse.

3.1.2. Analyse fréquentielle de la variabilité de fréquence cardiaque

Ce second type d'analyse est une méthode décelant les différentes oscillations d'un rythme. Elle décompose n'importe quel signal en composants sinusoïdaux et permet de tracer le graphique de la puissance de chacun de ces composants, comme la fonction de sa fréquence. Elle renseigne donc sur la distribution des ondes composant le signal en fonction de leur fréquence. Les premières études fréquentielles de la variabilité cardiaque datent de 1980 (Akselrod *et al.*, 1981 ; Pomeranz *et al.*, 1985). Il existe principalement trois méthodes d'analyse fréquentielle.

3.1.2.1. Transformée rapide de Fourier

La première méthode d'analyse repose sur les travaux de Jean-Baptiste Joseph Fourier, mathématicien français du XVIII^e siècle. Le principe de base de la transformée, qui prit son nom, veut qu'un signal stationnaire puisse être divisé en une somme de sinusoides simples. Cette opération mathématique permet donc de décomposer une fonction selon ses fréquences. Ainsi, une suite de valeurs dépendant du temps est transformée pour donner une autre suite de nombres, dépendant, cette fois, de la fréquence.

Il y a 50 ans, Cooley et Tukey proposèrent la transformée rapide de Fourier (FFT, de l'anglais « Fast Fourier Transform ») pour l'analyse des oscillations de la fréquence cardiaque. Cette analyse non-paramétrique, transforme un signal temporel en signal fréquentiel en économisant un nombre important d'opérations par rapport aux équations proposées par Fourier. Sa représentation est de forme spectrale, et le tracé de la puissance du signal s'exprime en fonction des fréquences exprimées en Hz (Figure 12).

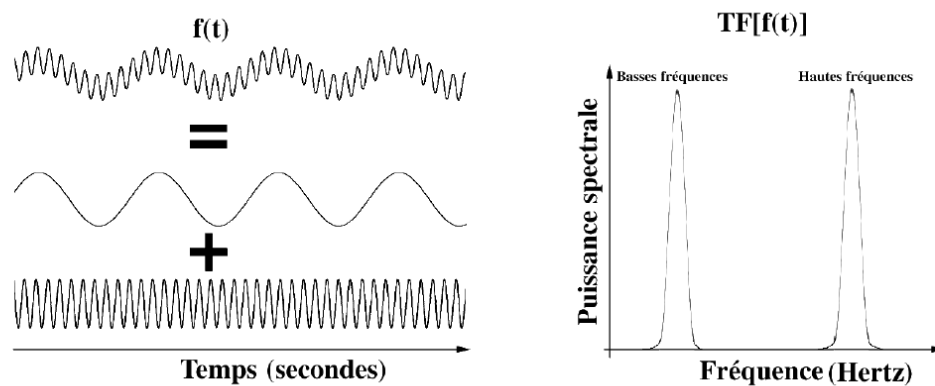


Figure 12 : A gauche, exemple de décomposition d'une fonction simple $f(t)$ en somme de sinusoïdes. A droite, représentation spectrale de sa transformée de Fourier $TF[f(t)]$

Pour une fonction temporelle $f(t)$, la transformée de Fourier se définit comme suit :

$$F(f): v \mapsto \hat{f}(v) = \int_{-\infty}^{+\infty} f(t) \cdot e^{-i2\pi vt} dt$$

Les ordinateurs étant devenus suffisamment puissants, Akselrod et ses collaborateurs furent les premiers, en 1981, à mettre en évidence chez le chien conscient la présence de pics de fréquences caractéristiques et observables sur le spectre de la variabilité cardiaque (Figure 13).

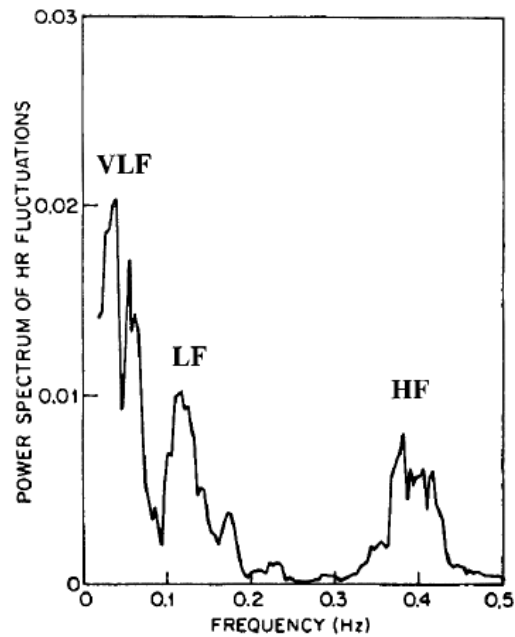


Figure 13 : Spectre de Fourier de la variabilité RR. Différents pics de fréquences sont observables sur le spectre. Les très basses fréquences (VLF) autour de 0,01 - 0,1 Hz, les basses fréquences (LF) autour de 0,1 - 0,15 Hz et les hautes fréquences (HF) autour de 0,3 - 0,5 Hz. (Akselrod et al., 1985)

Ces pics de fréquences semblaient refléter par leur amplitude et leur localisation sur le spectre, des phénomènes physiologiques résultant de l'activité régulatrice sympathique et parasympathique du SNA. Depuis lors, de très nombreuses études ont été réalisées dans le but de mieux comprendre les différentes corrélations entre puissance spectrale et influences sympathique et parasympathique. De nombreux résultats sont contradictoires, et les causes physiologiques de certains pics, ainsi que les effets de certaines situations ou substances, sont encore discutés aujourd'hui. Nous reviendrons ultérieurement et de manière plus précise sur ces résultats.

3.1.2.1.1. Avantages et inconvénients

Le principal avantage de la méthode d'analyse par FFT est la simplicité de son utilisation. L'algorithme utilisé permet des analyses très rapides sur les logiciels informatiques actuels.

Son principal inconvénient réside dans l'obligation d'utiliser des données enregistrées dans des conditions stationnaires. Les enregistrements de longues durées étant sujets aux variations que le corps humain traverse au cours de la journée, ils s'avèrent peu précis et demandent une vérification de la stationnarité de leur signal.

3.1.2.2. Modèle autorégressif

Une deuxième méthode, dite autorégressive, permet d'analyser de manière fréquentielle la variabilité cardiaque. Cette analyse paramétrique, mise en avant par Pagani *et al.* (1986), a pour principale différence la manière dont les données sont considérées. La série temporelle vue par la FFT est faite de composants déterministes alors que le modèle autorégressif traite les données comme un mélange de données déterministes et stochastiques (Berntson *et al.*, 1997). Plutôt que de reproduire précisément le spectre de puissance, comme le fait l'analyse FFT, le modèle autorégressif se concentre sur les pics les plus significatifs afin de s'ajuster de la meilleure manière à un modèle prédéterminé. Cela a pour effet d'atténuer le bruit du signal et d'éliminer ce qui peut être considéré comme des artéfacts. Ainsi, la FFT est considérée comme une méthode descriptive, alors que le modèle autorégressif découle plus d'un processus statistique. Malgré les avantages et inconvénients de chaque méthode (Task Force, 1996), il est admis que les méthodes paramétrique et non

paramétrique fournissent des résultats similaires, pour autant que le modèle autorégressif déterminé soit bon et que les signaux analysés soient stationnaires (Parati *et al.*, 1995)

3.1.2.2.1. Avantages et inconvénients

Le lissage énoncé précédemment permet une lecture facilitée des éléments principaux du spectre. De plus, même avec un échantillon de valeurs réduit, le modèle autorégressif permet une bonne analyse de la densité de puissance spectrale, pour autant que le signal soit stationnaire. Un autre avantage de cette méthode est la possibilité, en passant par un post-traitement des données spectrales, de séparer facilement les LF et les HF par des calculs automatisés.

Le fait de devoir vérifier la validité du modèle déterminé reste le principal inconvénient de la méthode autorégressive. En effet, les résultats pour la même bande de fréquence peuvent grandement varier en fonction du type de modèle autorégressif choisi (van de Louw, 2010). Cependant, Miranda Dantas *et al.* (2012) semblent avoir montré que le choix du modèle n'affectait pas les valeurs normalisées de LF et HF.

3.1.3. Composants spectraux

L'interprétation des paramètres issus de la variabilité cardiaque étant complexe, une standardisation des différentes fréquences a été mise en place. Dans le spectre de variabilité cardiaque, quatre pics ont été identifiés comme étant caractéristiques. Chacun de ces pics, situé dans une bande de fréquence

donnée, peut être quantifiée en intégrant l'air sous la courbe spectrale de la dite bande de fréquence. L'analyse de la densité de puissance spectrale de chacun de ces indices est exprimée en ms^2 . Les caractéristiques de ces indices sont listées dans le tableau II.

Le premier indice caractérise ce qu'on appelle les ultra-basses fréquences (ULF, Ultra Low Frequency). Elles ne sont mesurables qu'avec des enregistrements de très longues durées, car comprises en dessous de 0,003 Hz, ce qui représente des variations du R-R d'au minimum six minutes et jusqu'à 24 heures (Task Force, 1996). Ces fréquences pourraient refléter les cycles circadiens et des rythmes neuro-endocriniens (Barrett *et al.*, 2001), mais leur signification, à l'heure actuelle, reste encore floue.

Le second indice caractérise les très basses fréquences (VLF, Very Low Frequency), qui se mesurent entre 0,003 Hz et 0,04 Hz. Des résultats suggèrent une corrélation avec le système rénine angiotensine (Akselrod *et al.*, 1981 ; Taylor *et al.*, 1998), le système humoral de manière plus générale (Braga *et al.*, 2002 ; Porter et Rivkees, 2001) et la thermorégulation (Aoki *et al.*, 2001 ; Blumberg *et al.*, 2001 ; Overton *et al.*, 2001 ; Task Force, 1996). Cependant, les mécanismes physiologiques corrélés à cet indice sont encore discutés, et, tant les ULF que les VLF sont plus utilisés en cardiologie comme indicateurs de la mortalité post-infarctus (Bigger *et al.*, 1996) que lors d'analyses cherchant à expliquer l'influence de l'activité du SNA. C'est pourquoi nous ne les développerons pas davantage.

Viennent ensuite les basses fréquences (LF, Low Frequency), comprises entre 0,04 Hz et 0,1 Hz. Leur interprétation physiologique reste encore largement

discutée. Ces interprétations seront développées plus longuement dans un chapitre ultérieur.

Enfin, quatrième et dernière catégorie de fréquences, les hautes fréquences (HF, High Frequency) comprises entre 0,15 Hz et 0,4 Hz. Cette catégorie de fréquence a été associée à l'activité parasympathique (Pagani *et al.*, 1986 ; Task Force, 1996) et à la fréquence respiratoire. Tout comme pour les LF, nous reviendrons spécifiquement sur la signification de cette bande fréquentielle.

En intégrant l'ensemble des composants spectraux énumérés ci-dessus, il est facile de connaître la valeur totale de la puissance du spectre de variabilité cardiaque (P_{TOT}).

Il est aussi possible d'utiliser les valeurs normalisées des indices de variabilité cardiaque. En effet, les ULF et VLF ne pouvant être mesurées que durant des enregistrements de longues durées et pouvant représenter plus de 50% de la puissance totale du spectre avec la présence de bruits conséquents (Furlan *et al.*, 1990), il est utile de connaître la valeur relative de LF et HF (LFnu et HFnu, respectivement) afin de déterminer leur importance dans le spectre, en mettant de côté les fréquences en dessous de 0,03 Hz (Task Force, 1996 ; Malliani, 1991 ; Pagani, 1986). Ainsi, LFnu et HFnu s'obtiennent par les équations suivantes et s'expriment en pourcentage :

$$LFnu = 100 \cdot \frac{LF}{P_{TOT} - (ULF + VLF)}$$

$$HFnu = 100 \cdot \frac{HF}{P_{TOT} - (ULF + VLF)}$$

Tableau II

Variable	Unité	Bande de fréquence (Hz)	Description
P _{TOT}	Ms ²	0 - 0,5	Puissance totale des intervalles RR sur la totalité de l'enregistrement.
ULF	Ms ²	≤ 0,003	Puissance des intervalles RR dans la bande spectrale des ULF.
VLF	Ms ²	0,004 - 0,04	Puissance des intervalles RR dans la bande spectrale des VLF.
LF	Ms ²	0,05 - 0,14	Puissance des intervalles RR dans la bande spectrale des LF.
HF	Ms ²	0,15 - 0,4	Puissance des intervalles RR dans la bande spectrale des HF.
LFnu	%		Puissance relative des intervalles RR dans la bande spectrale des LF sur la puissance totale sans les ULF et VLF
HFnu	%		Puissance relative des intervalles RR dans la bande spectrale des HF sur la puissance totale sans les ULF et VLF.
LF/HF	-		Rapport entre la puissance dans la bande spectrale des LF sur celle des HF.

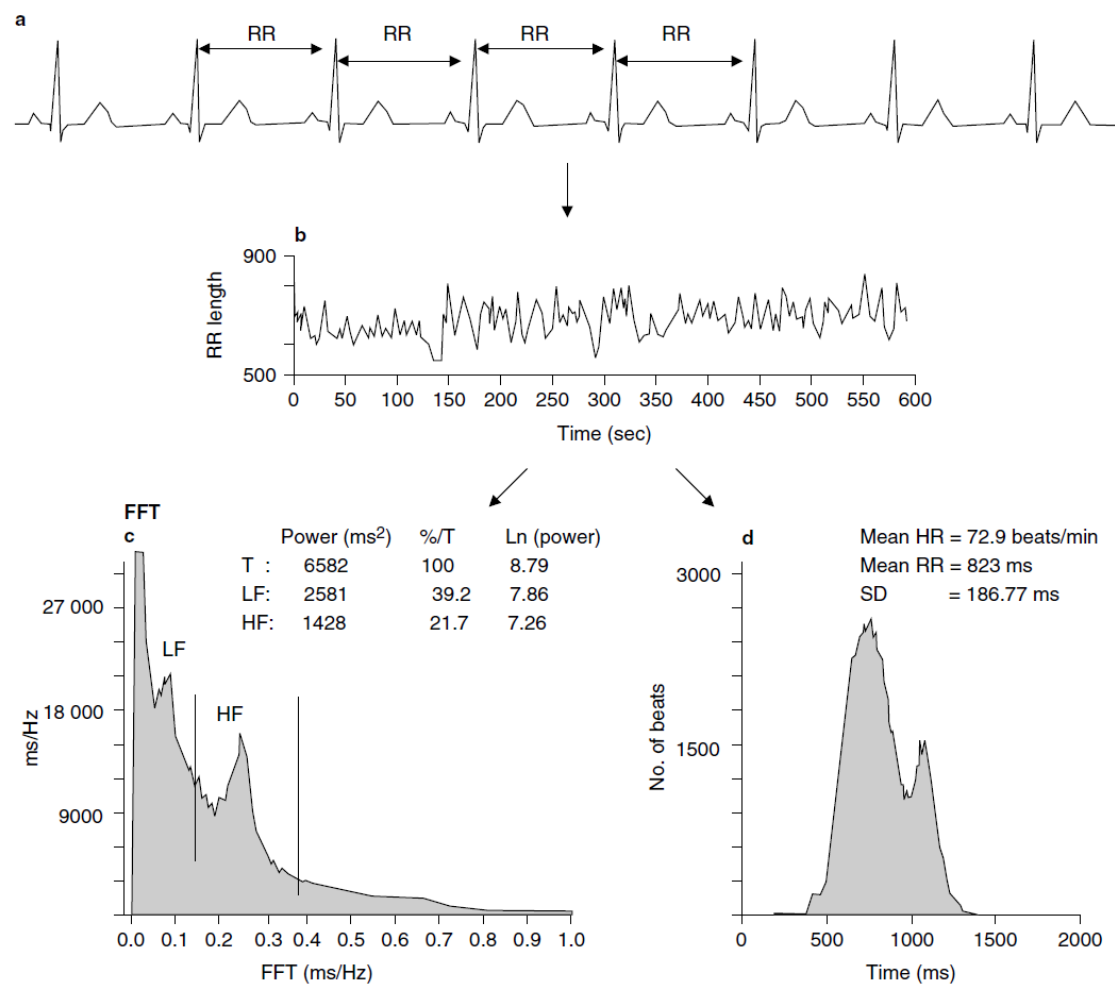


Figure 14 : Variabilité de la fréquence cardiaque. Le calcul des intervalles RR consécutifs (a) sur l'ECG résulte en une courbe sur le tachogramme (b) Cette dernière peut être analysée dans le domaine fréquentiel (c) et le domaine temporel (d). L'analyse spectrale (c) et l'histogramme (d) correspondent à des résultats provenant d'un enregistrement Holter sur 24 heures. L'histogramme montre deux pics : le premier, autour de 1100ms, correspond à la fréquence cardiaque nocturne moyenne, et le second, à environ 750ms, correspond à la fréquence cardiaque durant la journée. FFT : transformée de Fourier, HF : haute fréquence ; HR : fréquence cardiaque ; LF : basse fréquence ; Ln : logarithme naturel ; T : puissance totale. (Aubert et al., 2003).

3.1.3.1. Interprétation des composants spectraux

De nombreux travaux ont tenté d'interpréter l'ensemble des indices de la variabilité de la fréquence cardiaque. Comme mentionné dans le chapitre

précédent, nous ne nous intéresserons pas aux ULF et VLF, dont l'interprétation sur des enregistrements à court terme n'est pas possible (Task Force, 1996).

L'interprétation de la composante LF du spectre de puissance de la variabilité cardiaque reste encore de nos jours un sujet controversé de débats scientifiques. Longtemps, la composante LF fut présentée comme le miroir de l'activité sympathique par une partie importante de la communauté scientifique active dans le domaine (Kamath and Fallen, 1993 ; Malliani *et al.*, 1991 ; Pagani *et al.*, 1986). Cependant, d'autres chercheurs voient dans les LF non seulement un indicateur des activités sympathiques, mais aussi des activités parasympathiques, car les blocages pharmacologiques vagues ou sympathiques amènent, les deux, à une diminution des LF (Houle et Billman 1999 ; Pomeranz *et al.*, 1985 ; Persson *et al.*, 1992). D'autres études ont mis en évidence le lien entre les LF et l'activité nerveuse sympathique du muscle (MSNA) (Nakata *et al.*, 1998 ; Saul *et al.*, 1990). Cette dernière ne reflète qu'une activité périphérique et musculaire, mais en agissant sur la pression artérielle via des substances vaso-actives, on observe les mêmes effets sur les efférences cardiaques que sur le MSNA (Ninomiya *et al.*, 1971), ce qui démontre donc bien le lien entre les LF et l'activité sympathique.

Concernant la fenêtre des HF, son interprétation est plus claire, et l'ensemble de la communauté scientifique s'accorde pour dire qu'elle est un contributeur majeur de l'activité parasympathique. En effet, plusieurs études (Akselrod *et al.*, 1981, 1985 ; Pomerantz *et al.*, 1985) montrent le lien entre cet indice et l'activité de régulation du nerf vague sur le cœur. D'autres chercheurs se limitent à affirmer que le pic HF reflète les effets du rythme respiratoire sur la

fréquence cardiaque, d'autant plus que la localisation du pic HF dans le spectre de la variabilité cardiaque correspond à la fréquence respiratoire (0,25 Hz de norme au repos). Les HF se situant dans la même fourchette de fréquence, elles peuvent donc toujours être influencées par les variations mécaniques ou les changements de pression intra-thoracique, modifications anatomiques engendrées par la respiration. Cependant, lors d'administration d'atropine (Akselrod *et al.*, 1981) ou durant l'anesthésie (Latson, 1992), ces fréquences disparaissent sans que la ventilation ne s'arrête. De plus, des expériences cliniques et fondamentales avec manœuvres sur le SNA par blocage de récepteurs muscariniques, stimulation électrique du vague, ou encore vagotomie (Akselrod *et al.*, 1981 ; Malliani *et al.*, 1991 ; Pomeranz *et al.*, 1985) démontrent clairement le lien entre la composante HF et le système parasympathique.

Certains chercheurs ont proposé d'utiliser le rapport entre la composante LF et HF pour établir ce qu'ils ont défini comme la balance sympatho-vagale. En effet, si la composante LF est uniquement un marqueur de l'activité sympathique et la composante HF celui de l'activité vagale, le résultat du rapport de ces deux valeurs donnerait une information sur l'état du SNA, basé sur le concept voulant que lorsqu'une de ses branches augmente, l'autre diminue (Pagani *et al.*, 1986 ; Malliani, 1995). Ce rapport est couramment utilisé mais reste cependant critiqué par une partie de la communauté scientifique (Berntson *et al.*, 1997; Billman, 2009, 2011 ; Eckberg, 1997; Parati *et al.*, 2006) qui a pour principal argument que la composante LF ne peut se résumer à un indice de l'unique activité sympathique, mais est influencée par un mélange de paramètres bien plus complexes.

Comme les valeurs absolues des indices de la variabilité de fréquence cardiaque changent en fonction des conditions et d'une personne à l'autre, il peut être utile de calculer les valeurs relatives des composantes LF et HF afin de mieux se rendre compte de leur importance au sein du spectre de puissance. Cela permet d'écarter les ULF et VLF qui représentent une part importante de la puissance totale, mais ne sont pas interprétables sur de courts enregistrements (Task force, 1996). Contrairement à la fréquence cardiaque, les valeurs normalisées peuvent être influencées par les mouvements respiratoires, et leur utilisation reste limitée quand elles sont mesurées à l'exercice. Elles permettent de donner une indication sur l'influence relative des branches parasympathique et sympathique mais, une fois encore, la relation entre l'activité de chacune est plus complexe qu'une simple notion de vases communicants.

3.1.4. Transformée d'ondelette

Un désavantage des méthodes fréquentielles réside dans le manque d'informations temporelles. En effet, s'il est possible avec ces méthodes de détecter deux fréquences d'oscillation f et f' , il est cependant impossible de savoir quelle est la fréquence d'oscillation à un instant t , et il est donc impossible de détecter les changements de fréquence au cours du temps. De ce fait, d'autres méthodes dites « temps-fréquence » ont été développées pour permettre de rendre compte de ces changements de fréquences de variabilité de l'intervalle RR tout au long d'un enregistrement ECG.

La transformée d'ondelette a été introduite par Jean Morlet, chercheur français travaillant sur les signaux sismiques dans les années 80 (Morlet *et al.*, 1982). Tout comme la transformée de Fourier, il s'agit d'une transformée

intégrale. La décomposition d'un signal par cette méthode requiert une fonction Ψ régulière, nommée fonction mère. Partant de cette dernière, différentes fonctions sont construites par dilatation ou translation. La transformée d'ondelette d'un signal f est définie comme :

$$W_{f(a,b)} = \langle f, \Psi_{a,b} \rangle \quad a \in \mathbb{R} * b \in \mathbb{R}$$

où $\langle, \rangle$ est le produit scalaire L^2 de f et $\Psi_{a,b}$, i.e., $\int f(x) \cdot \Psi_{a,b}(x) \cdot dx$.

Contrairement à la transformée de Fourier, cette méthode ne demande pas à être réalisée sur un signal stationnaire. Il est donc possible d'analyser des enregistrements qui, de par leur durée ou leur protocole, impliquent des signaux de variabilité de fréquence cardiaque inconstants. (Pichot *et al.*, 1999)

3.2. Sensibilité du baroréflexe

Comme nous l'avons vu précédemment dans le chapitre des fondamentaux physiologiques, le baroréflexe est, par définition, un réflexe physiologique permettant de limiter, à très court terme, les changements de la pression artérielle.

En cas de chute de la pression artérielle, le SNA va stimuler ses efférences sympathiques en direction du cœur et du système vasculaire, avec pour résultat des effets chronotropes et inotropes positifs amenant à une tachycardie et une augmentation de la contraction du myocarde. De même, les résistances artérielles systémiques augmentent, d'où l'élévation de la pression artérielle.

Conjointement, la branche parasympathique du SNA inhibe ses efférences vagales. Le mécanisme inverse sera observé en cas d'augmentation brusque de la pression artérielle.

La respiration, parmi d'autres facteurs, influence de manière importante le baroréflexe. En effet, l'inspiration freine l'activation vagale alors que l'expiration l'augmente, au travers des centres respiratoires situés dans le bulbe rachidien (Eckberg, 2003).

3.2.1. Importance clinique de la sensibilité du baroréflexe

La pression artérielle change donc peu grâce au système de régulation du baroréflexe et ce, malgré les nombreuses causes de variation rencontrée par le corps humain. En effet, les émotions, le positionnement du corps, ou encore l'exercice sont autant de facteurs qui influencent de manière importante la pression artérielle et qui la modifieraient grandement sans la boucle de rétroaction du baroréflexe. Cette sensibilité est donc très importante. Les scientifiques et médecins ont rapidement compris l'importance de cette sensibilité et ont cherché à mieux la comprendre et à la quantifier. Ainsi, de nombreuses études (Benarroch, 2008 ; Lanfranchi et Somers, 2002 ; La Rovere *et al.*, 2008 ; Ormezzano *et al.*, 2008 ; Robinson *et al.*, 2003 ; Skrapari *et al.*, 2006) démontreraient que de nombreuses maladies sont corrélées à une baisse du BRS, et que les pronostics de vie chutent lorsque la sensibilité diminue. Ainsi, diabète, obésité, maladies coronariennes, âge, ou encore hypertension, impliquent tous une diminution du BRS. Après un épisode d'infarctus du myocarde ou d'insuffisance cardiaque congestive, les patients avec un BRS

réduit ont de plus fortes probabilités de mortalité cardiaque (La Rovere *et al.*, 2008). Au contraire, l'entraînement physique augmente le BRS de manière significative (Hua *et al.*, 2009 ; Joyner et Green, 2009 ; Pagani *et al.*, 1988).

3.2.2. Mesure de la sensibilité du baroréflexe

Mesurer le BRS est donc devenu primordial dans de nombreux domaines de la médecine. Différentes méthodes existent et sont utilisées selon le contexte d'investigation. Nous développerons brièvement les deux méthodes les plus performantes selon l'étude de Laude *et al.* (2004). Ces deux méthodes sont basées sur une investigation de type « boucle ouverte ».

3.2.2.1. Technique spectrale croisée

La technique spectrale croisée repose sur la mesure conjointe de la fréquence cardiaque et de la pression artérielle. En effet, en mesurant l'amplitude de leurs oscillations à une basse fréquence définie de 0,1 Hz, ou dans la fréquence ventilatoire, plus élevée, autour de 0,25 Hz, on obtient un rapport d'amplitude. Ces oscillations surviennent toujours avec un décalage de phase, la fréquence cardiaque étant précédée de la pression artérielle.

3.2.2.2. Méthode séquentielle

Cette technique se base sur la relation entre la fréquence cardiaque et la pression artérielle pour quantifier le BRS. En effet, dans des conditions physiologiques, lorsque la pression artérielle augmente ou diminue sur de très

courtes périodes, équivalentes à quelques battements cardiaques, l'intervalle RR change dans la même direction (la fréquence cardiaque dans le sens inverse), par régulation de la boucle de rétroaction du baroréflexe (Bertinieri *et al.*, 1988).

Ces auteurs ont proposé de détecter et de sélectionner chaque épisode d'au moins trois cycles cardiaques consécutifs où la pression augmente ou diminue d'au moins 1 mmHg et l'intervalle RR varie dans la même direction d'au moins 6 msec (Figure 15.A). En représentant graphiquement les trois points de la pression artérielle en fonction de l'intervalle RR, chaque séquence permet de tracer une régression linéaire, dont la moyenne des pentes représente, par définition, BRS (Figure 15.B).

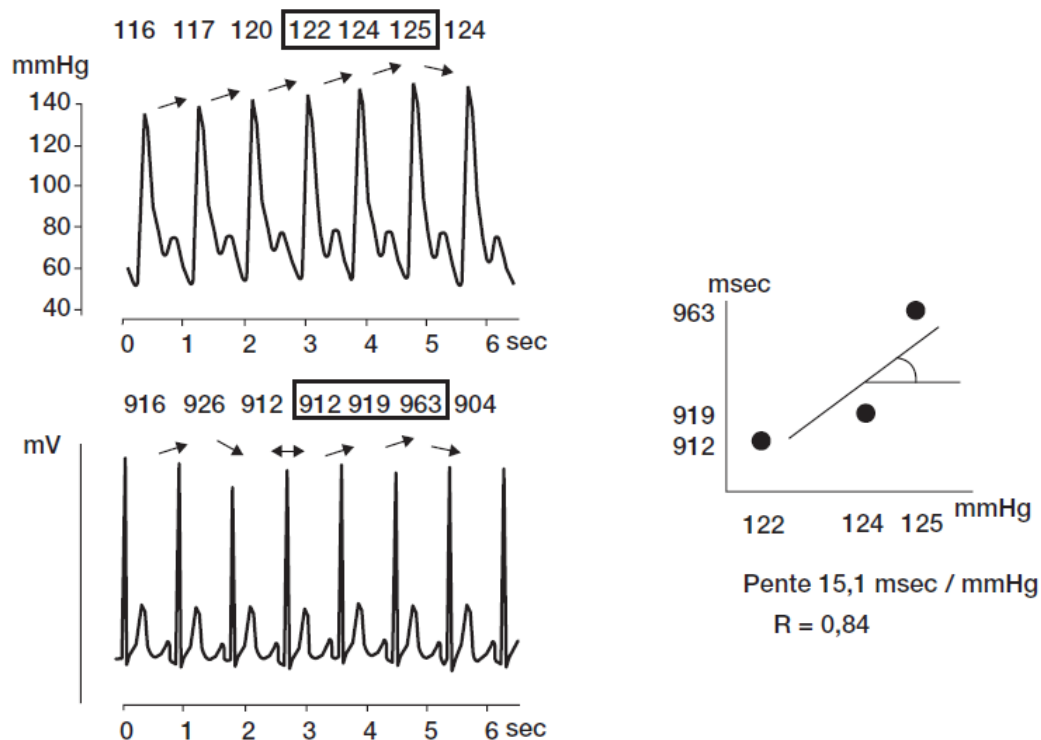


Figure 15 : Graphique de la pression artérielle, de la fréquence cardiaque et du rapport du premier en fonction du deuxième. On peut observer la diminution de la fréquence cardiaque concomitante à l'augmentation de la pression.

Cette méthode séquentielle a été principalement appliquée à l'étude du BRS au repos et pendant l'exercice à l'état stationnaire, essentiellement dans un

contexte d'étude de la fréquence cardiaque et de variabilité de la pression artérielle (Hartwich *et al.*, 2011 ; Iellamo *et al.*, 1994 ; 1997, Kardos *et al.*, 2001 ; La Rovere *et al.* 2008 ; Parati *et al.*, 2001 ; Vallais *et al.*, 2009). C'est avec cette méthode qu'ont été réalisées les analyses du BRS sur l'ensemble des études présentées dans cette thèse de doctorat.

Seconde Partie

4. Méthodologie commune

Les méthodes spécifiques à chacun des travaux de cette thèse sont expliquées précisément dans les articles présentés ultérieurement. Une partie de cette méthodologie étant commune à l'ensemble des travaux, nous la décrivons dans ce chapitre.

4.1. Acquisition des données

La fréquence cardiaque et l'intervalle R-R en découplant ont été mesurés par un système d'ECG (Elmed ETM 2000, Heiligenhaus, Allemagne).

La pression artérielle a été enregistrée de manière continue par un système de mesure placé sur la deuxième phalange de l'index droit. Ce système (Portapres, TNO, Eindhoven, Pays-Bas), se base sur le principe de la photopléthysmo-graphie (Figure 16). Le système enregistre en continu l'évolution du pulse de pression artérielle durant chaque cycle cardiaque. Les pressions artérielles systolique et diastolique ont été obtenues comme les plus hautes et plus basses valeurs identifiées durant chaque battement, à l'aide du programme « Beatscope » (FMS, Amsterdam, Pays-Bas). Ce procédé mesure la pression artérielle de manière non invasive sur une artère périphérique de l'index, et a des

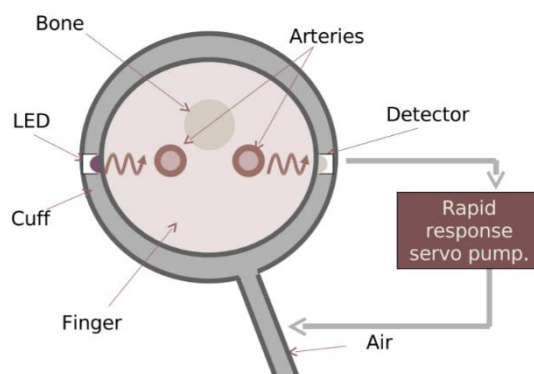


Figure 16 : Schéma descriptif de la méthode de mesure de la pression au doigt par le système Portapres.

caractéristiques mécaniques remarquablement différentes de celles mesurées sur une artère centrale (Remington et Wood, 1956). Cela implique une correction des valeurs absolues de pression artérielle systolique et diastolique (Azabji-Kenfack *et al.*, 2004) compte tenu des distorsions potentiellement introduites dans la forme du pulse de pression périphérique. Afin d'éviter des biais de mesure, le capteur situé au doigt ne se trouvant pas systématiquement à la même hauteur que le cœur, le Portapres calcule la différence de hauteur, puis soustrait ou additionne la différence de pression hydrostatique correspondante.

La respiration, et, plus précisément, la fréquence respiratoire, n'ont été mesurées sur les transplantés pulmonaires que dans le cadre du deuxième article. En effet, la thématique de cette étude se rapportant surtout au système respiratoire, la régulation et l'influence de ce dernier ont été investiguées de manière plus conséquente. La fréquence respiratoire a donc été mesurée par un appareillage à ultrason (Spiroson, Ecomedics, Suisse) fixé sur un masque respiratoire.

Afin d'être convenablement analysé, l'ensemble des signaux a été digitalisé en parallèle par un système d'acquisition multicanaux (MP150, Biopac Systems, Goleta CA, USA).

4.1.1. Logiciel *BRSanalysis*

Un programme, développé dans l'environnement MatLab, a permis de traiter de manière efficace l'ensemble des données qui composent les trois articles présentés dans cette thèse. Intitulé « BRSanalysis », ce programme informatique a été créé par le Dr Vincent Pichot, spécialiste du traitement de

signal au sein de l'unité de recherche SNA-EPIS de l'Université Jean Monnet de Saint-Etienne. Une version permettant uniquement l'analyse de la variabilité cardiaque, donc sans mesure de la pression artérielle et du BRS, a été mise en accès libre en fin d'année 2016. (Pichot *et al.*, 2016). Cette version, accessible à tous en téléchargement, varie en quelques points de la version utilisée pour les travaux présentés dans cette thèse, mais se base sur les mêmes procédures d'analyse décrites ci-dessous.

BRSanalysis permet de mesurer, dans une fenêtre prédéfinie de données, les différents indices de la variabilité de la fréquence cardiaque et de la pression artérielle, et donc du BRS. Il base son analyse de la variabilité de l'intervalle RR sur la méthode non paramétrique de la transformée de Fourier décrite dans le chapitre 3. Il est donc possible de connaître le spectre de puissance des indices de la variabilité de l'intervalle RR, et ce pour une fenêtre prédéfinie de données.

4.1.1.1. Traitement des données sur *BRSanalysis*

Le traitement des enregistrements d'intervalles RR comprend trois étapes. La première concerne l'importation des données, tirées, dans notre cas, d'enregistrements ECG. Ensuite, les pics R sont détectés par un algorithme largement inspiré des travaux de Chen *et al.* (2006). Pour ce faire, le signal ECG est échantillonné à 1000 Hz afin d'assurer une excellente résolution. Un filtre passe-haut y est appliqué afin de mettre en évidence le complexe QRS.

La deuxième étape du traitement du signal ECG concerne la correction et le formatage des données. Les enregistrements ECG contiennent souvent des battements ectopiques ou manquants dus aux arythmies cardiaques ou à la mauvaise qualité du signal. Induisant des erreurs de calcul des indices de

variabilité cardiaque, ces signaux doivent être corrigés afin d'éviter de mauvaises interprétations physiologiques (Saul *et al.*, 1988). La correction de ces battements inexacts est réalisée par l'algorithme de Kamath et Fallen (1995). Un filtre comportant des seuils de +32.5% et -24.5% est appliqué (Cheung, 1981). Ainsi repérés, le nombre de battements manquants est estimé en comparant la durée du battement qui précède à la durée totale de celui ayant été caractérisé comme anormal. Si le nombre de battements à recalculer est de trois ou moins, une interpolation spline cubique est effectuée. Cette configuration provient généralement d'un battement ectopique supraventriculaire ou ventriculaire unique, ou d'une détection erronée de pic R isolé. Pour quatre erreurs successives ou plus, les battements manquants sont interpolés en copiant et en insérant le même nombre de RR qu'entre le premier et le dernier RR valide. Bien que ces corrections aboutissent généralement à un signal RR propre, ce type d'algorithme automatique peut produire des valeurs incohérentes, par exemple, lorsque le signal ECG original est trop corrompu, avec de longues portions de faux successifs. Chaque série a donc été examinée visuellement avant d'effectuer les analyses de la variabilité cardiaque. Il est aussi possible d'effectuer une correction manuelle, soit en sélectionnant un intervalle RR et en changeant sa valeur, soit en éditant directement le tracé ECG, en ajoutant ou en enlevant un intervalle (Figure 17).

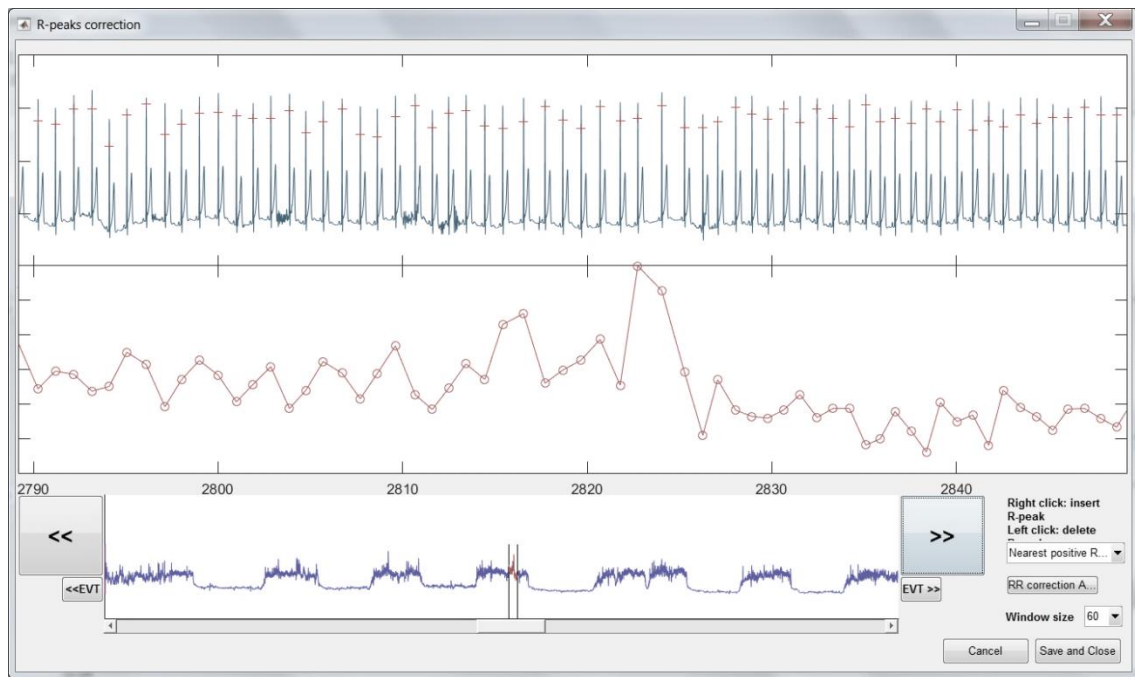


Figure 17 : Fenêtre de correction manuelle du tracé ECG tirée du programme de traitement de signal « BRSanalysis ».

Après ce nettoyage indispensable du signal, la troisième étape permet de calculer l'indice de variabilité cardiaque souhaité. BRSanalysis permet de sélectionner une fenêtre de données souhaitées sur l'ensemble du tracé ECG. Ainsi, les périodes de repos et d'exercice des différents protocoles ont pu être analysées précisément (Figure 18)

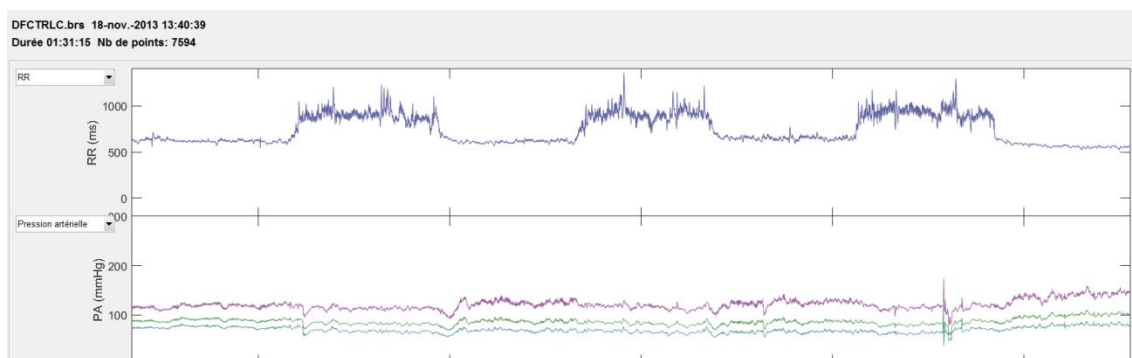


Figure 18 : Exemple de tracé ECG et de pression artérielle sur le programme BRSanalysis. Les phases de repos et d'exercice se distinguent clairement avec une diminution des intervalles R-R et de leur variabilité durant l'exercice.

Deux types d'analyses différentes ont été réalisés. Le premier permet d'analyser la puissance spectrale de la variabilité cardiaque par FFT et ainsi de recueillir des informations sur les indices de la variabilité cardiaque que sont P_{TOT} , LF, HF, LFnu, HFnu et LF/HF (Figure 19).

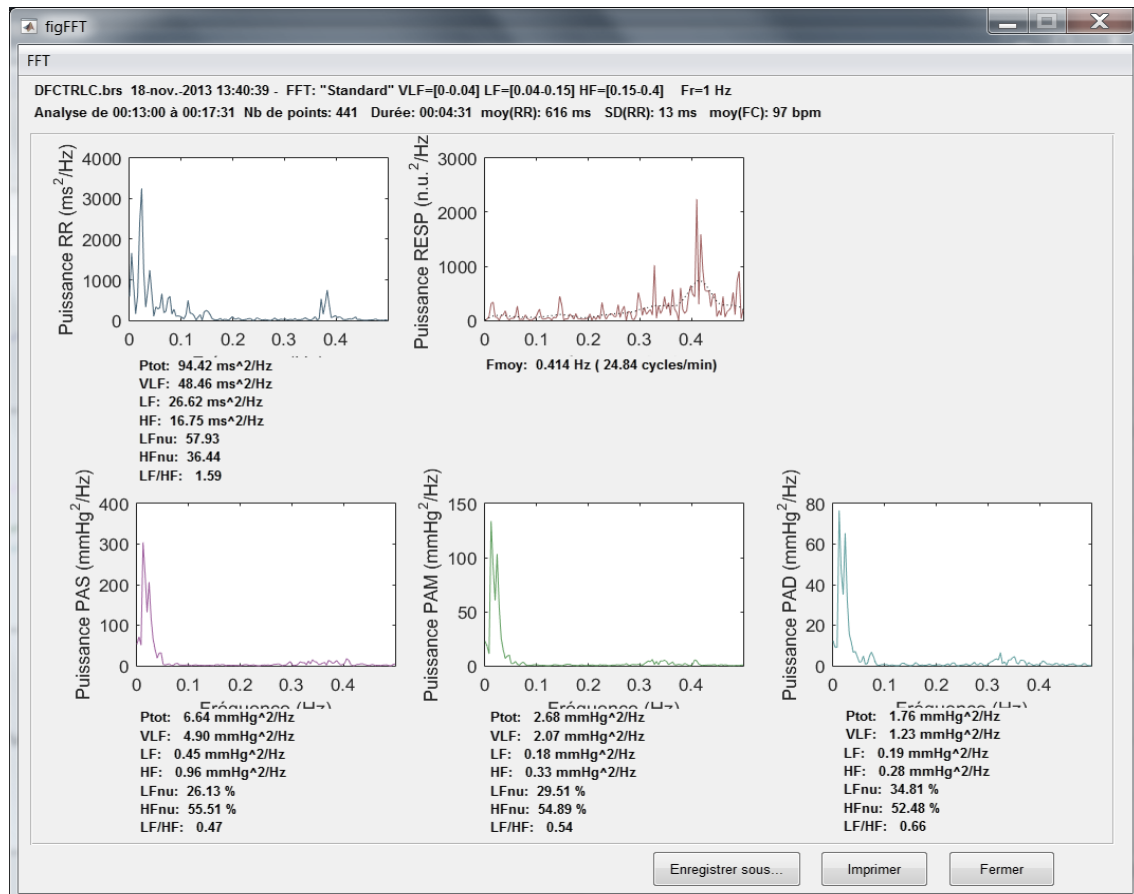


Figure 19 : Exemple d'analyse par FFT de la variabilité de l'intervalle R-R et de la pression artérielle par le programme « BRS analysis »

Le second permet de calculer le BRS de la section du tracé ECG sélectionné par la méthode séquentielle décrite précédemment. BRSanalysis permet de visualiser graphiquement les valeurs des pentes de chaque triplettes et d'enlever, au besoin, la plus grande ou la plus petite qui provient souvent d'une mesure erronée de la fréquence cardiaque ou de la pression artérielle (Figure 20). Ce travail de filtrage est indispensable afin d'éliminer les artéfacts et d'obtenir

une valeur moyenne de BRS permettant des interprétations physiologiques correctes.

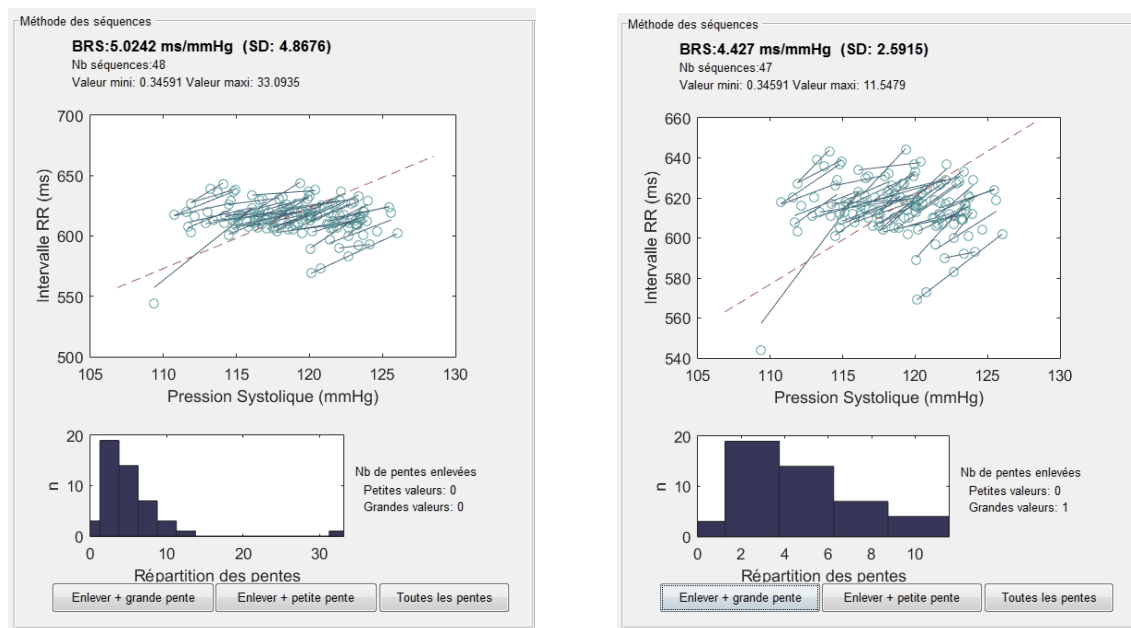


Figure 20 : Fenêtre d'analyse du BRS sur le programme BRSanalysis. Exemple d'élimination de données « absurdes ». La tripléte possédant une pente totalement hors de la distribution gaussienne (image de gauche) est éliminée (image de droite).

4.2. Analyses statistiques

Les données tirées de la variabilité cardiaque et du BRS ont été analysées statistiquement en suivant une méthode similaire dans les trois articles. De nombreuses méthodes d'analyses statistiques existent. Toutes nos expériences comptabilisent plusieurs variables dépendantes à expliquer, telles que les indices de variabilité cardiaque, le BRS, etc., ainsi que plusieurs facteurs de variabilité. Ces variables dépendantes ont, dans chacun des protocoles, été influencées par différents facteurs de variabilité.

C'est pourquoi, nous avons utilisé comme méthode de calcul statistique, l'analyse de variance, aussi communément appelée ANOVA. Cette dernière est

un test statistique mesurant la somme des carrés des écarts par rapport à la moyenne, divisé par le nombre de degrés de liberté. Ces expériences impliquant parfois deux facteurs de variabilité, une ANOVA à deux facteurs fut utilisée dans ces cas-là.

Par la suite, les ANOVA ne permettant pas de mesurer la significativité entre les moyennes de groupes, il est important d'appliquer un test « post-hoc », appelé aussi test de comparaisons multiples, afin de connaître la significativité des résultats d'un groupe de l'étude par rapport à un autre. Dans le cas de nos travaux, le choix s'est porté sur des tests de Tukey, reconnus pour être dans une moyenne de conservation raisonnable, et plus élevée que d'autres tests « post hoc » tel celui de Fisher, par exemple. (Winer *et al.*, 1991).

5. Travaux de recherche

5.1. Article I

Effects of gravitational acceleration on
cardiovascular autonomic control in resting
humans

5.1.1. Introduction

Ce premier travail de recherche fait suite aux travaux du laboratoire de physiologie intégrative du Professeur Ferretti, à l'Université de Genève. En effet, cette unité de recherche, concentrée sur le domaine de la physiologie cardio-respiratoire, a réalisé il y a quelques années un travail sur les effets de l'hypergravité sur la consommation d'oxygène (Bonjour *et al.*, 2010). Dans ce cadre et suite à une collaboration avec le Karolinska Institutet de Stockholm, en possession d'une centrifugeuse humaine permettant la réalisation du protocole de recherche, le laboratoire possédait des données très intéressantes et encore inutilisées dans le contexte de cette thèse.

Il est généralement reconnu que, lors d'une exposition à l'hypergravité, il y a, d'une part, augmentation de la fréquence cardiaque, de la pression artérielle moyenne, de la résistance périphérique totale ou encore de la ventilation et, d'autre part, diminution du volume d'éjection systolique et de la capacité de diffusion pulmonaire. (Bjurstedt *et al.*, 1968 ; Bonjour *et al.*, 2010, 2011 ; Burton et Smith, 2011; Girardis *et al.*, 1999 ; Linnarsson et Rosenhamer, 1967, 1968 ; Pendergast *et al.*, 2012 ; Rohdin and Linnarsson, 2002). Même si les effets de la microgravité furent étudiés par de nombreuses équipes de recherche (Ferretti *et al.*, 2009 ; Fortrat *et al.*, 2001 ; Hirayanagi *et al.*, 2004 ; Hughson *et al.*, 1995 ; Traon *et al.*, 1998), peu d'études, en revanche, se sont intéressées aux effets de l'hypergravité sur la variabilité cardiaque. Quelques travaux ont été réalisés en hypergravité ; mais ces derniers ne se focalisèrent que sur les réactions neuroendocrines en mesurant les concentrations de cortisol, d'adrénocorticotrophine (ACTH), de prolactine, d'épinéphrine et de norépinéphrine (Schneider *et al.*, 2008 ; Strempel *et al.*, 2008) ou sur les effets

de blocages bêta adrénergiques (Bjurstedt *et al.*,1974). Afin de pallier à ce manque d'information, nous avons donc réalisé le travail présenté ci-après.

5.1.2. Manuscrit

Effects of gravitational acceleration on cardiovascular autonomic control in resting humans

Timothée Fontolliet, Vincent Pichot, Guglielmo Antonutto, Julien Bonjour, Carlo Capelli, Enrico Tam, Jean-Claude Barthélémy and Guido Ferretti

Publié dans :

European Journal of Applied Physiology, volume 115, pages 1417-1427, 2015

Effects of gravitational acceleration on cardiovascular autonomic control in resting humans

Timothée Fontolliet, Vincent Pichot, Guglielmo Antonutto, Julien Bonjour, Carlo Capelli, Enrico Tam, Jean-Claude Barthélémy, et al.

**European Journal of Applied
Physiology**

ISSN 1439-6319
Volume 115
Number 7

Eur J Appl Physiol (2015) 115:1417-1427
DOI 10.1007/s00421-015-3117-9



Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Effects of gravitational acceleration on cardiovascular autonomic control in resting humans

Timothée Fontolliet · Vincent Pichot ·
Guglielmo Antonutto · Julien Bonjour · Carlo Capelli ·
Enrico Tam · Jean-Claude Barthélémy · Guido Ferretti

Received: 15 April 2014 / Accepted: 26 January 2015 / Published online: 14 February 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Purpose Previous studies of cardiovascular responses in hypergravity suggest increased sympathetic regulation. The analysis of spontaneous heart rate variability (HRV) parameters and spontaneous baroreflex sensitivity (BRS) informs on the reciprocal balance of parasympathetic and sympathetic regulations at rest. This paper was aimed at determining the effects of gravitational acceleration (a_g) on HRV and BRS.

Methods Eleven healthy subjects (age 26.6 ± 6.1) were studied in a human centrifuge at four a_g levels (1, 1.5, 2 and 2.5 g) during 5-min sessions at rest. We evaluated spontaneous variability of R–R interval (RR), and of systolic and diastolic blood pressure (SAP and DAP, respectively), by

power spectral analysis, and BRS by the sequence method, using the BRSanalysis[®] software.

Results At 2.5 g, compared to 1 g, (1) the total power (P_{TOT}) and the powers of LF and HF components of HRV were lower, while the LF/HF ratio was higher; (2) normalized units for LF and HF did not change significantly; (3) the P_{TOT} , LF and HF powers of SAP were higher; (4) the P_{TOT} and LF power of DAP were higher; and (5) BRS was decreased.

Conclusions These results do not agree with the notion of sympathetic up-regulation supported by the increase in HR and DAP (tonic indices), and of SAP and DAP LF powers (oscillatory indices). The P_{TOT} reduction leads to speculate that only the sympathetic branch of the ANS might have been active during elevated a_g exposure. The vascular response occurred in a condition of massive baroreceptive unloading.

Communicated by Massimo Pagani.

T. Fontolliet (✉) · J. Bonjour · G. Ferretti
Département de Neurosciences Fondamentales, Université de
Genève, 1, Rue Michel Servet, 1211 Genève 4, Switzerland
e-mail: timothee.fontolliet@unige.ch

T. Fontolliet · V. Pichot · J.-C. Barthélémy
EA SNA-Epis 4607, Université Jean-Monnet, Saint-Étienne,
France

G. Antonutto
Dipartimento di Scienze e Tecnologie Biomediche, Università di
Udine, Udine, Italy

C. Capelli · E. Tam
Dipartimento di Scienze Neurologiche e del Movimento,
Università di Verona, Verona, Italy

G. Ferretti
Dipartimento di Scienze Cliniche e Sperimentali, Università di
Brescia, Brescia, Italy

Keywords Heart rate variability · Arterial blood pressure · Baroreflexes · Human centrifuge

Abbreviations

a_g	Gravitational acceleration
ANOVA	Analysis of variance
ANS	Autonomic nervous system
BRS	Baroreflex sensitivity
DAP	Diastolic arterial pressure
FFT	Fast fourier transform
G_z	Vertical acceleration
HR	Heart rate
HF	High frequency
HFnu	Normalized high frequency
HRV	Heart rate variability
LF	Low frequency
LFnu	Normalized low frequency

LF/HF	Low and high frequency ratio
MAP	Mean arterial pressure
MSNA	Muscle sympathetic nerve activity
P_{TOT}	Total power
R_p	Total peripheral resistance
SAP	Systolic arterial pressure
VLF	Very low frequency

Introduction

A variety of studies have examined tolerance to high gravitational acceleration (a_g), both at rest and during exercise. Several cardiopulmonary variables were investigated, such as cardiac output, blood pressure, stroke volume and pulmonary diffusing capacity or oxygen consumption (Bjurstedt et al. 1968; Bonjour et al. 2010, 2011; Burton and Smith 2011; Girardis et al. 1999; Linnarsson and Rosenhamer 1967, 1968; Pendergast et al. 2012; Rohdin and Linnarsson 2002). These studies showed that at rest, when a_g [vertical acceleration (G_z) to be more specific] was increased, heart rate (HR), total peripheral resistance (R_p), mean arterial pressure (MAP) and ventilation increased linearly with it, whereas stroke volume and pulmonary diffusing capacity decreased in a linear fashion.

Tools for indirect non-invasive assessment of the autonomic nervous system (ANS) regulation, including the analysis of spontaneous heart rate variability (HRV) parameters and spontaneous baroreflex sensitivity (BRS), are thought to inform on the reciprocal balance of parasympathetic and sympathetic regulations at any given time, at rest (Drouin et al. 1997; Pagani et al. 1986; Rosenstock et al. 1999; Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). These tools were applied in microgravity (Ferretti et al. 2009; Fortrat et al. 2001; Hirayanagi et al. 2004; Hughson et al. 1995; Traon et al. 1998), along with the study of peripheral sympathetic nerve activity and catecholamine concentration (Arbeille et al. 2008; Barbe et al. 1999; Christensen et al. 2005; Eckberg and Neurolab Autonomic Nervous System Team 2003; Edgell et al. 2007; Ertl et al. 2002; Ferretti et al. 2009; Kamiya et al. 2004; Tanaka et al. 2013). In contrast, the studies carried out in hypergravity are scanty, and only stress hormones and the effects of beta-adrenergic blockade were analysed (Bjurstedt et al. 1974; Schneider et al. 2008; Stempel et al. 2008). These studies coherently suggest an increased regulation of the sympathetic system.

On this basis, we carried out the present study, the aim of which was to determine the effects of a_g on HRV and baroreflex sensitivity (BRS).

Methods

Subjects

Eleven young healthy subjects participated in the study. They were 26.6 ± 6.1 years old, their body mass was 76.5 ± 8.2 kg and they were 180 ± 5.1 cm tall. The aims, design, protocol and method of the study were thoroughly described to the volunteers who agreed by signing an informed consent form. They had no history of cardiopulmonary disease and were not taking medications at the time of the experiments. They had to abstain from coffee and from nicotine-containing products during 24 h before the experiment. The study was approved by the Regional Ethical Board, Stockholm, and it was conducted in accordance with the 1964 Declaration of Helsinki on experiments on human subjects.

Set-up

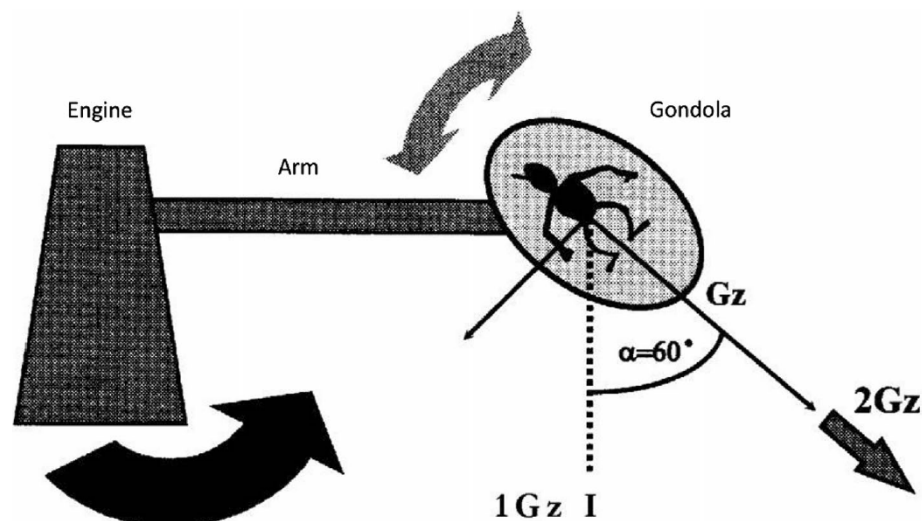
Experiments took place in the human centrifuge at the Karolinska Institute, Stockholm, Sweden. The subjects were located in the centrifuge gondola, sitting on a seat, which could be adjusted to be perpendicular to the resultant of the normal g vector and the centrifugal g vector. They were secured on the seat by a five-point safety belt, and their feet were fixed on an electrically braked cycle ergometer that was located in front of the seat. The rotational radius of the centrifuge was 7.2 m at the middle of the support surface. Slip rings at the centre of rotation allowed for audiovisual monitoring, power supply and transmission of physiological signals between the gondola and a control room.

Measurements

An electrocardiogram (ECG) was recorded and monitored throughout the experiments in order to look for abnormalities and to determine HR. Continuous recordings of arterial pulse pressure were obtained at a fingertip of the right arm by means of a non-invasive cuff pressure recorder (*Portapres*, TNO, Eindhoven, The Netherlands). The sensed fingertip was kept at the heart level. Systolic and diastolic blood pressure (SAP and DAP, respectively) values were obtained from each pulse pressure profile, using the *Beat-scope*[®] software package (FMS, Amsterdam, The Netherlands). Beat-by-beat mean arterial pressure (MAP) was computed as the integral mean of each pressure profile, using the same software package.

It is noteworthy that the blood pressure profiles in this study were obtained from non-invasive recordings from a peripheral finger artery. The pulse wave characteristics of a peripheral artery are, in fact, remarkably different from

Fig. 1 Schematic illustration of subject's positioning in the spinning centrifuge. The subject seated in the centrifuge gondola is exposed to acceleration equal to $1/\cos\alpha$, α being the angle formed by the vertical axis and the gondola axis



those of a central artery (Remington and Wood 1956), and different absolute SAP and DAP values are obtained (Azabji-Kenfack et al. 2004). Possible vasoconstriction (see “Results”) may blunt the pressure response as a_g is increased, although the growth of MAP with a_g was clearly demonstrated not only with the present method (Bonjour et al. 2011) but also with an invasive method (Linnarsson and Rosenhamer 1968).

Protocol

Experiments were conducted at four a_g levels, corresponding to 1, 1.5, 2 and 2.5 g. Control experiments at 1 g were also carried out in the gondola, using the same experimental set-up as for the hypergravity experiments. After having reached the appropriate spinning speed, 5 min at rest were allowed, during which all investigated parameters were continuously recorded. Then the protocol continued with an exercise session, which was carried out for other purposes (Bonjour et al. 2010). At the end of the exercise, the gondola was decelerated and parked. The reported values were obtained as the mean of all the beat-by-beat values determined during the last 2–3 min of the rest of each session with the centrifuge spinning. Overall, each subject underwent 16 experimental sessions as four different gravitational accelerations and four different workloads were used, which provided four repetitions of resting recordings at each investigated a_g for the sake of this study. A schematic drawing of the centrifuge arrangement during spinning, with indication of the gravitational vector, is shown in Fig. 1.

Data treatment

Examples at 1 and 2.5 g of the recordings and relevant analysis carried out in this study are presented in Fig. 2

for HRV and Fig. 3 for arterial pressure variability. After construction of the time series of R-wave to R-wave interval (RR), SAP and DAP from the continuous recordings of ECG and pulse pressure profiles, Fast Fourier transform (FFT) was used to evaluate spontaneous variability of RR, SAP and DAP (Pagani et al. 1986). The total power (P_{TOT}) of RR, SAP and DAP variabilities, corresponding to variance, was initially obtained. Subsequently, the powers of the low (0.03–0.14 Hz)- and the high (0.15–0.5 Hz)-frequency spectral components (low frequency, LF, and high frequency, HF, respectively) were computed and expressed in absolute units (ms^2), and their ratio (LF/HF ratio) was calculated. The very low frequency (VLF) component was neglected, as they are known to reflect the long-term regulatory mechanisms which are not relevant in our study. Moreover, our data were obtained during 5-min sessions, a condition in which the analysis of the very low frequency component provides dubious results (Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Normalized LF and HF (LFnu and HFnu, respectively) were also computed as

$$\frac{\text{LF} \times 100}{P_{TOT} - \text{VLF}} \quad (1)$$

and expressed in normalized units (nu) (Malliani et al. 1991; Pagani and Malliani 2000). The LF and HF central frequencies were also computed as the frequencies corresponding to the peak power value within the LF and HF bands of the HRV spectra.

The spontaneous baroreflex sensitivity (BRS, expressed in ms per mmHg) was estimated from SAP and RR by means of the sequence method (Bertinieri et al. 1988). In practice, we selected intermittent short sequences of

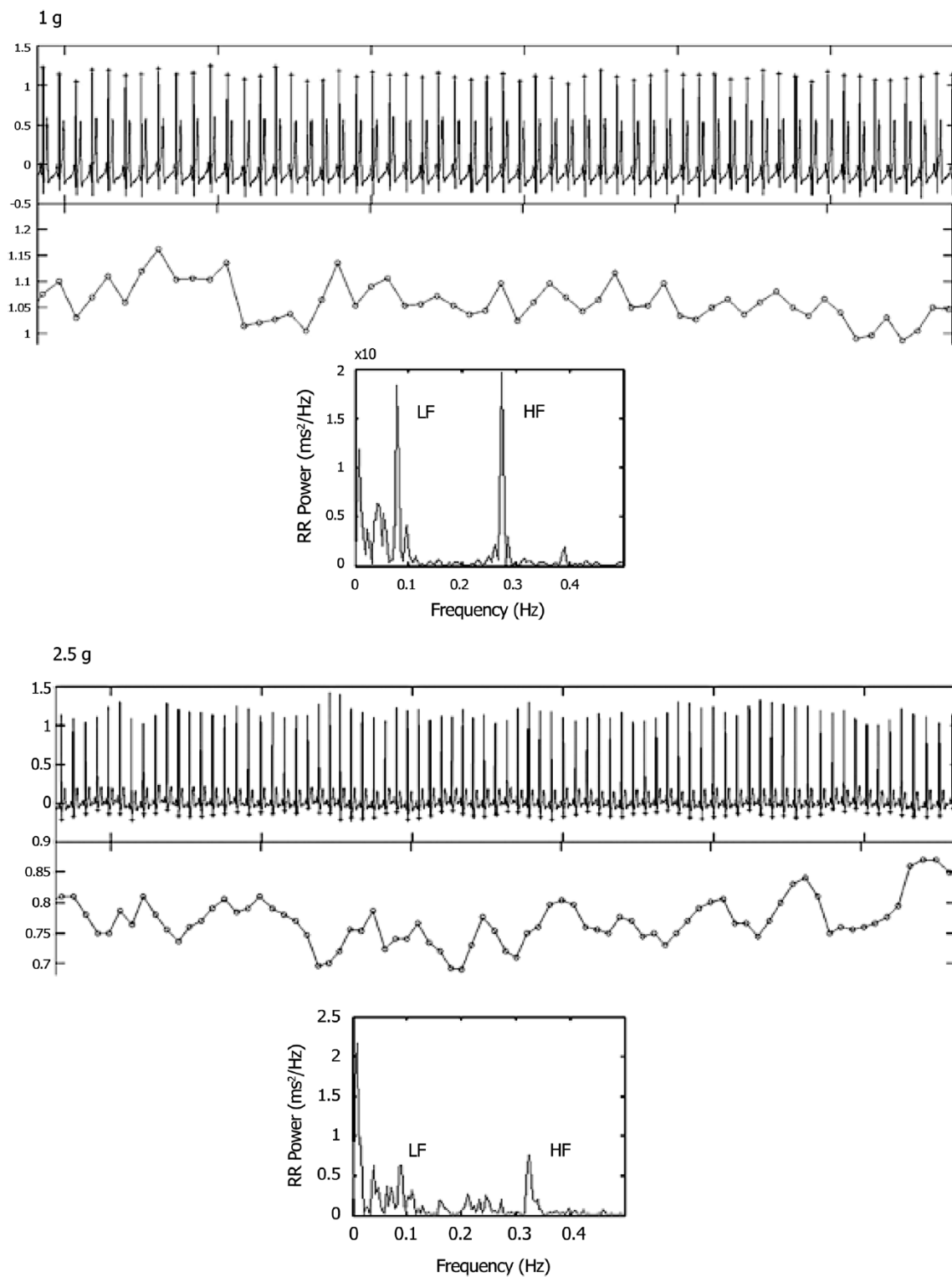


Fig. 2 Examples of a segment of ECG recording, with the corresponding RR time series, and the heart rate variability spectrum resulting from the experiment which the shown ECG segment belongs to *top* 1 g, *bottom* 2.5 g

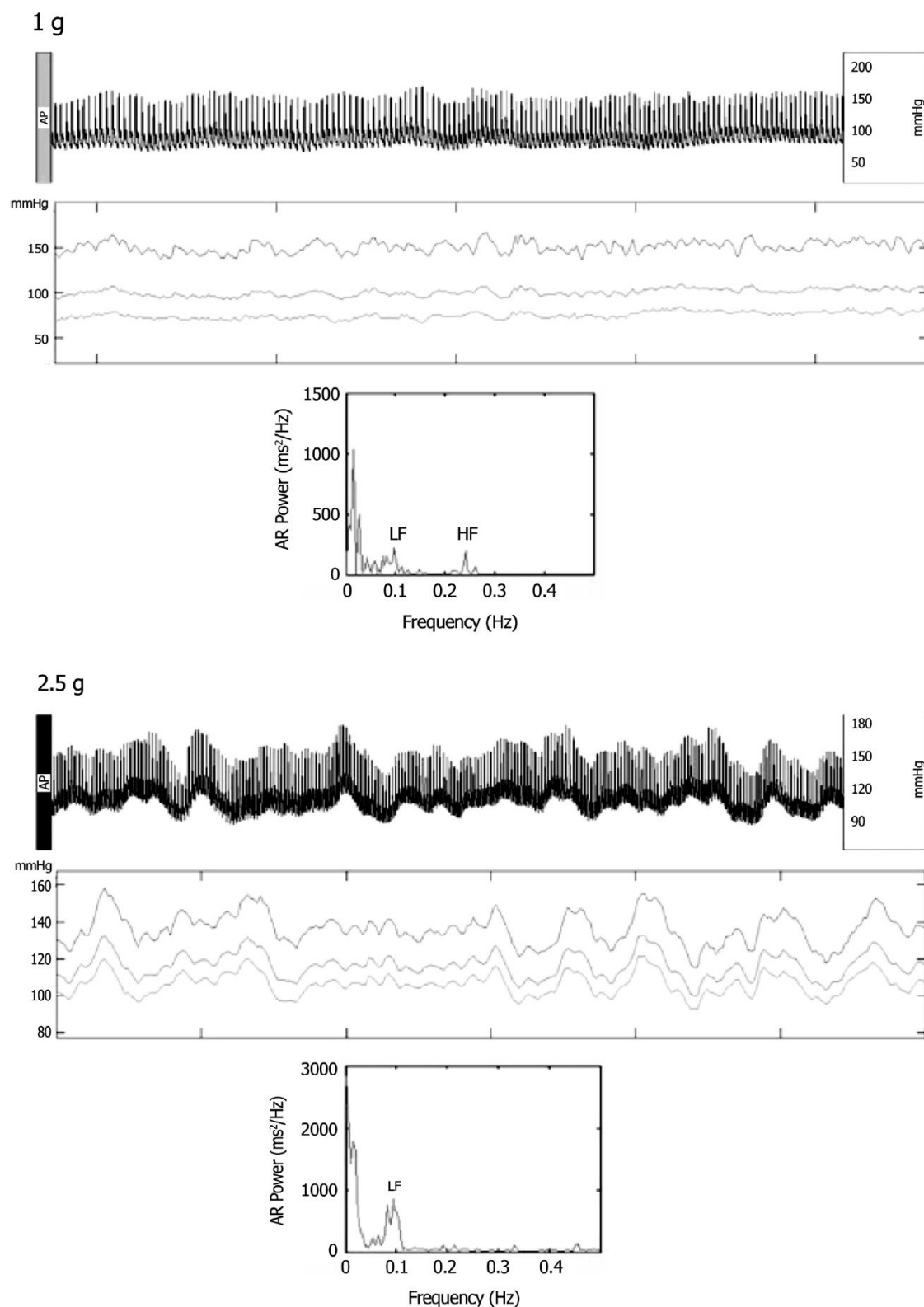


Fig. 3 Examples of a segment of arterial pressure recording, with the corresponding temporal series with SAP, MAP and DAP, and the SAP variability spectrum resulting from the experiment which the shown arterial pressure segment belongs to *top* 1 g, *bottom* 2.5 g

Table 1 Mean values measured at steady state and at each level of gravity acceleration (a_g)

Measured variables	1G	1.5G	2G	2.5G
HR (bpm)	70.20 ± 9.10	79.52 ± 6.19*	90.22 ± 13.64*	102.58 ± 15.25*
SAP (mmHg)	132.19 ± 12.46	127.98 ± 25.75	127.87 ± 16.41	132.59 ± 14.39
DAP (mmHg)	68.73 ± 7.01	75.03 ± 11.51	76.59 ± 10.87	82.04 ± 12.55*
MAP (mmHg)	89.88 ± 8.12	92.67 ± 15.62	93.69 ± 12.05	98.88 ± 12.05*

Data are given as means and standard deviations of all measured variables at steady state at each level of a_g

HR heart rate, SAP systolic arterial pressure, DAP diastolic arterial pressure, MAP mean arterial pressure

* Significantly different from 1G

Table 2 Means and standard deviations of all calculated variables at steady state at each level of gravitational acceleration (a_g)

Measured variables	1G	1.5G	2G	2.5G
RR (ms)	870.34 ± 114.29	762.88 ± 59.03*	680.04 ± 89.74*	598.75 ± 89.49*
f_{LF} RR (Hz)	0.096 ± 0.015	0.099 ± 0.014	0.094 ± 0.023	0.088 ± 0.015
f_{HF} RR (Hz)	0.294 ± 0.049	0.325 ± 0.057	0.302 ± 0.069	0.335 ± 0.090
BRS (ms mmHg ⁻¹)	11.57 ± 2.85	9.95 ± 6.19	7.46 ± 6.48*	4.88 ± 2.15*

Data are given as means and standard deviations of all calculated variables at steady state at each level of a_g

RR R–R interval, f_{LF} RR (Hz) central frequency of the LF component of heart rate variability, f_{HF} RR (Hz) central frequency of the HF component of heart rate variability, BRS Spontaneous baroreflex sensitivity

* Significantly different from 1G

at least three heart beats during which SAP increased or decreased resulting in a change in RR interval going to the same direction, resulting from a cardiovascular reflex. The software calculated the linear regression slope for each sequence, and only those sequences in which the value of r^2 was more than 0.85 were accepted and the slope retained. Then, BRS was calculated as the mean of the slopes of all sequences of each subject.

Spectral analysis and spontaneous baroreflex sensitivity were both analysed by means of BRSanalysis, a purposely laboratory-made programme written with Matlab® software (Version 7.9.1 R2009b) and produced by the Autonomic Nervous System Team at the University Jean-Monnet, Saint-Etienne, France (Assoumou et al. 2012; Costes et al. 2004; Dauphinot et al. 2013).

Statistical analysis

Data are reported as mean ± standard deviation for each experimental session. The effects of a_g on the investigated parameters were analysed by 2-way ANOVA for repeated measurements. Then, Tukey Post-hoc test has been applied to identify corresponding samples. a_g was fitted as parametric variable. All data were analysed with Statview 5©. (SAS Institute Inc., Cary, NC, USA) The results were considered significant if $p < 0.05$

Results

The mean values measured at steady state at each level of a_g are shown in Table 1. HR at 2.5 g was 47.1 ± 20.7 % higher than that at 1 g. Consistently, RR at 2.5 g was 30.76 ± 9.46 % lower than that at 1 g. No arrhythmic beats were observed, and in all cases a normal sinus beat was maintained. Differences were significant between each a_g level. SAP did not change with a_g , whereas DAP increased, being at 2.5 g significantly higher than that at 1 g ($+19.5 \pm 14.6$ %). Consistently, MAP at 2.5 g was also significantly higher than that at 1 g ($+10.2 \pm 10.81$ %).

The mean values calculated at steady state at each level of a_g are shown in Table 2. The central frequency of the HF component of RR, that is known to well represent the breathing frequency, did not change significantly with a_g . The same was the case for the central frequency of the LF component of RR. At 2.5 g, the BRS decrease attained 57.4 ± 17.7 %. This progressive BRS decrease led to significant changes at 2 and 2.5 g as compared to 1 g. The BRS at 2.5 g was also significantly lower than that at 1.5 g.

Means and standard deviations of all data calculated by means of HRV spectral analysis are shown in Table 3. P_{TOT} decreased progressively with increasing a_g from 1 g to 2.5 g. The P_{TOT} value at 2.5 g was significantly lower than the corresponding values at 1 and 1.5 g. Although the LF

Table 3 Means and standard deviations of all parameters calculated by means of heart rate variability, systolic arterial pressure and diastolic arterial pressure

	1G	1.5G	2G	2.5G
HRV				
P_{TOT} (ms ² Hz ⁻¹)	1984.1 ± 390.6	1745.0 ± 411.2	1421.9 ± 432.4	657.0 ± 157.8* [#]
LF (ms ² Hz ⁻¹)	702.9 ± 147.4	744.7 ± 190.7	640.8 ± 213.9	336.8 ± 105.4 [#]
HF (ms ² Hz ⁻¹)	314.4 ± 57.4	289.0 ± 115.7	186.9 ± 68.9	66.7 ± 15.4* [#]
LF/HF	2.60 ± 0.38	3.84 ± 0.55	4.41 ± 0.91	4.97 ± 1.34*
LFnu (%)	64.95 ± 3.42	73.35 ± 2.82	72.36 ± 4.18	69.17 ± 5.11
HFnu (%)	34.91 ± 3.42	26.21 ± 2.75	24.87 ± 3.42	24.34 ± 3.47
SAP				
P_{TOT} (ms ² Hz ⁻¹)	24.77 ± 3.94	33.77 ± 3.71	44.23 ± 4.87*	46.63 ± 5.81*
LF (ms ² Hz ⁻¹)	9.04 ± 1.61	11.38 ± 2.17	19.37 ± 3.96*	24.18 ± 5.02 [#]
HF (ms ² Hz ⁻¹)	2.80 ± 0.47	5.04 ± 1.18	7.83 ± 1.28*	8.24 ± 1.73*
LF/HF	4.99 ± 1.29	3.04 ± 0.40	3.28 ± 0.75	4.51 ± 1.00
DAP				
P_{TOT} (ms ² Hz ⁻¹)	8.17 ± 1.28	11.76 ± 2.01	15.73 ± 2.59	20.53 ± 3.23* [#]
LF (ms ² Hz ⁻¹)	3.41 ± 0.46	5.01 ± 1.05	8.15 ± 1.69	11.9 ± 2.33* [#]
HF (ms ² Hz ⁻¹)	1.25 ± 0.76	1.85 ± 0.86	2.69 ± 0.76	3.02 ± 0.64
LF/HF	9.45 ± 1.39	6.22 ± 0.95*	5.17 ± 1.06*	7.67 ± 1.2

Data are given as means and standard deviations of all parameters calculated at steady state at each level of a_g

HRV heart rate variability, SAP systolic arterial pressure, DAP diastolic arterial pressure, P_{TOT} total power, LF low frequency, HF high frequency, LF/HF low and high frequency ratio

* Significantly different from 1 g

[#] Significantly different from 1.5 g

power at 1 g did not change with increasing a_g up to 2 g, the value at 2.5 g resulted remarkably reduced. This change was significantly different with respect to the value at 1.5 g. HF power decreased progressively and significantly with increasing a_g , from 1 to 2.5 g. This last value was significantly lower than the corresponding values at 1 and 1.5 g. The LF/HF ratio thus increased significantly between 1 and 2.5 g, growing by 105.8 ± 156.1 %. LFnu and HFnu did not change significantly with a_g .

Means and standard deviations of all data calculated by means of SAP spectral analysis are shown in Table 3. P_{TOT} increased progressively and significantly from 1 to 2.5 g. The value at 2.5 g represented a 127.8 ± 132.9 % increase with respect to the corresponding value at 1 g and was significantly higher than the corresponding value at 1 g. The same was the case for the value at 2 g. LF power increased progressively with increasing a_g , to reach the maximum at 2.5 g. At this a_g level, LF power was 232.5 ± 257.3 % higher than that at 1 g and significantly different from that observed at 1 and 1.5 g. Also the value at 2 g was significantly higher than the corresponding value at 1G. HF power increased progressively with increasing a_g . Value at 2.5 g was 248.9 ± 230.5 % higher than that at 1 g. Values at 2 and 2.5 g were significantly higher than the corresponding

value at 1 g. The LF/HF ratio, as well as LFnu and HFnu, did not change significantly with increasing a_g .

Means and standard deviations of all data calculated by means of DAP spectral analysis are shown in Table 3. P_{TOT} increased progressively and significantly with increasing a_g until 2.5 g. This last value was 190.6 ± 146.6 % higher than and significantly different from the corresponding value at 1 g. It was also significantly higher than the value at 1.5 g. The value at 2 g was significantly higher than that at 1 g. LF power increased progressively with increasing a_g and significantly at 2 and 2.5 g. This represented a 275 ± 224.8 % increase. HF power did not change significantly between 1 g and 2.5 g. LF/HF ratio decreased progressively and significantly until 2 g. The rebound observed at 2.5 g was such as to have no significant differences with respect to any other a_g level. LFnu and HFnu did not change significantly with a_g .

Discussion

The absolute steady-state values of the measured variables conformed with previous observations (Bjurstedt et al. 1968; Bonjour et al. 2011; Burton and Smith 2011;

Pendergast et al. 2012; Rosenhamer 1967): as a_g was increased, HR and DAP grew and SAP did not change, which implied an increase in MAP. On the same subjects, in a previous study (Bonjour et al. 2011), a decrease in cardiac output and stroke volume as a function of a_g was found. The increase in DAP can be attributed to an increase in R_p , also reported by Bonjour et al. (2011), as a consequence of arteriolar vasoconstriction, possibly due to sympathetic stimulation of alpha-adrenergic receptors (Inagaki et al. 2011). We propose that the drop of venous return, as suggested by low stroke volume and cardiac output, induced a switch toward sympathetic up-regulation of the cardiovascular system at rest, leading to higher R_p and MAP (vascular regulation) and to higher HR (cardiac regulation). It was therefore logical to expect that HRV indexes in condition of high a_g change in the direction of sympathetic up-regulation.

We thus analysed for the first time the spontaneous variability of RR, SAP and DAP in hypergravity condition by means of power spectral analysis. The choice of FFT relied on two considerations: on one side, the implicit assumptions underlying the application of autoregressive methods are unwarranted at elevated a_g levels; on the other side, our study was carried out in steady-state conditions (Bonjour et al. 2011) and FFT is considered accurate when it is used during steady states (Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). We also comment a posteriori that the present results have shown a reduction of the total power with increasing a_g , a condition in which FFT was demonstrated to be a most reliable technique (Weippert et al. 2010). We nevertheless acknowledge that application of autoregressive analysis would have in principle allowed a more consistent and smoother spectral estimation, a spectral resolution independent of the number of samples and the possibility of avoiding windowing procedures (Pagani et al. 1986). Moreover, autoregressive models would have allowed decomposition of the autocorrelation function in single components, thus directly providing the individual centre frequency, which in this case had to be recalculated off-line.

The most common condition implying up-regulation of the sympathetic system is exercise. Already, the classical study of Robinson et al. (1966) demonstrated a progressively greater sympathetic activity as the power at which exercise was carried out increased. Moreover, a reduction of P_{TOT} of HRV typically occurs when only one branch of the ANS is active, as is the case during exercise. As soon as exercise starts, there is an immediate HR increase, which was attributed to sudden withdrawal of vagal regulation (Arai et al. 1989; Fagraeus and Linnarsson 1976; Lador et al. 2006; Perini et al. 1990). At 30 % of the maximal power, most of the decrease in P_{TOT} known to occur during

exercise has already taken place (Perini et al. 1990). The reduction of P_{TOT} that we found in hypergravity is thus in agreement with what is currently observed in exercise (Perini et al. 1990; Perini and Veicsteinas 2003). During exercise, there is also an increase of the power of the LF component of SAP variability (Perini and Veicsteinas 2003; Cottin et al. 2008), a finding that characterises also the present results and is coherent with the concept of an increased peripheral sympathetic activity. There is however a remarkable difference between exercise and hypergravity, namely that in the former case the up-regulation of the sympathetic system occurs at low R_p , while in the latter it occurs at elevated R_p . This entails differences in the peripheral (vascular) component of oscillatory regulation mechanisms. In fact, an exercising human undergoes a major increase in muscle blood flow with respect to rest, with consequent dramatic fall of R_p (Ferretti et al. 1995; Rådegran and Saltin 1998), whereas hypergravity at rest is characterised by strong peripheral vasoconstriction (Bonjour et al. 2011; Linnarsson and Rosenhamer 1968).

Respiratory rate is also a major difference between hypergravity and exercise. Previous work done with the same set-up and subjects showed that there were no significant changes of resting ventilation with increased a_g (Bonjour et al. 2011). Coherently, neither the power of the HF component of HRV nor its central frequency varied with increasing a_g . This is obviously not the case with exercise. Respiratory rate is well known to modulate HF component of HRV (Perini and Veicsteinas 2003), the power of which increases at exercise intensities above the so-called ventilatory threshold (Cottin et al. 2008). Other conditions characterised by reduced P_{TOT} of HRV are exposure to acute hypoxia (Brown et al. 2013; Buchheit et al. 2004) and chronic heart failure (Lombardi and Mortara 1998; Rydlewska et al. 2011). Also, these conditions, however, entail physiological differences with respect to hypergravity, as far as cardiac output is concerned.

In this study, the reduction of the HF component of HRV, at least at 2 and 2.5 g, was associated with a smaller decrease in LF. As a consequence, (1) P_{TOT} , decreased significantly; and (2) the LF/HF ratio increased significantly. However, an increase of the LF/HF ratio should imply significant increases in LFnu and decreases in HFnu. This was not the case, although a tendency toward a decrease is evident for HFnu. These results reveal dissociation between oscillatory (HRV parameters) and tonic (steady-state physiological variables) indices of cardiovascular regulation, as long as the latter, but not the former, are varied at elevated a_g levels (Bonjour et al. 2011). A similar dissociation was observed also after prolonged bed rest (Ferretti et al. 2009) and was related to the different meaning of these indices. The occurrence of changes in LFnu and HFnu is a crucial issue for the evaluation of the sympatho-vagal balance

concept (Pagani and Malliani 2000). These results rather suggest that the sympatho-vagal balance is not a key issue under elevated a_g conditions, considering also that in the present study the control condition was sitting instead of supine, in which case LFnu is already elevated.

In order to clarify the origin of the LF decrease for HRV, we analysed the spontaneous variability of SAP and DAP. Contrary to the LF component of HRV, the LF powers of both SAP and DAP increased progressively and significantly with increasing a_g , whereas the corresponding HF powers also significantly increased. This revealed the dissociation between the peripheral (arterial pressure control) and the cardiac (HRV) oscillatory components under the present circumstances. The increase in LF is coherent with the hypothesis that LF may have some links with the peripheral sympathetic activity (Pagani et al. 1997). Peripheral sympathetic activity can be investigated by analysing the muscle sympathetic nerve activity (MSNA). MSNA is increased in exercise (Victor et al. 1995) and head-down bed rest (Tanaka et al. 2013), all conditions that are generally associated with an increase in SAP LF power. Unfortunately, measuring MSNA was an impossible task in a spinning gondola due to space limitation and the exerted forces. The only data we are aware of, supporting these notions, were obtained during centrifugation at 3g and showed that both adrenaline and noradrenaline were increased (Schneider et al. 2008).

Mean BRS at 1 g was similar to the values reported with the same method in previous studies (Kardos et al. 2001). As long as a_g was increased, BRS decreased progressively and significantly. When MAP is artificially increased, e.g. by means of a bolus of phenylephrine (La Rovere et al. 2008), the ensuing MAP correction by arterial baroreflexes is mediated by increased cardiac parasympathetic outflow to the sinoatrial node. In a condition where the vagal branch of the ANS is inhibited, one would expect an impairment of such a MAP correction, as evidenced by a decrease in BRS. The hydrostatic force that is generated, e.g. by doubling a_g , unloads carotid baroreceptors remarkably—the exact amount depends in fact also on the position of the indifferent hydrostatic point in hypergravity, which is unknown in the present subjects—so that the subjects may move toward the functional limits of arterial baroreflexes, a fact which per se would imply an acute reduction of BRS. In fact, age, among many factors, is known to be a major determinant of BRS impairment (Kardos et al. 2001; Laitinen et al. 1998; Piccirillo et al. 2001). Lower BRS values than the present ones were found on older subjects than the present subjects (Parati et al. 1988; Tank et al. 2000). Similar effects on BRS were found also during head-up-tilt after 6 h of water immersion (Florian et al. 2013) or at the end of prolonged head-down bed rest (Ferretti et al. 2009; Sigaudo et al. 1998).

All these conditions entail an increased sympathetic regulation (Kamiya et al. 1999; O’Leary et al. 2003). The low BRS values represent an intriguing aspect of this study, as long as they are associated with higher LF values and elevated DAP values. In more general terms, the reduction of BRS in association with an increased vascular regulation response reveals the complexity of the autonomic regulation in conditions of acute a_g elevation, which would require joint perusal of several indices simultaneously.

Conclusion

The decrease in the power of the LF component of HRV, as well as in P_{TOT} , was associated, on one side, with a lack of significant changes in LFnu and HFnu and, on the other side, with significant changes in the LF/HF ratio. These results do not agree with the notion of sympathetic up-regulation, suggested by the increase in HR and DAP (tonic indices) and the increase in both SAP and DAP LF powers (oscillatory indices). Yet the P_{TOT} reduction leads us to speculate that only the sympathetic branch of the ANS might have been active during elevated a_g exposure. The finding that the vascular response, as revealed by the higher LF power of arterial pressure variability, occurred in a condition of massive baroreceptive unloading may indicate new tracks for the study of vascular regulation and, possibly, vascular to central interactions.

Acknowledgments This study was supported by Swiss National Science Foundation grants 3200B0 102181 and 32003B_143427 to Guido Ferretti, by a grant from the Access to Ground Based Facilities Initiative of the European Space Agency to Dag Linnarsson and by Italian Space Agency grant ASI/DCMC to Carlo Capelli.

References

- Arai Y, Saul JP, Albrecht P, Hartley LH, Lilly LS, Cohen RJ, Colucci WS (1989) Modulation of cardiac autonomic activity during and immediately after exercise. *Am J Physiol Heart Circ Physiol* 256:H132–H141
- Arbeille P, Kerbeci P, Mattar L, Shoemaker JK, Hughson R (2008) Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. *Am J Physiol Heart Circ Physiol* 295:H1846–H1854
- Assoumou H-GN, Bertholon F, Barthélémy J-C, Pichot V, Celle S, Gosse P, Gaspoz J-M, Roche F (2012) Alteration of baroreflex sensitivity in the elderly: the relationship with metabolic syndrome components. *Int J Cardiol* 155:333–335
- Azabji-Kenfack M, Lador F, Licker M, Moia C, Tam E, Capelli C, Morel D, Ferretti G (2004) Cardiac output by Modelflow method from intra-arterial and fingertip pulse pressure profiles. *Clin Sci* 106:365–369
- Barbe P, Galitzky J, Thalamos C, Langin D, Lafontan M, Senard JM, Berlan M (1999) Increase in epinephrine-induced responsiveness during microgravity simulated by head-down bed rest in humans. *J Appl Physiol* 87:1614–1620

- Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G (1988) Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol Heart Circ Physiol* 254:H377–H383
- Bjurstedt H, Rosenhamer G, Wigertz O (1968) High-g environment and responses to graded exercise. *J Appl Physiol* 25:713–719
- Bjurstedt H, Rosenhamer G, Tydén G (1974) Acceleration stress and effects of propranolol on cardiovascular responses. *Acta Physiol Scand* 90:491–500
- Bonjour J, Capelli C, Antonutto G, Calza S, Tam E, Linnarsson D, Ferretti G (2010) Determinants of oxygen consumption during exercise on cycle ergometer: The effects of gravity acceleration. *Respir Physiol Neurophysiol* 171:128–134
- Bonjour J, Bringard A, Antonutto G, Capelli C, Linnarsson D, Pendergast DR, Ferretti G (2011) Effects of acceleration in the G_z axis on human cardiopulmonary responses to exercise. *Eur J Appl Physiol* 111:2907–2917
- Brown SJ, Raman A, Barnes MJ, Mündel T (2013) Autonomic cardiovascular response to acute hypoxia and passive head-up tilting in humans. *Eur J Appl Physiol* 113:1731–1736
- Buchheit M, Richard R, Doutreleau S, Lonsdorfer-Wolf E, Brandenberger G, Simon C (2004) Effect of acute hypoxia on heart rate variability at rest and during exercise. *Int J Sports Med* 25:264–269
- Burton RR, Smith AH (2011) Adaptation to Acceleration Environments. *Compr Physiol Suppl* 14:943–970
- Christensen NJ, Heer M, Ivanova K, Norsk P (2005) Sympathetic nervous activity decreases during head-down bed rest but not during microgravity. *J Appl Physiol* 99:1552–1557
- Costes F, Roche F, Pichot V, Vergnon JM, Garet M, Barthelemy JC (2004) Influence of exercise training on cardiac baroreflex sensitivity in patients with COPD. *Eur Respir J* 23:396–401
- Cottin F, Médigue C, Papelier Y (2008) Effect of heavy exercise on spectral baroreflex sensitivity, heart rate, and blood pressure variability in well-trained humans. *Am J Physiol Heart Circ Physiol* 295:H1150–H1155
- Dauphinot V, Kossovsky MP, Gueyffier F, Pichot V, Gosse P, Roche F, Barthélémy J-C (2013) Impaired baroreflex sensitivity and the risks of new-onset ambulatory hypertension, in an elderly population-based study. *Int J Cardiol* 168:4010–4014
- Drouin E, Gournay V, Calamel J, Mouzard A, Rozé JC (1997) Assessment of spontaneous baroreflex sensitivity in neonates. *Arch Dis Child Fetal Neonatal Ed* 76:F108–F112
- Eckberg DL, Neurolab Autonomic Nervous System Team (2003) Bursting into space: alterations of sympathetic control by space travel. *Acta Physiol Scand* 177:299–311
- Edgell H, Zuj KA, Greaves DK, Shoemaker JK, Custaud M-A, Kerbeci P, Arbeille P, Hughson RL (2007) WISE-2005: adrenergic responses of women following 56-days, 6 degrees head-down bed rest with or without exercise countermeasures. *Am J Physiol Regul Integr Comp Physiol* 293:R2343–R2352
- Ertl AC, Diedrich A, Biaggioni I, Levine BD, Robertson RM, Cox JF, Zuckerman JH, Pawelczyk JA, Ray CA, Buckley JC Jr, Lane LD, Shiavi R, Gaffney FA, Costa F, Holt C, Blomqvist CG, Eckberg DL, Baisch FJ, Robertson D (2002) Human muscle sympathetic nerve activity and plasma noradrenaline kinetics in space. *J Physiol (Lond)* 538:321–329
- Fagraeus L, Linnarsson D (1976) Autonomic origin of heart rate fluctuations at the onset of muscular exercise. *J Appl Physiol* 40:679–682
- Ferretti G, Binzoni T, Hulo N, Kayser B, Thomet JM, Cerretelli P (1995) Kinetics of oxygen consumption during maximal exercise at different muscle temperatures. *Respir Physiol* 102:261–268
- Ferretti G, Iellamo F, Pizzinelli P, Kenfack MA, Lador F, Lucini D, Porta A, Narkiewicz K, Pagani M (2009) Prolonged head down bed rest-induced inactivity impairs tonic autonomic regulation while sparing oscillatory cardiovascular rhythms in healthy humans. *J Hypertens* 27:551–561
- Florian JP, Simmons EE, Chon KH, Faes L, Shyoff BE (2013) Cardiovascular and autonomic responses to physiological stressors before and after 6 h of water immersion. *J Appl Physiol* 115:1275–1289
- Fortrat JO, Sigaudo D, Hughson RL, Maillet A, Yamamoto Y, Gharib C (2001) Effect of prolonged head-down bed rest on complex cardiovascular dynamics. *Auton Neurosci* 86:192–201
- Girardis M, Linnarsson D, Moia C, Pendergast DR, Ferretti G (1999) Oxygen cost of dynamic leg exercise on a cycle ergometer: effects of gravity acceleration. *Acta Physiol Scand* 166:239–246
- Hirayanagi K, Iwase S, Kamiya A, Sasaki T, Mano T, Yajima K (2004) Functional changes in autonomic nervous system and baroreceptor reflex induced by 14 days of 6 degrees head-down bed rest. *Eur J Appl Physiol* 92:160–167
- Hughson RL, Maillet A, Gauquelin G, Arbeille P, Yamamoto Y, Gharib C (1995) Investigation of hormonal effects during 10-h head-down tilt on heart rate and blood pressure variability. *J Appl Physiol* 78:583–596
- Inagaki T, Sonobe T, Poole DC, Kano Y (2011) Progressive arteriolar vasoconstriction and fatigue during tetanic contractions of rat skeletal muscle are inhibited by α -receptor blockade. *J Physiol Sci* 61:181–189
- Kamiya A, Iwase S, Kitazawa H, Mano T (1999) Muscle sympathetic nerve activity (MSNA) after 120 days of 6 degrees head-down bed rest (HDBR). *Environ Med* 43:150–152
- Kamiya A, Michikami D, Iwase S, Hayano J, Kawada T, Sugimachi M, Sunagawa K (2004) Alpha-adrenergic vascular responsiveness to sympathetic nerve activity is intact after head-down bed rest in humans. *Am J Physiol Regul Integr Comp Physiol* 286:R151–R157
- Kardos A, Watterich G, de Menezes R, Csanády M, Casadei B, Rudas L (2001) Determinants of spontaneous baroreflex sensitivity in a healthy working population. *Hypertension* 37:911–916
- La Rovere MT, Pinna GD, Raczak G (2008) Baroreflex sensitivity: measurement and clinical implications. *Ann Noninvasive Electrocardiol* 13:191–207
- Lador F, Azabji Kenfack M, Moia C, Cautero M, Morel DR, Capelli C, Ferretti G (2006) Simultaneous determination of the kinetics of cardiac output, systemic O_2 delivery, and lung O_2 uptake at exercise onset in men. *Am J Physiol Regul Integr Comp Physiol* 290:R1071–R1079
- Laitinen T, Hartikainen J, Vanninen E, Niskanen L, Geelen G, Lämsmäies E (1998) Age and gender dependency of baroreflex sensitivity in healthy subjects. *J Appl Physiol* 84:576–583
- Linnarsson D, Rosenhamer G (1968) Exercise and arterial pressure during simulated increase of gravity. *Acta Physiol Scand* 74:50–57
- Lombardi F, Mortara A (1998) Heart rate variability and cardiac failure. *Heart* 80:213–214
- Malliani A, Pagani M, Lombardi F, Cerutti S (1991) Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84:482–492
- O’Leary DD, Kimmerly DS, Cechetto AD, Shoemaker JK (2003) Differential effect of head-up tilt on cardiovagal and sympathetic baroreflex sensitivity in humans. *Exp Physiol* 88:769–774
- Pagani M, Malliani A (2000) Interpreting oscillations of muscle sympathetic nerve activity and heart rate variability. *J Hypertens* 18:1709–1719
- Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell’Orto S, Piccaluga E (1986) Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 59:178–193

- Pagani M, Montano N, Porta A, Malliani A, Abboud FM, Birkett C, Somers VK (1997) Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation* 95:1441–1448
- Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Gropelli A, Pedotti A, Zanchetti A, Mancia G (1988) Evaluation of the baroreceptor-heart rate reflex by 24-h intra-arterial blood pressure monitoring in humans. *Hypertension* 12:214–222
- Pendergast DR, Olszowska A, Farhi LE (2012) Cardiovascular and pulmonary responses to increased acceleration forces during rest and exercise. *Aviat Space Environ Med* 83:488–495
- Perini R, Veicsteinas A (2003) Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. *Eur J Appl Physiol* 90:317–325
- Perini R, Orizio C, Baselli G, Cerutti S, Veicsteinas A (1990) The influence of exercise intensity on the power spectrum of heart rate variability. *Eur J Appl Physiol* 61:143–148
- Piccirillo G, Cacciafesta M, Viola E, Santagada E, Nocco M, Lionetti M, Bucca C, Moisè A, Tarantini S, Marigliano V (2001) Influence of aging on cardiac baroreflex sensitivity determined non-invasively by power spectral analysis. *Clin Sci* 100:267–274
- Rådegran G, Saltin B (1998) Muscle blood flow at onset of dynamic exercise in man. *Am J Physiol Heart Circ Physiol* 274:H314–H322
- Remington JW, Wood EH (1956) Formation of peripheral pulse contour in man. *J Appl Physiol* 9:433–442
- Robinson BF, Epstein SE, Beiser D, Braunwald E (1966) Control of heart rate by the autonomic nervous system: studies in man on the interrelation between baroreceptor mechanisms and exercise. *Circ Res* 19:400–411
- Rohdin M, Linnarsson D (2002) Differential changes of lung diffusing capacity and tissue volume in hypergravity. *J Appl Physiol* 93:931–935
- Rosenhamer G (1967) Influence of increased gravitational stress on the adaptation of cardiovascular and pulmonary function to exercise. *Acta Physiol Scand Suppl* 276:1–61
- Rosenstock E, Cassuto Y, Zmora E (1999) Heart rate variability in the neonate and infant: analytical methods, physiological and clinical observations. *Acta Paediatr* 88:477–482
- Rydlewska A, Jankowska EA, Ponikowska B, Borodulin-Nadzieja L, Banasiak W, Ponikowski P (2011) Changes in autonomic balance in patients with decompensated chronic heart failure. *Clin Auton Res* 21:47–54
- Schneider S, Guardiera S, Kleinert J, Steinbacher A, Abel T, Carnahan H, Strüder HK (2008) Centrifugal acceleration to 3Gz is related to increased release of stress hormones and decreased mood in men and women. *Stress* 11:339–347
- Sigaud D, Fortrat JO, Allevard AM, Maillet A, Cottet-Emard JM, Vouillarmet A, Hughson RL, Gauquelin-Koch G, Gharib C (1998) Changes in the sympathetic nervous system induced by 42 days of head-down bed rest. *Am J Physiol Heart Circ Physiol* 274:H1875–H1884
- Stempel S, Schroeder C, Hemmersbach R, Boese A, Tank J, Diedrich A, Heer M, Luft FC, Jordan J (2008) Norepinephrine transporter inhibition alters the hemodynamic response to hypergravitation. *J Appl Physiol* 104:756–760
- Tanaka K, Nishimura N, Sato M, Kanikowska D, Shimizu Y, Inukai Y, Abe C, Iwata C, Morita H, Iwase S, Sugeno Y (2013) Arterial pressure oscillation and muscle sympathetic nerve activity after 20 days of head-down bed rest. *Auton Neurosci* 177:266–270
- Tank J, Baevski RM, Fender A, Baevski AR, Graves KF, Ploewka K, Weck M (2000) Reference values of indices of spontaneous baroreceptor reflex sensitivity. *Am J Hypertens* 13:268–275
- Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) Heart rate variability. Standards of Measurement, physiological interpretation, and clinical use. *Circulation* 93:1043–1065
- Traon AP, Sigaud D, Vasseur P, Maillet A, Fortrat JO, Hughson RL, Gauquelin-Koch G, Gharib C (1998) Cardiovascular responses to orthostatic tests after a 42-day head-down bed-rest. *Eur J Appl Physiol Occup Physiol* 77:50–59
- Victor RG, Secher NH, Lyson T, Mitchell JH (1995) Central command increases muscle sympathetic nerve activity during intense intermittent isometric exercise in humans. *Circ Res* 76:127–131
- Weippert M, Kumar M, Kreuzfeld S, Arndt D, Rieger A, Stoll R (2010) Comparison of three mobile devices for measuring R–R intervals and heart rate variability: Polar S810i, Suunto t6 and an ambulatory ECG system. *Eur J Appl Physiol* 109:779–786

5.1.3. Conclusion

Les résultats de ce travail de recherche nous amenèrent à tirer plusieurs conclusions. Premièrement, l'augmentation de la puissance de la composante LF de la variabilité de la pression artérielle systolique et diastolique nous incite à penser que ces LF sont liées à l'activité périphérique sympathique. En effet, les résultats obtenus vont dans le même sens que ceux observés lors d'enregistrements à l'exercice, condition connue d'une augmentation de cette activité sympathique (Robinson *et al.*, 1966).

Deuxièmement, la réduction de la puissance de la composante LF de la variabilité de fréquence cardiaque montre que la régulation de la variabilité cardiaque est dissociée du contrôle de la pression artérielle et de l'activité sympathique périphérique.

Troisièmement, la réduction observée du BRS en condition d'hypergravité va dans le même sens que les deux premières conclusions. Cependant, ce point représente un aspect intrigant de nos résultats, ces derniers montrant clairement une augmentation de la régulation vasculaire. Nous aurions donc pu imaginer une augmentation plutôt qu'une diminution du BRS. Dans ce contexte, la chute du BRS révèle la complexité des mécanismes de régulation cardiovasculaire en condition d'hypergravité et, plus largement, de manière générale.

Un autre point de discussion intéressant concerne les résultats de puissance de la variabilité cardiaque. En effet, la baisse de la composante HF de la variabilité de fréquence cardiaque s'accompagne aussi d'une baisse de LF ayant pour conséquence une baisse logique de la puissance totale et une augmentation du ratio LF/HF. Cette augmentation du ratio LF/HF devrait être

logiquement s'accompagner d'une augmentation de LFnu et d'une diminution de HFnu. Cependant, aucune de ces valeurs normalisées n'a significativement changé sous hypergravité. Ces valeurs étant des points cruciaux de l'évaluation de la balance sympatho-vagale (Pagani and Malliani, 2000), elles remettent en question, sous hypergravité du moins, cette théorie développée par Pagani et ses collaborateurs (Pagani *et al.*, 1986).

5.2. Article II

Heart rate variability and baroreflex sensitivity in
bilateral lung transplant recipients

5.2.1. Introduction

La transplantation pulmonaire bilatérale est une opération impliquant une dénervation des afférences pulmonaires. Sachant que la puissance des HF de la variabilité cardiaque est influencée par la fréquence respiratoire et est synchrone avec elle, analyser la variabilité cardiaque de ces patients pourrait permettre de répondre à certaines questions importantes.

Les études sur le sujet sont peu nombreuses et mélangent souvent différentes conditions, comme l'analyse de la VFC chez des transplantés pulmonaires partiels et complets, des transplantés uniquement pulmonaires, et d'autres, à la fois pulmonaires et cardiaques. Certaines autres études portent sur un nombre de sujets très faible. Il était donc intéressant de clairement observer les effets d'une transplantation complète des poumons sur une douzaine de patients adultes issus du centre de transplantation pulmonaire des Hôpitaux Universitaires de Genève.

Le but de cette étude était d'analyser les effets de la dénervation chez des transplantés pulmonaires, en les comparant à des sujets sains. L'hypothèse formulée dans cet article soutenait une disparition de la composante HF du spectre de la variabilité de fréquence cardiaque suite au sectionnement des afférences pulmonaires durant la transplantation.

5.2.2. Manuscrit

Heart rate variability and baroreflex sensitivity in bilateral lung transplant recipients

Timothée Fontolliet, Pietro Gianella, Vincent Pichot, Jean-Claude Barthélémy, Paola M. Soccal, Guido Ferretti, Frédéric Lador

Submitted : American Journal of Respiratory and Critical Care Medicine, avril 2017

Heart rate variability and baroreflex sensitivity in bilateral lung transplant recipients

Fontolliet T^{1,2}, Gianella P³, Pichot V², Barthélémy JC², Soccal PM^{3,4}, Ferretti G^{1,4}, Lador F^{3,4}

1. Départements d'Anesthésiologie, de Pharmacologie et des Soins Intensifs / des Neurosciences Fondamentales, Université de Genève, Geneva, Switzerland
2. EA SNA-Epis 4607, Université Jean-Monnet, Saint-Étienne, France
3. Service de Pneumologie, Hôpitaux Universitaires de Genève, Geneva, Switzerland
4. Faculté de Médecine, Université de Genève, Geneva, Switzerland
5. Dipartimento di Medicina Molecolare e Traslazionale, Università di Brescia, Brescia, Italy

Corresponding author

Timothée Fontolliet

Département des Neurosciences Fondamentales

Université de Genève - CMU

1, Rue Michel Servet

CH-1211 Genève 4, Switzerland

timothee.fontolliet@unige.ch

Phone: 0041 22 3795365

Fax: 0041 22 3795402

Author's contributions

Fontolliet T: Experiment execution, data treatment, preparation of the first draft of the manuscript.

Gianella P: Patient selection and experiment execution.

Pichot V: Preparation of data treatment software, statistical analysis.

Barthélémy JC: Rereading of the manuscript with special references to heart rate variability technics.

Soccal PM: Experiment conception, study design, rereading of the manuscript.

Ferretti G: Experiment conception, study design, rereading of the manuscript.

Lador F: Preparation of ethical committee folder, experiment conception, study design, patient selection, rereading of the manuscript.

Supports

Research project of national interest, Ministry of Instruction, University and Research, Republic of Italy, protocol 2007SEESE8_005.

Swiss National Science Foundation grant 32003B_143427

Running title

Heart rate variability after lung transplant

Descriptor number

16.2 Transplantation

Total word

3213

At a glance Commentary

Heart-lung interactions, a well-known component of spontaneous heart rate variability, may have either mechanical or neural origin. In the latter case, since lung transplant recipients have denervated lungs, transplantation may seriously impact the autonomic modulation of heart activity. In this study, lung transplant recipients showed extremely low heart rate variability indexes as compared with healthy age-matched controls. These results, suggesting that the effects of the lungs on heart rate variability are neurally mediated, indicate that, in the follow-up of patients after transplantation, particular attention must be given to the prevention and treatment of potentially altered cardiac responses to various stresses.

Abstract

Rational

The effects of lung afferents denervation on cardiovascular regulation can be assessed on bilateral lung transplantation patients. The high frequency component of heart rate variability is known to be synchronous with breathing frequency.

Objective

Then, if heart beat is neurally modulated by breathing frequency, in bilateral lung transplantation patients we may expect disappearance of high frequency of heart rate variability.

Methods

On 11 patients, and 11 matching healthy controls, we measured R-R interval (electrocardiography), blood pressure (Portapres®) and breathing frequency (ultrasonic device) in supine rest, during 10-min free breathing, 10-min cadenced breathing (0.25Hz), and 5-min handgrip. We analysed heart rate variability and spontaneous variability of arterial blood pressure, by power spectral analysis, and baroreflex sensitivity, by the sequence method.

Measurements and Main Results

Concerning heart rate variability, with respect to controls, transplant recipients had lower total power, low and high frequencies power. The low frequency/high frequency ratio was higher. Concerning systolic, diastolic and mean arterial pressure variability, transplant recipients had lower total power (only for cadenced breathing), low frequency and low frequency/high frequency ratio during free and cadenced breathing. Baroreflex sensitivity was decreased.

Conclusions

Denervated lungs implied strong heart rate variability reduction. The higher low frequency/high frequency ratio suggested that the total power drop was mostly due to high frequency. These results support the hypothesis that neural modulation from lung afferents contributes to the high frequency of heart rate variability.

Number of words: 231

Key words: Lung denervation. Autonomic nervous system. Heart rate variability. Arterial blood pressure. Baroreflex

Introduction

Bilateral lung transplantation (BLT) implies denervation of lung afferent cardiovascular sensitive nerves, so that the graft is no longer connected to the autonomic nervous system (ANS)(1). Thus, BLT is an excellent experimental model for the study of cardiovascular regulation without modulation of heart activity by parasympathetic and/or sympathetic lung afferents. Breathing frequency (BF) is synchronous with and modulates the high frequency peak (HF) of spontaneous heart rate variability (HRV)(2–4). If the ANS is the main modulator of HRV, then the HF of HRV would have to disappear after BLT.

The studies of autonomic modulation of HRV in BLT recipients (BLTR) are scanty(1,5). Some were on children(6,7) and pointed to a demonstration of possible lung reinnervation after transplantation. Single lung transplant recipients were compared with either BLTR(8) or lung and heart-lung transplant recipients(9). No clear conclusion emerged on this subject from those works.

The aim of this study was to analyse the effects of lung denervation on HRV in BLTR as compared with healthy controls. The hypothesis was that the modulation of the HF component of HRV by the breathing frequency is mediated by the ANS. This hypothesis would be supported by the results if HF of HRV is suppressed in BLTR.

Methods

Subjects

Eleven BLTR (6 women, 5 men) participated in the experiments. They were all clinically stable, without evidence of chronic or acute rejection or any other disease affecting the ANS. Patients with diabetes, either induced by pancreatic manifestations of cystic fibrosis or by immunosuppressant treatment, were excluded from the study. The patients' age ranged from 26 to 68 years (mean value 49.0 ± 14.5 years). They were 168 ± 10 cm tall, and weighed

62.6±12.0 kg. Their transplant surgery took place between 6 months and 12 years (mean 3.9±3.5 years) before their inclusion in the study. All their anti-rejection medications are reported in Table 1.

Eleven healthy non-smoking subjects were recruited as controls, so that each BLTR was coupled with a matching control for gender, age (±2years), height (±5cm) and weight (±5kg). Their age ranged from 25 to 69 years (47.8±14.1). They were 168±10 cm tall and weighed 63.6±8.9 kg. They had no history of cardiopulmonary disease and were not taking medications at the time of the experiments.

All subjects abstained from coffee-containing products during the 12 hours before the experiment. They were preliminarily informed of aims, design, protocol, procedures and risks associated with the experiments. Informed consent was obtained from each volunteer, who was aware of the right of withdrawing from the study at any time without jeopardy. The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional ethical committee.

Measurements

The R-R interval (RR) was continuously measured by electrocardiography (Elmed ETM 2000, Heiligenhaus, Germany). Continuous recordings of arterial pulse pressure were obtained at the middle phalanx of the left hand's second finger, using a non-invasive cuff pressure recorder (Portapres, FMS, Amsterdam, The Netherlands). Systolic and diastolic blood pressure (SAP and DAP, respectively) were computed from each pulse pressure profile, using the Beatscope® software package (FMS, Amsterdam, The Netherlands). Beat-by-beat mean arterial pressure (MAP) was computed as the integral mean of each pressure profile, using the same software package. The BF was measured by an ultrasound device (Spiroson, Ecomedics, Suisse) plugged on a respiratory mask.

All the signals were digitalized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta CA, USA) and stored on a computer. The acquisition rate was 400 Hz.

Protocol

After instrumentation, the subject took the supine posture. After 5 minutes of quiet rest, the protocol consisted of 10 min of free breathing, followed by 10 min with cadenced breathing (0.25 Hz), then by further 5 min of handgrip contractions, following 30 to 50 contractions per minute without controlled BF, in order to stimulate and increase sympathetic activity. All investigated variables were continuously recorded during the entire protocol.

Data treatment

The time series of RR, from the continuous recordings of ECG, and that of SAP and DAP, from pulse pressure profiles, were constructed. On these time series, power spectral analysis was performed to evaluate spontaneous variability of RR, SAP and DAP(10). The total power (P_{TOT}) of RR, SAP and DAP variabilities, corresponding to the signal variance in the 0.0 – 0.5 Hz frequency range, was initially obtained. Subsequently, the powers and frequencies of the low (LF, 0.04–0.15 Hz) and the high (HF, 0.15–0.4 Hz) frequency spectral components were computed and expressed in absolute units. The LF/HF ratio was also calculated. The very low frequency (VLF) component was neglected, since it can be measured only during very long recordings and it is a source of noise in the present experimental circumstances (11).

Therefore, in order to infer on the relative weight of LF and HF in the spectra, they were normalized with respect to their sum made equal to 100% (LFnu et HFnu, respectively) after exclusion of the frequencies lower than 0,03 Hz (2,10,12). LFnu and HFnu were computed as:

$$\frac{LF \times 100}{P_{tot-VLF}} \quad \text{or} \quad \frac{HF \times 100}{P_{tot-VLF}} \quad (1)$$

and expressed in normalized units (nu) (2).

At rest and exercise steady states, we also computed Baroreflex sensitivity (BRS, expressed in ms mmHg^{-1}) by means of the sequence method (Bertinieri *et al.* 1988). Briefly, sequences of three or more consecutive beats in which SAP and RR changed in the same direction, either increasing or decreasing, were identified. A phase shift of one beat was introduced between the SAP and the RR

values of each sequence, as in previous studies (13). Within each individual sequence, the RR versus SAP relationship was analysed by linear regression, to compute the slope and the corresponding coefficient of determination (r^2). Only slopes showing r^2 values higher than 0.85 were retained (14,15). For each subject, the mean slope of the RR versus SAP relationships was then computed and taken as a measure of individual BRS, at rest and at exercise, respectively.

Spectral analysis and BRS were analysed by means of a purposely laboratory-made program written with Matlab® software (Version 7.9.1 R2009b)(16–19).

Statistics

Data are reported as mean $\pm$ standard deviation (SD) for each experimental session. The effects of transplantation on the investigated parameters were analysed by 2-way ANOVA (20). When applicable, a Tukey post-hoc test was used to locate significant differences. The Statistica 12® software package (StatSoft, Inc., Tulsa, OK, USA) was used. Differences were considered significant when $p < 0.05$.

Results

The mean values of measured and calculated cardiovascular variables in all investigated conditions are shown in Figure 1. The heart rate was higher, and thus RR lower, in BLTR than in controls, in all conditions. Cadenced breathing and handgrip did not affect RR. SAP, DAP and MAP were the same in patients and controls. With handgrip, SAP and DAP increased in control group and MAP increased in both groups. BRS was lower in BLTR than in controls, in every condition. BF was higher in BLTR than in controls during free breathing (see Figure 2).

The results of power spectral analysis for HRV are shown in Table 2, top panel, for all subjects. P_{TOT} was lower in BLTR than in controls, because both the

LF and the HF powers were lower. LFnu was higher and HFnu was lower in BLTR than in controls, in all investigated conditions. The LF/HF ratio was higher in BLTR than in controls in all conditions.

The absolute LF and HF powers of BLTR were characterised by large SD, because two patients were outliers: in fact their LF and HF powers were so much higher than those of all other patients, that they resulted more than two SD above the mean group value (Figure 3). These patients were thus extracted from the group and analysed separately in comparison with their respective control counterparts.

The bottom panel of Table 2 reports the results of power spectral analysis for HRV, for the remaining patients group (n=9). All differences with respect to the control group were significant, as they were for n=11, except for the LF power during handgrip. For n = 9, in BLTR compared to controls, P_{TOT} was $93.3 \pm 7.1\%$ lower during free breathing, $89.7 \pm 13.1\%$ lower during cadenced breathing and 88.3 ± 8.9 lower during handgrip. The LF power was $82.7 \pm 24.9\%$ lower during free breathing and $94.3 \pm 7.2\%$ lower during cadenced breathing. The HF power was $95.1 \pm 5.6\%$ lower during free breathing, $97.6 \pm 4.2\%$ lower during cadenced breathing and 95.3 ± 3.2 lower during handgrip. Conversely, LFnu was higher, and thus HFnu lower in BLTR than in controls, in all conditions. The LF/HF ratio was higher in the three investigated conditions.

The results of power spectral analysis for SAP, DAP and MAP are shown in Table 3. Concerning SAP, P_{TOT} was lower in BLTR than in controls, in cadenced breathing ($-51.0 \pm 36.4\%$). The LF power was lower in BLTR than in controls, during free breathing ($-41.8 \pm 42.2\%$) and cadenced breathing ($-72.6 \pm 21.6\%$), but not during handgrip. HF power in patients was the same than in controls. The LF/HF ratio was lower in BLTR than in control, both in free and in cadenced breathing.

Concerning DAP, P_{TOT} and LF power were lower in patients than in controls in cadenced breathing (respectively, $-42.2 \pm 41.9\%$ and $-22.9 \pm 84.7\%$) and also in free breathing for LF power ($-37.2 \pm 72.6\%$). HF power was not different between BLTR and controls in any conditions. The LF/HF ratio was lower in BLTR than in controls in free breathing ($-50.2 \pm 46.2\%$) and cadenced breathing ($-9.6 \pm 3.3\%$). Concerning MAP, P_{TOT} and LF power were lower in patients than in

controls in cadenced breathing (respectively, $-23.2 \pm 49.8\%$ and $-11.3 \pm 114.4\%$) and also in free breathing for LF power ($-20.1 \pm 85.2\%$). The LF/HF ratio was lower in BLTR than in controls ($-76.2 \pm 77.2\%$ in free breathing, $-76.9 \pm 66.4\%$ in cadenced breathing and $-50.7 \pm 62.0\%$ in handgrip).

The results of power spectral analysis for HRV of the two outliers and their respective control counterparts are shown in Table 4. All data were closer to controls' results than for the rest of the patients. Results were still lower for the two outliers than for their respective control counterparts.

Discussion

In this study, we tested the hypothesis that the modulation of the HF component of HRV by BF is mediated by afferent fibres from the lungs that are interrupted after BLT. To this aim, we investigated the effects of BLT on HRV. The tested hypothesis would be fully supported by the results if the HF component of HRV was suppressed after BLT. The data show that P_{TOT} and the LF and HF powers were strongly reduced in BLTR, a finding in substantial agreement with the tested hypothesis. We can, therefore, state that most of the spontaneous HR oscillations at rest are affected by neural afferents coming from the lungs. Nevertheless, some degree of spontaneous HRV remained in BLTR, suggesting that some of the overall HRV is related to other mechanisms than a modulation of cardiovascular centres by lung afferents. Moreover, if we look at normalised data, LF_{nu} resulted higher, and HF_{nu} lower in BLTR than in controls, suggesting that the remaining variability in BLTR might have been under predominant sympathetic modulation.

We note, however, that in two BLTR spontaneous HRV was only partially reduced. Their LF and HF powers were more than two standard deviations above the mean group value. If the tested hypothesis was correct, this would mean that the neural efflux through lung afferents was present in these patients, implying the possibility of at least partial re-innervation after complete lung denervation. This was not a direct effect of time after intervention, since the two patients who had, by far, the oldest surgery had very low HRV values, compared to other

patients with more recent intervention. Anti-rejection medication cannot explain the different behaviour of the two outliers, as they had the same treatment as the other patients. In particular, they were both under tacrolimus, which if any would have prevented the resurgence of HRV due to its neurotoxic effects. In fact the outliers were the two youngest patients, who were operated when they were 22 and 28 years old, respectively 3.5 and 2.7 years before our study. Since nerve plasticity is more efficient in younger than in later life (21–23), we speculate that three years may have been enough, in these two patients, to recover afferent neural fibres between lungs and cardio-respiratory centres. It would be interesting to repeat the experiments on these patients in 2-4 years: if the hypothesis of a gradual re-innervation of transplanted lungs holds, we would expect a progressive increase in the power of the HF peak.

The HF peak of HRV was centred around a 0.30 Hz frequency in BLTR, while it was located around 0.20 Hz in controls, coherently with the finding that spontaneous BF was higher in BLTR than in controls. During cadenced breathing, the HF peak of HRV in controls was higher than during free breathing. These observations confirm that the HF peak is modulated by the breathing cycle mechanism, as pointed out by several authors(2–4). By contrast, in BLTR, the power of the HF peak was extremely low, whether during free breathing or during cadenced breathing, more than a 100 times lower than in controls. This is compatible with the notion that lung denervation suppressed most of the HF peak.

Also the LF power was smaller in BLTR than in controls, but not as much as the HF power. During handgrip, no difference between BLTR and controls was found, whereas in free and cadenced breathing, the LF power was some 10 times smaller in BLTR than in controls. This suggests that the LF peak was less affected than the HF peak by the breathing patterns.

Although the hypothesis that a neural modulation from lung afferents determines most of the HF component of HRV is essentially supported by the present results, there remains a component of spontaneous HRV that must be related to different mechanisms. We speculate that oscillations in heart filling, and thus in central venous pressure, following changes in lung volumes may stimulate atrial low-pressure cardiac baroreceptor. This might correct the oscillations in central venous pressure synchronous with changes in lung volume by means of

compensatory heart rate oscillations, whence a surviving LF and HF component in BLTR.

Concerning spontaneous variability of SAP and DAP, differences between BLTR and controls were smaller than for HRV. The HF power did not differ between BLTR and controls in any condition. No differences between patients and controls were observed during handgrip for any of the investigated parameters. This means that blood pressure variability was poorly affected, much less than HRV, by lung denervation. The LF power of SAP is linked to peripheral sympathetic modulation(24). Notwithstanding, The LF/HF ratio was higher, and consistently LFnu was higher and HFnu lower in BLTR than in controls, suggesting predominant sympathetic modulation of HR in patients. Thus, the lower LF power of SAP in BLTR would confirm the dissociation between modulation mechanisms involved in arterial pressure variability, representing the peripheral oscillatory component, and HRV, representing the cardiac oscillatory component. We speculate that the lung afferents to the cardiovascular centres affect specifically the central (heart) component of cardiovascular modulation by the ANS. Yet we acknowledge that meaning and links between LF and fluctuation in the modulation of the ANS are highly disputed in literature(25–30), so that some authors(26,30–32) proposed that the LF power is a poor indicator of sympathetic heart modulation.

The analysis of HRV is a potentially useful tool in different clinical settings(33). Cardiac autonomic dysfunction changes HRV and is a risk factor of cardiovascular diseases(25,34). An evaluation of cardiac autonomic dysfunction by HRV helps predicting cardiac issues. Low HRV is, for example, associated with higher risk of heart failure(35,36), sudden cardiac death(37) and mortality after myocardial infarction(38–40). In the case of BLTR, atherosclerotic diseases are potentially accelerated by immunosuppressive therapy(41,42). Then, coronary artery diseases are not uncommon in BLTR(43,44).

The remarkable differences between BLTR and controls imply that HRV analysis may represent a sensitive tool for assessing autonomic improvements in BLTR follow-up. For example, exercise training, improving exercise capacity also in BLTR (45), carries along an increased vagal modulation of the heart (46), which may be reflected by a decrease in the LF/HF ratio. Moreover, progressive

heart reinnervation after surgery may translate into an increased P_{TOT} , LF and HF of HRV, making the BLTR spectra closer to those of healthy subjects. Under this respect, a stimulating perspective is opened by the two young patients who showed P_{TOT} values more than two standard deviations higher than the group mean. These patients were the two youngest investigated BLTR, and also the youngest at the time of surgery. A follow-up study on young BLTR from operation would inform on whether indexes of HRV progress toward normalisation as time after operation goes on.

Concerning BRS, we observed a drastic reduction in BLTR with respect to controls in every condition. Autonomic alterations with reduced vagal or increased sympathetic nerve activity are always accompanied by decreased BRS(47–49). This is especially true during exercise(4). The reduced BRS is therefore consistent with the observed increase in LFnu in BLTR. As physical training increases BRS(50), it would be relevant for BLTR to systematically enter a training program and useful for clinical teams to exactly know how they respond to it.

A limitation of this study concerns the fact that this is a transversal study, wherein we compared BLTR to healthy subjects, instead of a longitudinal study, with the same patients being studied before and after BLT. Thus, we acknowledge that the attribution to lung denervation of the observed effects of HRV and BRS was indirect, not direct: performance of a longitudinal study would be needed to further reinforce the present interpretation. Another point is the transplant medication that can cause side effects on both the central and peripheral nervous system. For example, tacrolimus, used on nine of our eleven patients, is well known to have neuropathies associated with it. Thus, we cannot totally exclude that the lack of HRV may be due to a neurotoxic effect of tacrolimus. Nevertheless, the fact that the two outliers were also under tacrolimus speaks against this second hypothesis.

Conclusion

From a physiological viewpoint, lung denervation carried along an important reduction of HRV, indicating that neural modulation from lung afferents contributes largely to HRV. The higher LF/HF ratio implies that the P_{TOT} drop was mostly due to the HF component. In contrast, the effects of lung denervation on blood pressure variability were much smaller than on HRV. This suggests that the effects of lung denervation were specific to HRV modulation and confirms that blood pressure variability and HRV are under different control mechanisms.

Clinically speaking, it is very important for physicians to be aware that BLTR have a decreased cardiac autonomic regulation with a global and remarkable reduction of all parameters related to HRV spectral analysis. Those patients need an appropriate training program to be strengthened and it would be very useful to quantify exactly how they respond to it. Further research could evaluate HRV indices as sensitive tool for assessing autonomic improvements in BLTR follow-up.

Acknowledgments

This study was supported by grant from Research project of national interest, Ministry of Instruction, University and Research, Republic of Italy, protocol 2007SEESE8_005, and by Swiss National Science Foundation grant 32003B_143427 to Guido Ferretti.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

References

1. Schaeffers HJ, Waxman MB, Patterson GA, Frost AE, Maurer J, Cooper JD. Cardiac innervation after double lung transplantation. Toronto Lung Transplant Group. *J Thorac Cardiovasc Surg*. 1990 Jan;99(1):22–9.
2. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation*. 1991 Aug;84(2):482–92.
3. Taha BH, Simon PM, Dempsey JA, Skatrud JB, Iber C. Respiratory sinus arrhythmia in humans: an obligatory role for vagal feedback from the lungs. *J Appl Physiol Bethesda Md* 1985. 1995 Feb;78(2):638–45.
4. Perini R, Veicsteinas A. Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. *Eur J Appl Physiol*. 2003 Oct;90(3–4):317–25.
5. Iber C, Simon P, Skatrud JB, Mahowald MW, Dempsey JA. The Breuer-Hering reflex in humans. Effects of pulmonary denervation and hypocapnia. *Am J Respir Crit Care Med*. 1995 Jul;152(1):217–24.
6. Dalla Pozza R, Kleinmann A, Bechtold S, Netz H. Hypertension in heart and heart-lung transplanted children: does impaired baroreceptor function play a role? *Transplantation*. 2006 Jan 15;81(1):71–5.
7. Pozza RD, Kleinmann A, Bechtold S, Fuchs A, Netz H. Reinnervation after heart transplantation in children: results of short-time heart rate variability testing. *Pediatr Transplant*. 2006 Jun;10(4):429–33.
8. Berakis A, Williams TJ, Naughton MT, Martin JH, Muhlmann M, Krum H. Altered sympathetic and parasympathetic activity in lung transplantation patients at rest and following autonomic perturbation. *Chest*. 2002 Oct;122(4):1192–9.
9. Seals DR, Suwarno NO, Joyner MJ, Iber C, Copeland JG, Dempsey JA. Respiratory modulation of muscle sympathetic nerve activity in intact and lung denervated humans. *Circ Res*. 1993 Feb;72(2):440–54.
10. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res*. 1986 Aug 1;59(2):178–93.
11. Furlan R, Guzzetti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, et al. Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. *Circulation*. 1990 Feb;81(2):537–47.
12. Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards

- of Measurement, physiological interpretation, and clinical use. *Circulation*. 1996;(93):1043–65.
13. Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G. Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol*. 1988 Feb;254(2 Pt 2):H377-383.
 14. Iellamo F, Hughson RL, Castrucci F, Legramante JM, Raimondi G, Peruzzi G, et al. Evaluation of spontaneous baroreflex modulation of sinus node during isometric exercise in healthy humans. *Am J Physiol*. 1994 Sep;267(3 Pt 2):H994-1001.
 15. Iellamo F, Legramante JM, Raimondi G, Peruzzi G. Baroreflex control of sinus node during dynamic exercise in humans: effects of central command and muscle reflexes. *Am J Physiol*. 1997 Mar;272(3 Pt 2):H1157-1164.
 16. Assoumou H-GN, Bertholon F, Barthélémy J-C, Pichot V, Celle S, Gosse P, et al. Alteration of baroreflex sensitivity in the elderly: the relationship with metabolic syndrome components. *Int J Cardiol*. 2012 Mar 8;155(2):333–5.
 17. Costes F, Roche F, Pichot V, Vergnon JM, Garet M, Barthelemy JC. Influence of exercise training on cardiac baroreflex sensitivity in patients with COPD. *Eur Respir J*. 2004 Mar;23(3):396–401.
 18. Dauphinot V, Kossovsky MP, Gueyffier F, Pichot V, Gosse P, Roche F, et al. Impaired baroreflex sensitivity and the risks of new-onset ambulatory hypertension, in an elderly population-based study. *Int J Cardiol*. 2013 Oct 9;168(4):4010–4.
 19. Fontolliet T, Pichot V, Antonutto G, Bonjour J, Capelli C, Tam E, et al. Effects of gravitational acceleration on cardiovascular autonomic control in resting humans. *Eur J Appl Physiol*. 2015 Jul;115(7):1417–27.
 20. McDonald JH. Handbook of Biological Statistics. In: Handbook of Biological Statistics. 3rd ed. Baltimore, Maryland.: Sparky House Publishing,; 2014. p. 173–9.
 21. Kumar A, Rani A, Tchigranova O, Lee W-H, Foster TC. Influence of late-life exposure to environmental enrichment or exercise on hippocampal function and CA1 senescent physiology. *Neurobiol Aging*. 2012 Apr;33(4):828.e1-17.
 22. Kempell AT, Fieber LA. Age-related deficits in synaptic plasticity rescued by activating PKA or PKC in sensory neurons of *Aplysia californica*. *Front Aging Neurosci* [Internet]. 2015 Sep 3 [cited 2016 Sep 21];7. Available from: <http://journal.frontiersin.org/Article/10.3389/fnagi.2015.00173/abstract>
 23. Burke SN, Barnes CA. Neural plasticity in the ageing brain. *Nat Rev Neurosci*. 2006 Jan;7(1):30–40.

24. Pagani M, Montano N, Porta A, Malliani A, Abboud FM, Birkett C, et al. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation*. 1997;95(6):1441–1448.
25. Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of Measurement, physiological interpretation, and clinical use. *Circulation*. 1996;(93):1043–65.
26. Eckberg DL. Sympathovagal Balance : A Critical Appraisal. *Circulation*. 1997 Nov 4;96(9):3224–32.
27. Malliani A, Pagani M, Montano N, Mela GS. Sympathovagal balance: a reappraisal. *Circulation*. 1998 Dec 8;98(23):2640–3.
28. Notarius CF, Atchison DJ, Rongen GA, Floras JS. Effect of adenosine receptor blockade with caffeine on sympathetic response to handgrip exercise in heart failure. *Am J Physiol Heart Circ Physiol*. 2001 Sep;281(3):H1312-1318.
29. Billman GE. Heart rate variability - a historical perspective. *Front Physiol*. 2011;2:86.
30. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol* [Internet]. 2013 [cited 2015 Nov 26];4. Available from: <http://journal.frontiersin.org/article/10.3389/fphys.2013.00026/abstract>
31. Goldstein DS, Benth O, Park M-Y, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes: Low-frequency power of heart rate variability. *Exp Physiol*. 2011 Dec;96(12):1255–61.
32. Heathers JAJ. Everything Hertz: methodological issues in short-term frequency-domain HRV. *Front Physiol*. 2014;5:177.
33. La Rovere MT, Pinna GD, Maestri R, Mortara A, Capomolla S, Febo O, et al. Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. *Circulation*. 2003 Feb 4;107(4):565–70.
34. Pipilis A, Flather M, Ormerod O, Sleight P. Heart rate variability in acute myocardial infarction and its association with infarct site and clinical course. *Am J Cardiol*. 1991 May 15;67(13):1137–9.
35. Tuininga YS, van Veldhuisen DJ, Brouwer J, Haaksma J, Crijns HJ, Man in't Veld AJ, et al. Heart rate variability in left ventricular dysfunction and heart failure: effects and implications of drug treatment. *Br Heart J*. 1994 Dec;72(6):509–13.

36. Sandercock GRH, Brodie DA. The role of heart rate variability in prognosis for different modes of death in chronic heart failure. *Pacing Clin Electrophysiol PACE*. 2006 Aug;29(8):892–904.
37. Kudaiberdieva G, Görennek B, Timuralp B. Heart rate variability as a predictor of sudden cardiac death. *Anadolu Kardiyol Derg AKD Anatol J Cardiol*. 2007 Jul;7 Suppl 1:68–70.
38. Wolf MM, Varigos GA, Hunt D, Sloman JG. Sinus arrhythmia in acute myocardial infarction. *Med J Aust*. 1978 Jul 15;2(2):52–3.
39. Kleiger RE, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol*. 1987 Feb 1;59(4):256–62.
40. Dougherty CM, Burr RL. Comparison of heart rate variability in survivors and nonsurvivors of sudden cardiac arrest. *Am J Cardiol*. 1992 Aug 15;70(4):441–8.
41. Kasiske BL. Risk factors for accelerated atherosclerosis in renal transplant recipients. *Am J Med*. 1988 Jun;84(6):985–92.
42. Hruban RH, Beschoner WE, Hutchins GM. Lymphocytic bronchitis and lung allograft rejection. *Transplantation*. 1990 Oct;50(4):723.
43. Reed RM, Eberlein M, Girgis RE, Hashmi S, Iacono A, Jones S, et al. Coronary artery disease is under-diagnosed and under-treated in advanced lung disease. *Am J Med*. 2012 Dec;125(12):1228.e13-1228.e22.
44. Castleberry AW, Martin JT, Osho AA, Hartwig MG, Hashmi ZA, Zanotti G, et al. Coronary revascularization in lung transplant recipients with concomitant coronary artery disease. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2013 Nov;13(11):2978–88.
45. Wickerson L, Mathur S, Brooks D. Exercise training after lung transplantation: A systematic review. *J Heart Lung Transplant*. 2010 May;29(5):497–503.
46. Levy WC, Cerqueira MD, Harp GD, Johannessen KA, Abrass IB, Schwartz RS, et al. Effect of endurance exercise training on heart rate variability at rest in healthy young and older men. *Am J Cardiol*. 1998 Nov 15;82(10):1236–41.
47. Billman GE, Schwartz PJ, Stone HL. Baroreceptor reflex control of heart rate: a predictor of sudden cardiac death. *Circulation*. 1982 Oct;66(4):874–80.
48. Schwartz PJ, Zaza A, Pala M, Locati E, Beria G, Zanchetti A. Baroreflex sensitivity and its evolution during the first year after myocardial infarction. *J Am Coll Cardiol*. 1988 Sep;12(3):629–36.

49. La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, et al. Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: implications for clinical trials. *Circulation*. 2001 Apr 24;103(16):2072–7.
50. La Rovere MT, Bersano C, Gnemmi M, Specchia G, Schwartz PJ. Exercise-induced increase in baroreflex sensitivity predicts improved prognosis after myocardial infarction. *Circulation*. 2002 Aug 20;106(8):945–9.

Figure legends

Figure 1

The mean cardiac values and standard deviations measured and calculated at steady state in supine position in all investigated conditions (n=11). HR: heart rate. RR: R-R interval. SAP: systolic arterial pressure. DAP: diastolic arterial pressure. MAP: mean arterial pressure. BRS: Baroreflex sensitivity. FB: Free Breathing. CB: Cadenced Breathing. HG: Handgrip. *: significantly different from Controls. #: significantly different from FB for the same group.

Figure 2

The mean breathing values and standard deviations measured at steady state in supine position in supine position in all investigated conditions (n=11). FB: Free Breathing. CB: Cadenced Breathing. HG: Handgrip. *: significantly different from Controls. #: significantly different from FB for the same group.

Figure 3

LF and HF power values for HRV during free breathing for every patient, from the most recent to the oldest surgery (6 months to 12 years). X axis is shorten for a better reading.

Table 1

Anti-rejection medication in every bilateral lung transplant recipient

Patients	Medication		
1	Everolimus	Mycophénolate mofétil	Prednisolone
2	Tacrolimus		Prednisolone
3	Tacrolimus		Prednisolone
4	Tacrolimus		Prednisolone
5	Tacrolimus		Prednisolone
6	Tacrolimus		Prednisolone
7	Tacrolimus		Azathioprine
8	Everolimus		Prednisolone
9	Tacrolimus		Prednisolone
10	Tacrolimus		Prednisolone
11	Tacrolimus		Prednisolone

Table 2

Mean and standard deviations of all parameters calculated by mean of heart rate variability, in supine position in the three investigated conditions.

HRV (n=11)		FB	CB	HG
<i>P_{tot} [ms²Hz⁻¹]</i>	P	128.3 ± 144.3*	126.6 ± 118.3*	243.6 ± 229.8*
	C	1799.1 ± 1303.3	927.5 ± 448.5	1735.9 ± 947
<i>LF [ms²Hz⁻¹]</i>	P	86.1 ± 163.8*	37.3 ± 48.14*	133.2 ± 205 *
	C	480 ± 377.6	221.5 ± 197.1	381 ± 237.8
<i>HF [ms²Hz⁻¹]</i>	P	30.5 ± 53.6*	11 ± 11.21*	57.8 ± 81.1*
	C	346 ± 230	663.9 ± 646.7	522.5 ± 508.2
<i>LF/HF</i>	P	3.2 ± 2.1*	2.5 ± 2.1*	3.6 ± 3.5*
	C	1.5 ± 1.3	0.9 ± 0.8	1 ± 0.9
<i>LFnu [%]</i>	P	69.8 ± 9.4*	61.3 ± 16.5*	62.1 ± 21.7*
	C	48.4 ± 20.1	38.9 ± 19.6	40 ± 19.8
<i>HFnu [%]</i>	P	29 ± 16.2*	35.5 ± 19.45*	26.07 ± 10.8*
	C	45 ± 16.4	55.5 ± 16.4	51.9 ± 19.3

HRV (n=9)		FB	CB	HG
<i>P_{tot} [ms²Hz⁻¹]</i>	P	66.4 ± 52.2*	68.6 ± 61.6*	168.5 ± 147.8*
	C	1804.6 ± 932.8	932.8 ± 500.1	1597.9 ± 819.1
<i>LF [ms²Hz⁻¹]</i>	P	15.6 ± 13.9*	18.8 ± 21.63*	187.2 ± 312.3
	C	375.8 ± 295.7	224.4 ± 212.6	356 ± 211.8
<i>HF [ms²Hz⁻¹]</i>	P	7.4 ± 4.9*	5.3 ± 3.16*	11.2 ± 7.8*
	C	334.7 ± 236	741.6 ± 694.2	457.6 ± 499
<i>LF/HF</i>	P	3.3 ± 2.3*	2.7 ± 2.3*	3.1 ± 2.4*
	C	1.1 ± 0.7	1 ± 0.1	1.1 ± 0.9
<i>LFnu [%]</i>	P	63.5 ± 20.7*	58.2 ± 23.3*	65.1 ± 23.1*
	C	46.7 ± 20	39.2 ± 21.8	41.6 ± 21.8
<i>HFnu [%]</i>	P	29.4 ± 18*	33.2 ± 19.4*	24.4 ± 11.3*
	C	45.5 ± 14.8	54.1 ± 17.9	50.8 ± 21.3

*Top panel: eleven patients and controls. Bottom panel: nine patients and controls. HRV: heart rate variability. P_{TOT}: total power. LF: low frequency power. HF: high frequency power. LF/HF: low-to-high frequency ratio. LFnu: normalized LF power. HFnu: normalized HF power. P: Patients. C: Controls. FB: Free Breathing. CB: Cadenced Breathing. HG: Handgrip. *: significantly different from controls.*

Table 3

Mean and standard deviations of all parameters calculated by mean of systolic arterial pressure, diastolic arterial pressure, mean arterial pressure at steady state, in supine position in the three investigated conditions.

SAP		Free Breathing	Cadenced Breathing	Handgrip
<i>P_{tot}</i> [ms2Hz-1]	P	14.5 ± 11.4	7.8 ± 4.4*	18.1 ± 11.4
	C	24.7 ± 16.6	15.6 ± 8.9	18.7 ± 15.3
<i>LF</i> [ms2Hz-1]	P	3.5 ± 3.2*	1.6 ± 1.1*	3.7 ± 2.9
	C	6.4 ± 3.8	4.4 ± 2.7	5.2 ± 3.1
<i>HF</i> [ms2Hz-1]	P	2.0 ± 1.3	2.2 ± 1.9	3.6 ± 4
	C	1.2 ± 1.0	1.2 ± 1.1	2.0 ± 1.7
<i>LF/HF</i>	P	2.0 ± 1.8*	1.2 ± 1*	2.7 ± 2
	C	5.3 ± 3.5	3.8 ± 4.3	3.4 ± 2.9
DAP				
<i>P_{tot}</i> [ms2Hz-1]	P	4.5 ± 3.7	2.6 ± 1.5*	7.8 ± 5.6
	C	6.9 ± 4	6.2 ± 4.6	9 ± 7.6
<i>LF</i> [ms2Hz-1]	P	1.5 ± 1.5*	0.6 ± 0.7*	2.1 ± 1.9
	C	2.7 ± 1.6	1.9 ± 1.4	2.3 ± 1.2
<i>HF</i> [ms2Hz-1]	P	1.1 ± 1.4	0.7 ± 0.5	2.1 ± 2.7
	C	1.1 ± 1	1.3 ± 1.3	1.6 ± 2.3
<i>LF/HF</i>	P	1.8 ± 1.4*	1.4 ± 1.4*	2.4 ± 3.5
	C	4.1 ± 3.2	3 ± 2.8	2.6 ± 1.5
MAP				
<i>P_{tot}</i> [ms2Hz-1]	P	5.5 ± 3.5	3.5 ± 1.8*	8.6 ± 5.1
	C	7.5 ± 4.2	5.4 ± 2.2	8.3 ± 5
<i>LF</i> [ms2Hz-1]	P	1.5 ± 1.2*	0.8 ± 0.7*	2.8 ± 3.5
	C	2.8 ± 1.5	1.9 ± 1.1	2.8 ± 1.7
<i>HF</i> [ms2Hz-1]	P	0.8 ± 0.6	0.9 ± 0.7	1.9 ± 2.1
	C	0.5 ± 0.4	0.6 ± 0.7	1 ± 1.3
<i>LF/HF</i>	P	2.2 ± 1.8*	1.4 ± 1.3*	2.7 ± 2.7*
	C	9.3 ± 9	6 ± 6.7	5.4 ± 4.1

SAP: systolic arterial pressure. *DAP*: diastolic arterial pressure. *MAP*: mean arterial pressure. *P_{TOT}*: total power. *LF*: low frequency power. *HF*: high frequency power. *LF/HF*: low-to-high frequency ratio. *LFnu*: normalized *LF* power. *HFnu*: normalized *HF* power. *P*: Patients. *C*: Controls. *: significantly different from controls.

Table 4

Parameters calculated by means of heart rate variability, in supine position in the three investigated conditions, for the two outliers and respective control counterparts.

HRV		Patient 6	Control 6	Patient 10	Control 10
<i>P_{tot}</i> [<i>ms</i> ² <i>Hz</i> ⁻¹]	FB	1338.8	1942.4	503.9	1606.7
	CB	1049.6	866.1	272.2	941.8
	HG	774.1	3509.9	389.4	1204.1
<i>LF</i> [<i>ms</i> ² <i>Hz</i> ⁻¹]	FB	542.9	725.2	219.2	322.7
	CB	155.7	93.4	85.6	323.2
	HG	302.6	789.9	121.8	197.3
<i>HF</i> [<i>ms</i> ² <i>Hz</i> ⁻¹]	FB	165.8	200.3	103.9	592.8
	CB	260.4	193.2	37.9	435.5
	HG	236.9	1251.5	73.5	377.4
<i>LF/HF</i>	FB	3.3	3.6	2.1	0.5
	CB	0.6	0.5	2.3	0.7
	HG	1.3	0.6	1.7	0.5
<i>LFnu</i> [%]	FB	76.1	76.8	67.1	35.1
	CB	36.8	31.9	66.3	42.5
	HG	44.9	32.8	52.5	32.3
<i>HFnu</i> [%]	FB	23.3	21.2	31.8	64.4
	CB	61.6	66.0	29.4	57.3
	HG	35.1	52.0	31.7	61.8

HRV: heart rate variability. *P_{TOT}*: total power. *LF*: low frequency power. *HF*: high frequency power. *LF/HF*: low-to-high frequency ratio. *LFnu*: normalized *LF* power. *HFnu*: normalized *HF* power. *FB*: Free Breathing. *CB*: Cadenced Breathing. *HG*: Handgrip.

Figure 1

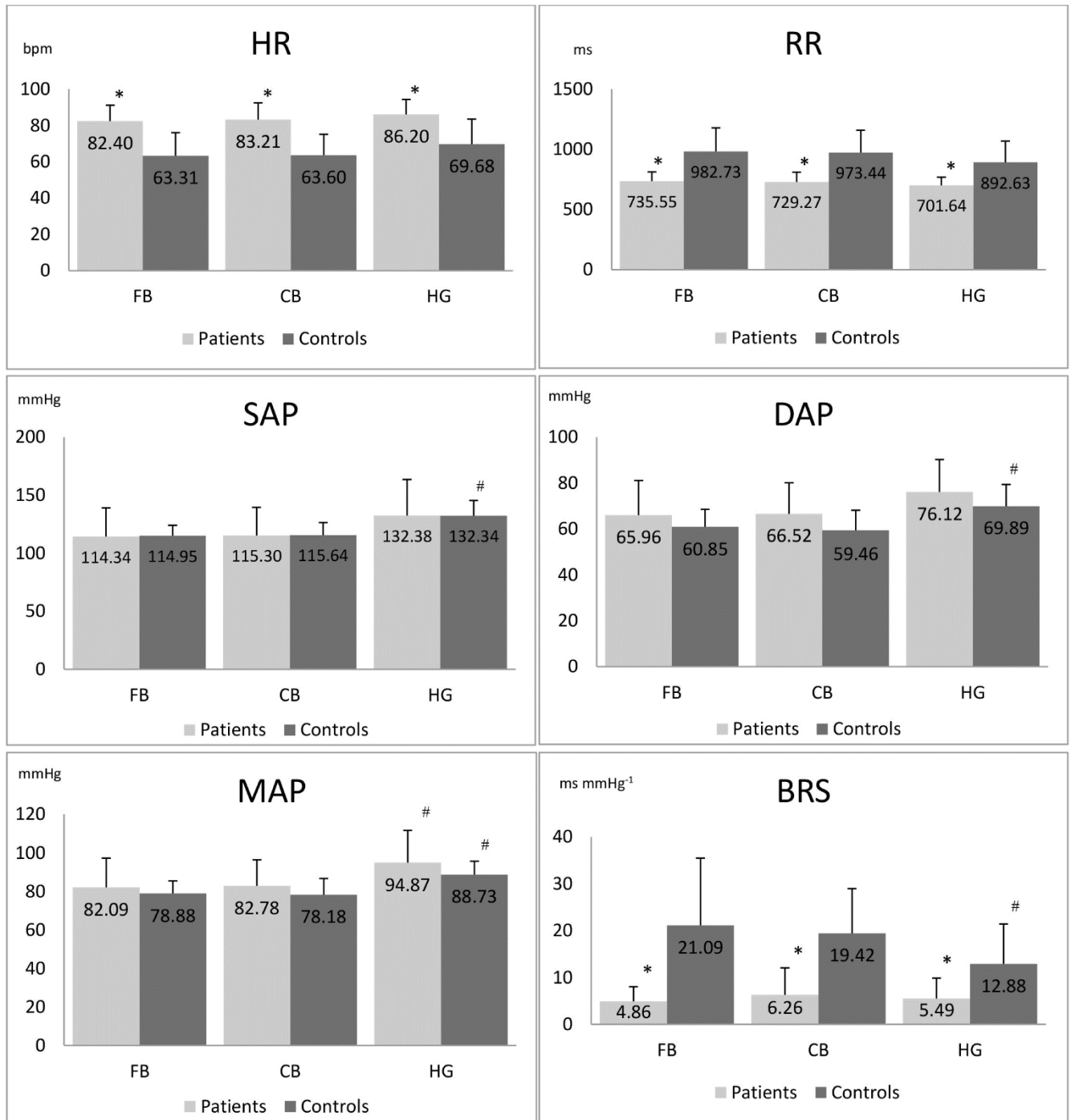


Figure 2

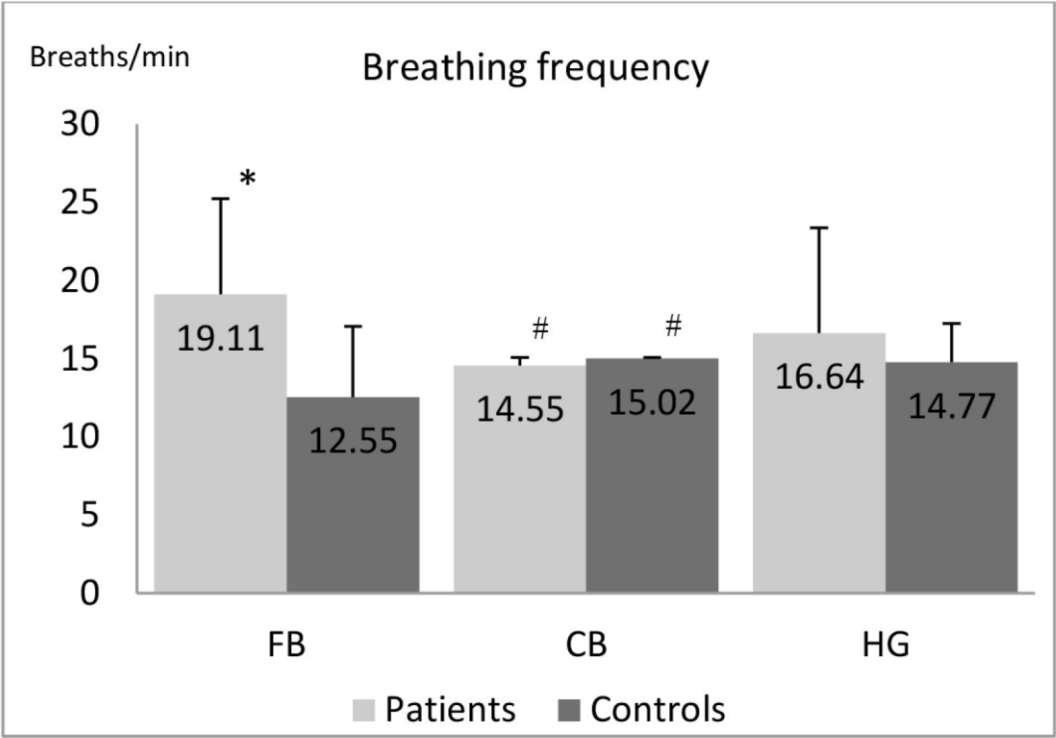
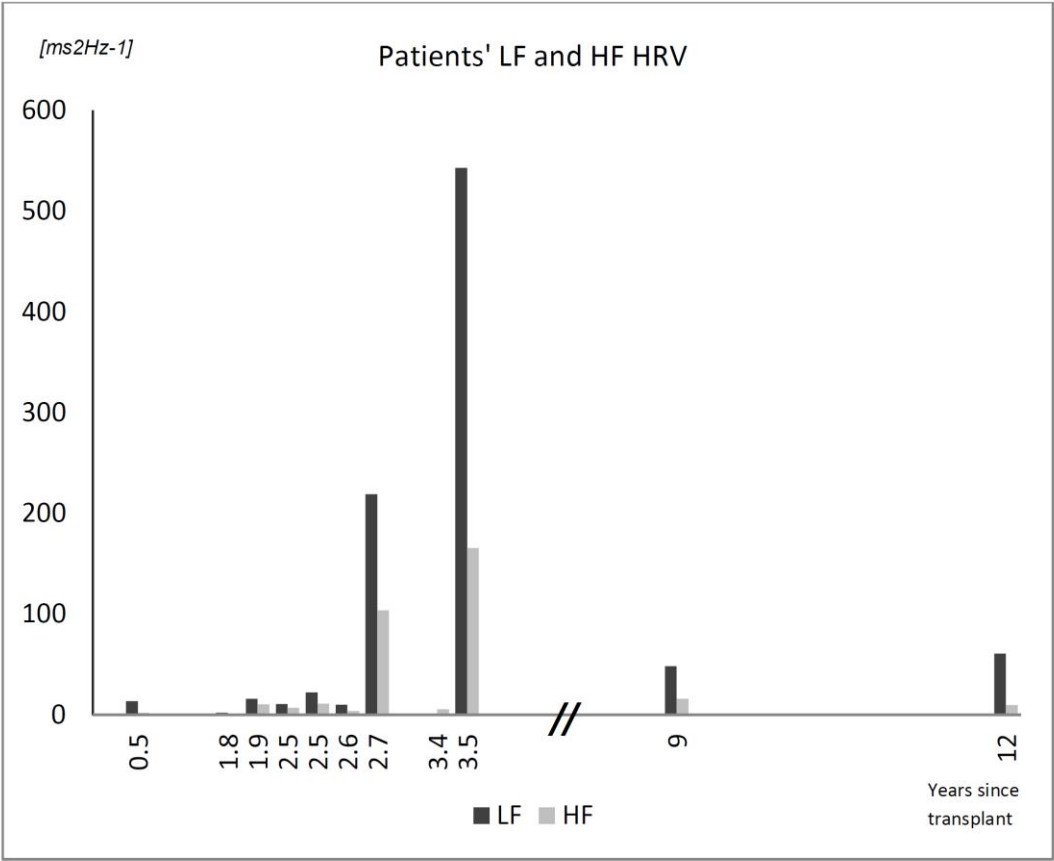


Figure 3



5.2.3. Conclusion

Les résultats obtenus apportent des éléments intéressants pour la compréhension des mécanismes de régulation de la variabilité cardiaque et de la pression artérielle. La dénervation des poumons, suite à l'acte chirurgical de transplantation, implique une forte réduction de la VFC. L'hypothèse formulée soutenant la contribution des afférences pulmonaires à la modulation de la composante HF du spectre de puissance de la variabilité cardiaque, les résultats obtenus vont dans ce sens et valident cette hypothèse. Nous mettons donc en lumière une autre contribution, indépendante, influençant la régulation chronotrope du cœur. Les résultats de variabilité de la pression artérielle entre patients transplantés et sujets contrôles n'étant pas significativement différents, ils démontrent une fois encore la différenciation entre régulation cardiovasculaire centrale et périphérique.

D'un point de vue clinique, cette étude nous conforte dans l'idée qu'une réadaptation physique est essentielle pour tous les transplantés pulmonaires, quelle que soit leur condition physique préopératoire. En effet, les valeurs d'indices de variabilité cardiaque et du BRS chutant suite à la transplantation, les patients s'exposent de manière plus importante aux risques de mortalité liés à de nombreux troubles cardiovasculaires (Bigger *et al.*, 1992 ; Kudaiberdieva *et al.*, 2007 ; Pipilis *et al.*, 1991 ; Ponikowski *et al.*, 1997 ; Sandercock *et al.*, 2006 ; Singh *et al.*, 1996 ; Tuininga *et al.*, 1994 ; Zuanetti *et al.*, 1996). L'activité physique augmentant significativement les valeurs de VFC (Aubert *et al.*, 2003 ; Malik *et al.*, 1996 ; Perini *et al.*, 2002 ; Sandercock and Brodie, 2005) et de BRS (Hua *et al.*, 2009 ; Pagani *et al.*, 1988), c'est un excellent moyen de limiter les

risques d'événements cardiovasculaires fatals et, bien évidemment, d'améliorer les performances cardio-respiratoires de ces patients.

La deuxième partie du protocole de recherche de cette étude inclut déjà un programme de réentraînement, avec une analyse transversale des données récoltées avant et après cette phase de réentraînement. Cette étude, toujours en partenariat avec le département de pneumologie des Hôpitaux Universitaires de Genève, est en projet et permettrait de connaître et de chiffrer, d'un point de vue de la variabilité cardiaque et du BRS, les effets du réentraînement qui est déjà administré en post-opératoire à chaque patient transplanté. Ainsi, les indices de VFC pourraient être potentiellement validés comme outil de suivi des capacités de modulation du SNA et de manière plus générale, des capacités cardiovasculaires en post-opératoire.

5.3. Article III

Effects of autonomic blockade on
human cardiovascular control, at rest
and during exercise

5.3.1. Introduction

Après l'observation de la variabilité de fréquence cardiaque chez des patients ayant subi une transplantation pulmonaire complète, nous nous sommes intéressés aux blocages du système nerveux autonome par substance pharmacologique. La deuxième étude a montré que les afférences pulmonaires jouent un rôle important dans la régulation de la composante HF de la variabilité cardiaque. Par la suite, et indépendamment de cela, nous voulions confirmer ce qu'il en était des efférences cardiaques. Nous savons que la fréquence cardiaque est modulée par le SNA et ses branches sympathique et vagale (Lombardi *et al.*, 1996 ; Pagani *et al.*, 1986 ; Perini and Veicsteinas, 2003 ; Saul, 1990 ; Task force, 1996). Afin de confirmer quels étaient leurs rôles précis dans la régulation de la variabilité de la fréquence cardiaque, nous avons administré des substances bloquant les flux efférents sympathiques et parasympathiques en provenance des centres cardiorégulateurs au sein du bulbe rachidien. Ces blocages furent soit sympathiques, soit vagues, soit complets en inhibant l'ensemble des influx des branches parasympathique et sympathique du SNA.

En suivant l'hypothèse que les branches afférentes du SNA représentent le seul modulateur neural de la fréquence cardiaque, le blocage sympathique ou vagal devrait réduire la VFC alors que le blocage complet pourrait, lui, faire disparaître toute puissance spectrale de la variabilité cardiaque. Afin de tester cette hypothèse, nous avons investigué les effets de blocage de deux substances différentes, au repos et à l'exercice. Le metoprolol est une molécule β -bloquante cardiosélective bloquant les récepteurs adrénergiques β_1 au niveau cardiaque. Il stoppe donc les informations nerveuses circulant à travers les efférences sympathiques en direction du cœur. L'atropine est un antagoniste cholinergique

qui bloque les informations parasympathiques en se fixant sur les récepteurs muscariniques de l'acétylcholine et ce, autant dans le système nerveux central que périphérique. En utilisant ces deux substances conjointement, nous avons bloqué simultanément les deux branches du SNA.

De nombreuses études ont cherché à mettre en évidence les effets des blocages pharmacologiques (Beckers *et al.*, 2006 ; Castiglioni *et al.*, 2011 ; Challapalli *et al.*, 1999 ; Formes *et al.*, 2005 ; Kamimori *et al.*, 2009 ; Krediet and Dunning, 1979 ; Montano *et al.*, 1998 ; Mullen *et al.*, 1997 ; Ng *et al.*, 2009 ; Warren *et al.*, 1997). Les effets du blocage vagal apparaissent assez clairement, cependant, ceux du blocage sympathique semblent être plus ambigus. En effet, différents β -bloquants cardiosélectifs et non-cardiosélectifs, tel le propranolol, ont été utilisé à différents dosages dans ces nombreuses études. Ces travaux n'ont donc pas pu différencier les effets des blocages centraux des blocages périphériques. De plus, les degrés de blocage des récepteurs variaient d'une étude à l'autre. Nous avons donc mené l'étude présentée ci-après afin d'affirmer ou d'infirmer notre hypothèse de départ et de répondre aux ambiguïtés mises en lumière par l'étude de la littérature sur cette thématique.

5.3.2. Manuscrit

Effects of autonomic blockades on human cardiovascular control, at rest and during exercise.

Timothée Fontolliet, Vincent Pichot, Aurélien Bringard, Nazzareno Fagoni, Alessandra Adami, Enrico Tam, Raffaello Furlan, Jean-Claude Barthélémy, Guido Ferretti

Submitted :

American Journal of Physiology, Regulatory, Integrative and Comparative Physiology.

EFFECTS OF AUTONOMIC BLOCKADES ON HUMAN CARDIOVASCULAR CONTROL, AT REST AND DURING EXERCISE.

Timothée Fontolliet ^{1,2}, Vincent Pichot ², Aurélien Bringard ¹, Nazzareno Fagoni ³, Alessandra Adami ^{1,4}, Enrico Tam ⁵, Raffaello Furlan ⁶, Jean-Claude Barthélémy ², Guido Ferretti ^{1,3}

1. Départements d'Anesthésiologie, de Pharmacologie et des Soins Intensifs / des Neurosciences Fondamentales, Université de Genève, Geneva, Switzerland
2. EA SNA-Epis 4607, Université Jean-Monnet, Saint-Étienne, France
3. Dipartimento di Scienze Cliniche e Sperimentali, Università di Brescia, Brescia, Italy
4. Division of Respiratory and Critical Care Physiology and Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA, USA
5. Dipartimento di Scienze Neurologiche e del Movimento, Università di Verona, Verona, Italy
6. Division of Internal Medicine, Humanitas Clinical and Research Center, Rozzano Humanitas University, Rozzano (MI), Italy

Corresponding author :

Timothée Fontolliet

Départements d'Anesthésiologie, de Pharmacologie et des Soins Intensifs / de Neurosciences Fondamentales

Université de Genève

1, Rue Michel Servet

CH-1211 Genève 4, Switzerland

timothee.fontolliet@unige.ch

Phone: 0041 22 3795365

Fax: 0041 22 3795402

Running head: Autonomic blockade and heart rate variability

ABSTRACT

Introduction. The autonomic nervous system (ANS) is a key source of spontaneous heart rate variability (HRV). If ANS is the only modulator of the heartbeat, HRV should decrease with vagal or sympathetic blockades, and disappear during simultaneous blockade of both ANS branches. To test this hypothesis, we investigated the effects of autonomic blockades on HRV and blood pressure variability in resting and exercising humans.

Methods. 7 males (age 24.3 ± 2.6 years) performed 5-min exercise at 80 W, preceded by 5 min at rest, under control (C, no drugs), atropine (A), metoprolol (M) and atropine plus metoprolol (double blockade, DB). On continuous recordings of heart rate and arterial blood pressure, we determined HRV and spontaneous blood pressure variability by power spectral analysis.

Results. At rest, the total power (P_{TOT}), and the powers of the low (LF) and high (HF) frequency components of HRV were dramatically decreased in A and DB compared to C and M, with practically no effects on the LF/HF ratio and on the normalised LF (LFnu) and HF (HFnu). During exercise, P_{TOT} , LF and HF varied as at rest. Blood pressure variability followed similar patterns as HRV, but with much smaller changes.

Conclusions. The results are in contrast with the tested hypothesis, suggesting that ANS is not the only responsible of heartbeat modulation. The parasympathetic outflow to the heart appears to be a major determinant of. The observed effects appear specific to HRV.

Key words

Heart rate variability · Arterial blood pressure · Baroreflexes · Metoprolol · Atropine

INTRODUCTION

The heartbeat is modulated by both the sympathetic and the vagal branches of the autonomic nervous system (ANS) (33, 43, 46, 57, 59). The interaction between the two branches of the ANS is considered a key source of spontaneous heart rate variability (HRV) and was extensively investigated (6, 8, 31, 33, 34, 36, 43, 59). Several investigators tried to understand the neural modulation of the heartbeat by means of pharmacological blockade of either the vagal or the sympathetic branch of the ANS, or both, at rest (5, 10, 11, 13, 20, 26, 29, 38, 40, 41, 43, 63). The data show that vagal blockade reduced the total power (P_{TOT}) of HRV, due to reduction of both its high frequency (HF) component and its low frequency (LF) component. More ambiguous results were obtained under β -adrenergic blockade, partly as a consequence of the type of drug that was used and of the degree of receptor blockade. Under atenolol, a shift toward HF was considered to contradict a concomitant increase in muscle sympathetic nerve activity (13)

Warren et al. (1997) investigated the effects of vagal blockade with glycopyrrolate, selective β -1 blockade with esmolol and simultaneous double blockade with both drugs, on HRV at rest and exercise on the same subjects. The results showed that the power of the HF peak decreased in response to exercise and was attenuated by glycopyrrolate, as they expected. Muscarinic blockade, however, and contrary to their expectations, reduced also the power of the LF peak of HRV, whereas esmolol had no effects on it. The authors concluded that the HF of HRV is a valid index of cardiac vagal activity, as they hypothesized, but the absence of effects of selective β -1 blockade on the LF peak was in contrast with their hypothesis. The very short half time of esmolol may suggest that β -1 receptors were not blocked anymore at the time of their measurements, which may undermine the meaning of their study. Moreover, Warren et al (1997) did not discuss their results in terms of responses to exercise in the four experimental conditions. They neither analysed blood pressure variability, another important indirect feature of sympathetic modulation of the cardiovascular system.

At exercise start, there is an almost immediate attenuation, if not suppression, of vagal activity (18, 30), while the degree of sympathetic activation increases (54, 56, 64) and the modulation of the heartbeat by the sympathetic efferents becomes predominant (47, 53), in apparent contrast with the reported decrease of the LF peak in humans (46, 48). ANS activity during exercise differs from that at rest, and the data at exercise show that further, deeper investigations, possibly including an analysis not only of HRV, but also of blood pressure variability, are necessary to achieve a better understanding of the mechanisms modulating the heartbeat during exercise.

Let us assume that the vagal withdrawal that occurs at exercise start, an analogous of vagal blockade, generates most of the HRV patterns observed during exercise. If this hypothesis were correct, we should expect an analysis of HRV to show: i) no differences in P_{TOT} , LF and HF between rest and exercise under full parasympathetic blockade; ii) a fall in P_{TOT} , LF and HF between rest and exercise under selective β_1 -sympathetic blockade; iii) no differences in P_{TOT} , LF and HF between rest and exercise under blockade of the two branches of the ANS. Moreover, these predictions should not apply to arterial blood pressure variability, which reflects more the peripheral vascular modulation than the central cardiac modulation.

To test the hypothesis formulated here above, we carried out the present study, the aim of which was to investigate the effects of selective β_1 -sympathetic blockade, of vagal blockade and of simultaneous blockade of both branches of the ANS, at rest and during exercise, on HRV and blood pressure variability.

METHODS

Subjects

Seven healthy non-smoking young participants volunteered for the experiments. They were (mean $\pm$ SD) 24.3 ± 2.6 years old, 181.2 ± 3.1 cm tall, and weighed 78.9 ± 6.1 kg. Exclusion criteria were: presence of history of cardiopulmonary disease and regular medication usage at the time of the study.

Participants were instructed to avoid caffeine consumption 24 hours before the visit, and to refrain from performing strenuous exercise the day before.

All participants were preliminarily informed on the design and risks associated with the experimental testing, and they signed a written informed consent. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the institutional ethical committee.

Protocol and measurements

The volunteers reported to the laboratory four times, on different days, with at least a 48-hour break in between. Experiments were performed in supine posture. After instrumentation, a 20-gauge catheter was placed in the antecubital vein of the right arm to administer medications. A 5-min period of monitoring at rest preceded a series of three 5-min square wave exercises at a 80-W intensity. Between each repetition a 5-min recovery was allowed.

During the entire protocol, the electrocardiogram was continuously recorded (Elmed ETM 2000, Heiligenhaus, Germany), and continuous recordings of arterial pulse pressure were obtained at a fingertip of the left arm by means of a non-invasive cuff pressure recorder (Portapres, FMS, Amsterdam, The Netherlands).

The R-R interval (RR) and its reciprocal, HR, were computed beat-by-beat. Systolic and diastolic blood pressure (SAP and DAP, respectively) values were obtained from each pulse pressure profile, using the Beatscope® software package (FMS, Amsterdam, The Netherlands). Beat-by-beat mean arterial pressure (MAP) was computed as the integral mean of each pressure profile, using the same software package.

All the signals were digitalized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta CA, USA) and stored on a computer. The acquisition rate was 400 Hz.

This protocol was performed under four experimental conditions, administered in random order: i) control (C), i.e. without any drug administration, ii) parasympathetic blockade with atropine administration (A), ii) selective β_1 -adrenergic blockade with metoprolol administration (M), and iv) double blockade

of both branches of the ANS with simultaneous atropine and metoprolol administration (DB).

Drug administration

Parasympathetic blockade was achieved with a unique dose of 0.04 mg/kg (mean 3.06 ± 0.23 mg, range 2.7 – 3.4 mg) of atropine (21, 39).

For adrenergic blockade, we used metoprolol tartrate (Loprésor, Novartis, Switzerland): after an initial bolus of 15 mg, metoprolol was continuously infused at a rate of 45 mg per hour, by means of an infusion pump. The efficacy of adrenergic blockade along time was evaluated on a separate session, by analysing the heart rate response following isoprenaline injection. The correct metoprolol maintenance dose was identified as the dose ensuring an 80% reduction of the HR response to isoprenaline for the entire protocol duration. This dose was then administered during the experimental test in M condition.

During the DB experiments, we used the same doses of atropine and metoprolol as in A and M sessions, respectively. After injection of atropine, metoprolol was administered using the same procedure described here above.

Data treatment

After construction of the time series of RR, SAP and DAP from the continuous recordings of electrocardiogram and pulse pressure profiles, means of power spectral analysis were used to evaluate spontaneous variability of RR, SAP and DAP (43). The total power (P_{TOT}) of RR, SAP and DAP variabilities, corresponding to variance, was initially obtained. Subsequently, the powers and frequencies of LF low (0.03–0.149 Hz) and HF (0.15–0.5 Hz) components of the power spectrum were computed and expressed in absolute units (ms^2). The very low frequency component was neglected. The LF/HF ratio was also calculated. Normalized LF and HF (LFnu and HFnu, respectively) were computed as:

$$\frac{LF \times 100}{P_{tot} - VLF} \quad (1)$$

and expressed in normalized units (36).

The spontaneous baroreflex sensitivity (BRS, expressed in ms mmHg⁻¹) was estimated from SAP and RR by means of the sequence method (7). We selected intermittent short sequences of at least three heartbeats during which SAP increased or decreased resulting in a change in RR interval going to the same direction. On these sequences we applied linear regression analysis, and the calculated slope was retained. BRS was then calculated as the mean of the slopes of all sequences per each participant in each condition. Only sequences showing a coefficient of determination of at least 0.85 were analysed.

Spectral analysis and BRS were both performed using software that purposely-written in the Matlab® environment (49).

Statistics

Data are reported as group means $\pm$ standard deviation. The effects of drugs and exercise on the investigated parameters were analysed by two-way ANOVA for repeated measurements. When applicable, a Tukey post-hoc test was used to locate significant differences. All data were analysed with Statistica 12 ©. (StatSoft, Inc., Tulsa, OK). Differences were considered significant if $p < 0.05$.

RESULTS

The mean values of measured and calculated variables at rest and during exercise for the four investigated conditions are shown in Table 1. No arrhythmic beats were observed, and in all cases a normal sinus beat was maintained.

At rest, HR was $62.7 \pm 8.5 \text{ min}^{-1}$ in C; it was $43.5 \pm 8.26\%$ higher in A than in C and $33.3 \pm 10.2\%$ higher in DB than in C. HR in DB was also $36.5 \pm 6\%$ higher than in M and $18.2 \pm 9.1\%$ lower than in A. In M, no differences with respect to C were observed. During exercise, HR was $105.0 \pm 12.4 \text{ beats} \cdot \text{min}^{-1}$ in C; it was higher in A ($+20.7 \pm 14.6\%$), M ($+28.1 \pm 21.3\%$) and DB ($+22.0 \pm 13.8\%$) than in C. With respect to the corresponding values at rest, HR during exercise increased in C, A and M, but not in DB.

No differences were observed at rest for either SAP or DAP, in any of the investigated conditions, with respect to C. Nevertheless, DAP tended to be higher in DB than in C, and was significantly higher in DB than in M ($25.8 \pm 15.6\%$). During exercise, SAP was lower in A ($-28.9 \pm 20.8\%$), M ($-28.1 \pm 21.3\%$) and DB ($22.0 \pm 13.8\%$) than in C. No differences were observed for DAP. No significant differences for MAP were found at rest, except that it was higher in DB than in M ($16.9 \pm 15.1\%$). At exercise, it did not vary significantly between conditions. MAP during exercise was higher than at rest only in C.

HRV data at rest and exercise are shown in Table 2. Whereas P_{TOT} was not affected by M with respect to C, it was largely and significantly decreased in A and DB, both at rest and at exercise. The decrease in P_{TOT} in A and DB was due to the drop of both LF and HF powers. As a consequence, no differences for LF and HF powers between M and C, and between A and DB were observed.

The HF/LF ratio at rest was unaffected by drug treatment, the only significant difference being between A and DB; at exercise, all pharmacological treatments provided different results with respect to C. As for the LF/HF ratio, LFnu at rest was higher in A than in DB; at exercise, it was lower in DB than in any other condition. Coherently, HFnu did not change significantly at rest; during exercise, it was higher in DB than in any other condition. With respect to rest, HFnu was decreased during exercise in C and in M.

Means and standard deviations of all data calculated by means of SAP and DAP spectral analysis at rest and during exercise are also shown in Tab 2.

Concerning SAP, no differences among conditions for P_{TOT} were observed at rest. During exercise, P_{TOT} was lower in A ($-91.8 \pm 6.7\%$), M ($-88.9 \pm 9.3\%$) and DB ($-91.6 \pm 6.4\%$), compared to C. At rest, the LF power followed the same patterns as in HRV, with lower values in A and DB than in C ($-70.5 \pm 20.2\%$ and $-62.0 \pm 27.4\%$, respectively) and βB ($-63.7 \pm 19.7\%$ and $-52.9 \pm 34.4\%$, respectively), while the HF power was lower in A ($-49.5 \pm 43.4\%$) and DB ($-62.7 \pm 22.3\%$) than in C. No differences among conditions were observed during exercise for both the LF and the HF powers. The LF power was higher in C and A with respect to the same condition at rest. No differences among conditions or

compared to rest were observed for the HF power. The LF/HF ratio was unchanged in all conditions.

Concerning DAP, no changes in P_{TOT} were found in any pharmacological condition with respect to C. At rest, the HF power did not vary among conditions, while the LF power was lower in A and DB than in C. The LF/HF ratio was lower in all conditions than in C. During exercise, there were no significant differences among conditions or with respect to the same condition at rest.

The BRS values at rest and exercise are shown in Figure 1. At rest, BRS was $91.4 \pm 1.93\%$ lower in A and $86.2 \pm 7.4\%$ lower in DB than in C. It was also lower in DB ($-86.8 \pm 9.5\%$) and in A ($-91.5 \pm 4.4\%$) than in M. During exercise, BRS was lower in A than in C ($-58.2 \pm 28.0\%$) and M ($-72.4 \pm 11.0\%$). BRS was lower at exercise than at rest in C, A and M, but not in DB.

Discussion

Main findings

The analysis of spontaneous heart rate variability at rest showed that: 1. atropine administration at rest drastically reduced P_{TOT} , due to the fall of both LF and HF powers, with respect to control; 2. simultaneous double blockade with atropine and metoprolol at rest provided the same results as atropine administration only; 3. metoprolol administration had no effects on heart rate variability at rest.

A comparison of exercise data versus rest data in the four conditions showed that exercise: 4. did not change P_{TOT} , LF and HF following parasympathetic blockade and simultaneous double blockade with respect to rest; 5. decreased P_{TOT} , and the LF and HF powers, by the same extent under metoprolol as in control. In spite of this, at exercise, P_{TOT} , and the LF and HF powers were lower in A and in DB than in C or in M.

All drug administrations provided ambiguous results concerning LFnu and HFnu, whether at rest or at exercise. The results concerning arterial blood pressure variability were similar to those obtained for HRV, but with much smaller

changes. Baroreflex sensitivity was drastically reduced both at rest and during exercise by atropine administration and by double blockade.

These results agree with the tested hypothesis that vagal activity suppression is the main determinant of the reduction of P_{TOT} during light steady-state exercise, as long as the three predictions on P_{TOT} that we formulated were supported by the results. Yet we still found effects on arterial blood pressure variability, which followed similar patterns as those for HRV, although of much smaller size. No clear cut conclusions can be formulated concerning the relative weight of LF and HF and their balance at rest and at exercise.

Heart rate variability

At rest, HR was significantly increased after A administration, in line with previous studies (12, 26, 28, 58, 60); in M, despite a slight decrease, HR in M did not change significantly. These results, although similar in size to those obtained in a previous study with the same drug (58), are at variance with those of other studies, carried out in upright posture, showing a significant HR reduction at rest with beta-blockade (19, 20, 22). This may suggest that the β_1 -adrenergic blockade might not have been complete in the present study. It is of note, however, that the same procedure and dose of metoprolol administration was used in a previous study (20), generating a significant resting HR decrease both in normoxia and in acute hypoxia. In supine posture, the predominance of vagal modulation of HR (23, 43) may explain the lack of changes in HR in M.

Concerning HRV, the present P_{TOT} and LF and HF powers in C were in line with previous results (6, 36, 43, 46, 51, 59). M failed in changing P_{TOT} , LF and HF at rest indicating that a selective blockade of cardiac β -adrenergic receptors has no effects on spontaneous HR oscillations. This would mean that the sympathetic outflow to the heart may not be a main determinant of HRV, although the P_{tot} values after DB appear lower (just a tendency) than after A. These results are in agreement with some previous studies (20, 63). However, different results were obtained by others (13, 49), who remarked a decrease of LF_{nu} and LF/HF index, an increase in P_{TOT} mostly due to an increase in HF, a finding that suggests a stronger cardio-respiratory coupling under atenolol than

in control. Spontaneous HR oscillations were almost suppressed in A, as observed in all previous studies (11, 20, 26, 38, 40, 41, 63), indicating that the parasympathetic outflow to the heart is the major determinant of HRV at rest in the present population. This was so also under simultaneous sympathetic and vagal blockade, indicating that the most of the effect observed in DB was due to the suppression of parasympathetic modulation of the heartbeat only.

Passing from rest to exercise implied a large change in LF and HF powers in C and in M, whereas no changes with respect to rest were found in A and in DB. These results indicate that the well-known fall of HRV usually observed during exercise (46), and found also in this study, is mostly due to the withdrawal of the vagal outflow to the heart occurring at exercise onset (18, 30). If indeed vagal withdrawal occurs at exercise onset, these results would indicate at the investigated powers vagal withdrawal is not complete, because the LF and HF powers in C were still higher than in A or in DB, the two conditions in which a full blockade of muscarinic receptors was attained.

When we analyse LFnu, HFnu and LF/HF ratio, the results appear more ambiguous. Since the effects of drug administration on the LF and HF powers were similar, the LF/HF ratio did not change significantly. In spite of this, during exercise, there was a tendency toward a lower HF power than LF power. This tendency was such as to provide, at exercise compared to rest, significantly reduced HFnu values in C and M, though not in A and in DB; as a consequence, LF/HF ratio turned higher at exercise than at rest, at least in C and in M. This suggests that the withdrawal of vagal tone at exercise onset might have had greater effects on the HF than on the LF component of HRV. Alternatively, an initial increase of the cardiac sympathetic modulation is already present, as suggested by the relative increase of the LF component of RR variability. Nevertheless, at exercise, LFnu in DB was significantly lower and HFnu significantly higher than in C, despite the lack of differences in the LF/HF ratio. This may be due to the non-autonomic effect of an increase in ventilation that is reflected on HRV through changes in venous return. A similar condition can be observed in a neurodegenerative disease such as the Pure Autonomic Failure. This condition is characterized by both a cardiac sympathetic and parasympathetic denervation leading to Ptot values mimicking high dosage

atropine administration, in which a HF component of HRV, non-autonomic in origin, is present (48). Notwithstanding, these apparently contradictory results prevent from arriving at clear-cut conclusions concerning the mechanisms at the basis of relative powers in this study.

Blood pressure variability

At rest, arterial blood pressure was not affected by drugs, consistently with previous studies (20, 26). On one side, the vagal branch of the ANS affects the central cardiovascular function (with significant differences for HR) but not the peripheral one (with no differences in blood pressure), coherently with the notion that atropine does not act on systemic arterioles, which are normally devoid of muscarinic receptors. On the other side, metoprolol is a selective blocker of β_1 -adrenergic receptors that are expressed specifically in the heart, not in arterioles, so it was not expected to induce changes in blood pressure.

SAP variability was much less affected by A and DB than HRV. Most remarkable are the changes observed when passing from rest to exercise: the LF for SAP power increased in C (coherently with previous results, see 46 and 48) and A, but not in M and in DB. This indicates that the increase in LF power for SAP may be a consequence of increased sympathetic modulation during exercise. No effects were observed under any drug on the HF power: this means that the HF power of SAP is independent of the activity of the two branches of the ANS. The lack of exercise effects on HF power under drug stimulation explains why the P_{TOT} did not differ significantly at exercise with respect to rest in A.

DAP variability was unaffected by drug administration: this suggests that the exercise effect on the LF power of SAP, related to a selective blockade of β_1 adrenergic receptors, is mediated by a central (cardiac) rather than a peripheral (arteriolar muscle vasodilation) action of the sympathetic branch of the ANS.

Baroreflex sensitivity

At rest, BRS was drastically reduced in A and DB as compared to C, but not in M, in agreement with previous observations (1, 13, 65). This agrees with the concept that BRS is mainly modulated by the parasympathetic efferent branch on the ANS. The changes in BRS are consistent with the changes in arterial blood pressure variability, suggesting that baroreflexes may modulate the LF power of arterial blood pressure. The reduction of BRS observed at exercise with respect to rest (60) agrees also with the alfa-index changes observed in previous studies (44). In the present study, the BRS reduction at exercise was observed only in C and in M, but not in A and in DB. It can well be attributed to the withdrawal of vagal tone at exercise onset, as proposed (42). As a consequence, no differences in BRS among the four investigated conditions were observed during exercise.

Conclusion

The results of this study support the hypothesis that the main determinant of spontaneous heart rate variability is the parasympathetic outflow to the heart. The observed effects on arterial blood pressure variability are indirectly related to the action of the administered drugs, supporting the notion that blood pressure and heart rate variability are only partially-associated phenomena, subject to somewhat independent regulation mechanisms. These conclusions have no impact on the meaning and on the physiological mechanisms behind LFnu and HFnu.

Acknowledgments

This study was supported by Swiss National Science Foundation grants 3200B0 102181 and 32003B_143427 to Guido Ferretti.

References

1. **Ahmed MW, Kadish AH, Parker MA, Goldberger JJ.** Effect of physiologic and pharmacologic adrenergic stimulation on heart rate variability. *J Am Coll Cardiol* 24: 1082–1090, 1994.
2. **Airaksinen KE, Niemelä MJ, Huikuri HV.** Effect of beta-blockade on baroreflex sensitivity and cardiovascular autonomic function tests in patients with coronary artery disease. *Eur Heart J* 15: 1482–1485, 1994.
3. **Assoumou H-GN, Bertholon F, Barthélémy J-C, Pichot V, Celle S, Gosse P, Gaspoz J-M, Roche F.** Alteration of baroreflex sensitivity in the elderly: the relationship with metabolic syndrome components. *Int J Cardiol* 155: 333–335, 2012.
4. **Azabji Kenfack M, Lador F, Licker M, Moia C, Tam E, Capelli C, Morel D, Ferretti G.** Cardiac output by Modelflow method from intra-arterial and fingertip pulse pressure profiles. *Clin Sci* 106: 365–369, 2004.
5. **Beckers F, Verheyden B, Ramaekers D, Swynghedauw B, Aubert AE.** Effects of autonomic blockade on non-linear cardiovascular variability indices in rats. *Clin Exp Pharmacol Physiol* 33: 431–439, 2006.
6. **Berntson GG, Bigger JT, Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, van der Molen MW.** Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34: 623–648, 1997.
7. **Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G.** Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol Heart Circ Physiol* 254: H377–383, 1988.
8. **Billman GE.** Heart rate variability - a historical perspective. *Front Physiol* 2:86, 2011.
9. **Bonjour J, Bringard A, Antonutto G, Capelli C, Linnarsson D, Pendergast DR, Ferretti G.** Effects of acceleration in the Gz axis on human cardiopulmonary responses to exercise. *Eur J Appl Physiol* 111: 2907–2917, 2011.
10. **Castiglioni P, Parati G, Di Rienzo M, Carabalona R, Cividjian A, Quintin L.** Scale exponents of blood pressure and heart rate during autonomic blockade as assessed by detrended fluctuation analysis: Cardiovascular scale exponents and autonomic blockade. *J Physiol* 589: 355–369, 2011.
11. **Challapalli S, Kadish AH, Horvath G, Goldberger JJ.** Differential effects of parasympathetic blockade and parasympathetic withdrawal on heart rate variability. *J Cardiovasc Electrophysiol* 10: 1192–1199, 1999.
12. **Chamberlain DA, Turner P, Sneddon JM.** Effects of atropine on heart rate in healthy man. *The Lancet* 290: 12–15, 1967.
13. **Cogliati C, Colombo S, Gneccchi Ruscone T, Gruosso D, Porta A, Montano N, Malliani A, Furlan R.** Acute beta-blockade increases muscle sympathetic activity and modifies its frequency distribution. *Circulation* 110: 2786–2791, 2004.

14. **Costes F, Roche F, Pichot V, Vergnon JM, Garet M, Barthelemy JC.** Influence of exercise training on cardiac baroreflex sensitivity in patients with COPD. *Eur Respir J* 23: 396–401, 2004.
15. **Dauphinot V, Kossovsky MP, Gueyffier F, Pichot V, Gosse P, Roche F, Barthélémy J-C.** Impaired baroreflex sensitivity and the risks of new-onset ambulatory hypertension, in an elderly population-based study. *Int J Cardiol* 168: 4010–4014, 2013.
16. **Drouin E, Gournay V, Calamel J, Mouzard A, Rozé JC.** Assessment of spontaneous baroreflex sensitivity in neonates. *Arch Dis Child Fetal Neonatal Ed* 76: F108–112, 1997.
17. **Eckberg DL.** Sympathovagal Balance : A Critical Appraisal. *Circulation* 96: 3224–3232, 1997.
18. **Fagraeus L, Linnarsson D.** Autonomic origin of heart rate fluctuations at the onset of muscular exercise. *J Appl Physiol* 40: 679–682, 1976.
19. **Ferretti G, Licker MJ, Anchisi S, Moia C, Susta D, Morel DR.** The effects of beta1-adrenergic blockade on cardiovascular oxygen flow in normoxic and hypoxic humans at exercise. *Eur J Appl Physiol* 95: 250–259, 2005.
20. **Formes KJ, Wray DW, O-Yurvati AH, Weiss MS, Shi X.** Sympathetic cardiac influence and arterial blood pressure instability. *Auton Neurosci* 118: 116–124, 2005.
21. **Goldberger JJ, Challapalli S, Tung R, Parker MA, Kadish AH.** Relationship of heart rate variability to parasympathetic effect. *Circulation* 103: 1977–1983, 2001.
22. **Hawkins MN, Barnes Q, Purkayastha S, Eubank W, Ogoh S, Raven PB.** The effects of aerobic fitness and beta1-adrenergic receptor blockade on cardiac work during dynamic exercise. *J Appl Physiol* 106: 486–493, 2009.
23. **Hughson RL, Yamamoto Y, McCullough RE, Sutton JR, Reeves JT.** Sympathetic and parasympathetic indicators of heart rate control at altitude studied by spectral analysis. *J Appl Physiol* 77: 2537–2542, 1994.
24. **Iellamo F, Legramante JM, Pigozzi F, Spataro A, Norbiato G, Lucini D, Pagani M.** Conversion from vagal to sympathetic predominance with strenuous training in high-performance world class athletes. *Circulation* 105: 2719-2724, 2002.
25. **Just A, Faulhaber J, Ehmke H.** Autonomic cardiovascular control in conscious mice. *Am J Physiol Regul Integr Comp Physiol* 279: R2214–2221, 2000.
26. **Kamimori GH, Bellar D, Fein HG, Smallridge RC.** Hormonal and cardiovascular response to low-intensity exercise with atropine administration. *Mil Med* 174: 253–258, 2009.
27. **Karlen-Amarante M, da Cunha NV, de Andrade O, de Souza HCD, Martins-Pinge MC.** Altered baroreflex and autonomic modulation in monosodium glutamate-induced hyperadipose rats. *Metab Clin Exp* 61: 1435–1442, 2012.
28. **Kelbaek H, Marving J, Hvid-Jacobsen K, Nielsen SL.** Effects of atropine on left ventricular volumes and ejection and filling rates at rest and during exercise. *Br J Clin Pharmacol* 32: 585-589, 1991.

29. **Krediet RT, Dunning AJ.** Baroreflex sensitivity in hypertension during beta-adrenergic blockade. *Br Heart J* 41: 106–110, 1979.
30. **Lador F, Azabji Kenfack M, Moia C, Cautero M, Morel DR, Capelli C, Ferretti G.** Simultaneous determination of the kinetics of cardiac output, systemic O₂ delivery, and lung O₂ uptake at exercise onset in men. *Am J Physiol Regul Integr Comp Physiol* 290: R1071–1079, 2006.
31. **Lanfranchi VA, Somers P.** Arterial baroreflex function and cardiovascular variability: interactions and implications. *Am J Physiol Reg Integr Comp Physiol* 283: R815–R826, 2003.
32. **Linnarsson D, Rosenhamer G.** Exercise and arterial pressure during simulated increase of gravity. *Acta Physiol Scand* 74: 50–57, 1968.
33. **Lombardi F, Malliani A, Pagani M, Cerutti S.** Heart rate variability and its sympatho-vagal modulation. *Cardiovasc Res* 32: 208–216, 1996.
34. **Lombardi F, Stein PK.** Origin of heart rate variability and turbulence: an appraisal of autonomic modulation of cardiovascular function. *Front Physiol* 2: 95, 2011.
35. **Lucini D, Pagani M, Mela GS, Malliani A.** Sympathetic restraint of baroreflex control of heart period in normotensive and hypertensive subjects. *Clin Sci* 86: 547–556, 1994.
36. **Malliani A, Pagani M, Lombardi F, Cerutti S.** Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84: 482–492, 1991.
37. **Merati G, Maggioni MA, Invernizzi PL, Ciapparelli C, Agnello L, Veicsteinas A, Castiglioni P.** Autonomic modulations of heart rate variability and performances in short-distance elite swimmers. *Eur J Appl Physiol* 115: 825–835, 2015.
38. **Montano N, Cogliati C, Porta A, Pagani M, Malliani A, Narkiewicz K, et al.** Central vagotonic effects of atropine modulate spectral oscillations of sympathetic nerve activity. *Circulation* 6; 98(14):1394–9, 1998.
39. **Morikami Y, Yasue H, Okumura K, Horio Y, Fujii H, Matsuyama K.** Effects of phentolamine and atropine on angina pectoris induced by handgrip test in patients with variant angina. *Am J Cardiol* 61: 71–76, 1988.
40. **Mullen TJ, Appel ML, Mukkamala R, Mathias JM, Cohen RJ.** System identification of closed-loop cardiovascular control: effects of posture and autonomic blockade. *Am J Physiol Heart Circ Physiol* 272: H448–461, 1997.
41. **Ng J, Sundaram S, Kadish AH, Goldberger JJ.** Autonomic effects on the spectral analysis of heart rate variability after exercise. *Am J Physiol Heart Circ Physiol* 297: H1421–1428, 2009.
42. **Ogoh S, Fisher JP, Dawson EA, White MJ, Secher NH, Raven PB.** Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans: Arterial baroreflex function in exercise. *J Physiol* 566: 599–611, 2005.
43. **Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell’Orto S, Piccaluga E.** Power spectral analysis of heart rate and arterial

pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 59: 178–193, 1986.

44. **Pagani M, Somers V, Furlan R, Dell'Orto S, Conway J, Baselli G, et al.** Changes in autonomic regulation induced by physical training in mild hypertension. *Hypertension* 12(6): 600–10, 1988.
45. **Parlow J, Viale JP, Annat G, Hughson R, Quintin L.** Spontaneous cardiac baroreflex in humans. Comparison with drug-induced responses. *Hypertension* 25: 1058–1068, 1995.
46. **Perini R, Veicsteinas A.** Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. *Eur J Appl Physiol* 90: 317–325, 2003.
47. **Persson P. B.** Modulation of Cardiovascular Control Mechanisms and Their Interaction. *Physiol Rev.* 76: 193–244, 1996
48. **Piazza S, Furlan R, Dell'Orto S, Porta A, Lombardi F, Pagani M, Malliani A.** Mechanical effects of respiration and stepping on systolic arterial pressure variability during treadmill exercise. *J Hypertens* 13: 1643-1647, 1995.
49. **Pichot, V, Roche F, Celle S, Barthélémy J-C, Chouchou F.** “HRVanalysis: A Free Software for Analyzing Cardiac Autonomic Activity.” *Front Physiol* 7 : 557, 2016
50. **Pickering TG, Gribbin B, Petersen ES, Cunningham DJ, Sleight P.** Effects of autonomic blockade on the baroreflex in man at rest and during exercise. *Circ Res* 30: 177–185, 1972.
51. **Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS.** Heart rate variability: a review. *Med Biol Eng Comput* 44: 1031–1051, 2006.
52. **Remington JW, Wood EH.** Formation of peripheral pulse contour in man. *J Appl Physiol* 9: 433–442, 1956
53. **Rimoldi O, Furlan R, Pagani MR, Piazza S, Guazzi M, Pagani M, Malliani A.** Analysis of neural mechanisms accompanying different intensities of dynamic exercise. *Chest* 101: 226S-230S, 1992.
54. **Robinson BF, Epstein SE, Beiser GD, Braunwald E.** Control of heart rate by the autonomic nervous system. Studies in man on the interrelation between baroreceptor mechanisms and exercise. *Circ Res* 19: 400–411, 1966.
55. **Rosenstock E, Cassuto Y, Zmora E.** Heart rate variability in the neonate and infant: analytical methods, physiological and clinical observations. *Acta Pædiatrica* 88: 477–482, 1999.
56. **Rowell LB.** Human Cardiovascular Control. Oxford University Press, New York, NY; Oxford. 1993
57. **Saul JP.** Beat-to-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *News Physiol Sci* 5: 32–37, 1990.
58. **Smith ML, Hudson DL, Graitzer HM, Raven PB.** Blood pressure regulation during cardiac autonomic blockade: effect of fitness. *J Appl Physiol* 65: 1789-1795, 1988.
59. **Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.** Heart rate variability. Standards of Measurement, physiological interpretation, and clinical use. *Circulation*: 1043–1065, 1996.

60. **Toska K, Eriksen M.** Respiration-synchronous fluctuations in stroke volume, heart rate and arterial pressure in humans. *J Physiol* 472: 501-512, 1993.
61. **Vallais F, Baselli G, Lucini D, Pagani M, Porta A.** Spontaneous baroreflex sensitivity estimates during graded bicycle exercise: a comparative study. *Physiol Meas* 30: 201–213, 2009.
62. **Vesalainen R, Kantola I, Airaksinen K, Tahvanainen K, Kaila T.** Vagal Cardiac Activity in Essential Hypertension: The Effects of Metoprolol and Ramipril. *Am J Hypertens* 11: 649–658, 1998.
63. **Warren JH, Jaffe RS, Wraa CE, Stebbins CL.** Effect of autonomic blockade on power spectrum of heart rate variability during exercise. *Am J Physiol* 273: R495–502, 1997.
64. **White DW, Raven PB.** Autonomic Neural Control of Heart Rate during Dynamic Exercise: Revisited. *J Physiol* 592: 2491–2500, 2014.
65. **Wray DW, Formes KJ, Weiss MS, O-Yurvati AH, Raven PB, Zhang R, Shi X.** Vagal cardiac function and arterial blood pressure stability. *Am J Physiol Heart Circ Physiol* 281: H1870–1880, 2001.
66. **Ylitalo A, Airaksinen KEJ, Sellin L, Huikuri HV.** Effects of combination antihypertensive therapy on baroreflex sensitivity and heart rate variability in systemic hypertension. *Am J Cardiol* 83: 885–889, 1999.

Figure legend

*Figure 1: Mean values of BRS in each investigated condition (control / atropine / metoprolol / double blockade) at rest and during exercise. N=7. Statistics: two-way ANOVA and Tukey post-hoc. BRS: Spontaneous baroreflex sensitivity. CTRL: Control. ATR: Atropine. β B: Metoprolol DB: Double blockade. *: significantly different from CTRL. #: significantly different from DB. §: significantly different from ATR. \$: significantly different from the same condition at rest.*

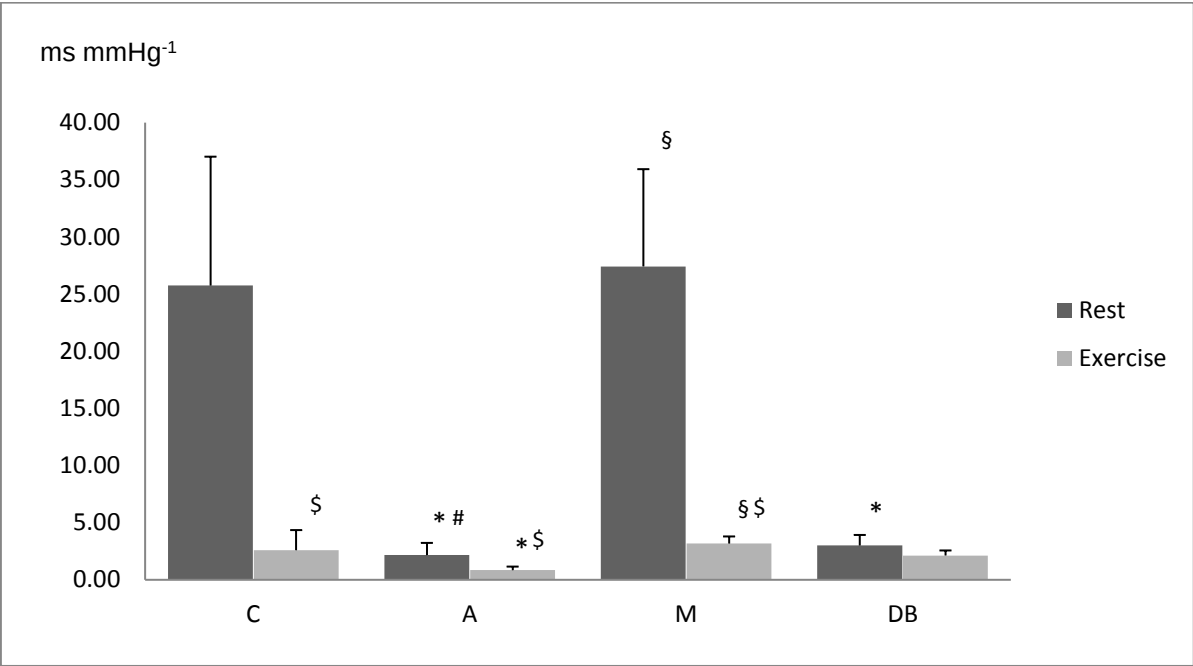
Table 1: Mean steady state values for the cardiovascular variables monitored during rest (R) and exercise (E) in the 4 experimental conditions: control (C), atropine (A), metoprolol (M), and double blockade (DB). HR: heart rate; RR: R-R interval; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; BRS: spontaneous baroreflex sensitivity. *: $p < 0.05$ compared to C. #: $p < 0.05$ compared to DB. §: $p < 0.05$ compared to A. *Italic: Exercise significantly different from Rest*

Measured variables		C	A	M	DB
HR [min^{-1}]	R	62.67±8.47	111.17±17.75*#	59.58±7.11#§	93.71±5.48*
	E	105.04±12.39	135.04±20.56*	93.53±8.17§	103.19±8.06§
RR [ms]	R	985.3 ± 185.7	548.6 ± 79.5 *#	1017.7 ± 104.4§	642.1 ± 38.2 *
	E	577.9 ± 66.2	455.9 ± 89.3*	645.3 ± 51.3§	584.2 ± 41.1§
SAP [mmHg]	R	111.97±9.52	112.96±11.83	109.75±13.89	119.48±14.29
	E	138.51±17.53	108.73±15.94*	113.58±15.21*	107.70±14.76*
DAP [mmHg]	R	54.95±9.64	60.95±9.10	48.96±10.81#	66.16±8.43
	E	60.94±7.48	54.21±7.72	53.35±13.55	54.34±6.92
MAP [mmHg]	R	73.95±8.59	78.28±7.76	69.22±10.42#	83.93±7.78
	E	86.79±9.88	72.58±10.03	73.42±13.53	72.13±9.41
BRS [ms mmHg^{-1}]	R	25.74 ± 11.28	2.17 ± 1.06 *#	27.42 ± 8.51§	3.00 ± 0.92 *
	E	2.59 ± 1.76	0.85 ± 0.31*	3.17 ± 0.62 §	2.13 ± 0.44

Table 2: Mean and standard deviations of all parameters calculated by means of heart rate variability, and of the spontaneous variability of systolic arterial pressure, diastolic arterial pressure, at steady state, in supine position and at every condition (control/atropine/metoprolol/double blockade). C: Control. A: Atropine. M: Metoprolol; DB: Double blockade. HRV: heart rate variability. SAP: systolic arterial pressure. DAP: diastolic arterial pressure. P_{TOT} : total power. LF: low frequency. HF: high frequency. LF/HF: low and high frequency ratio. R: Rest. E: Exercise*: significantly different from C. # significantly different from DB. §: significantly different from A. *Italic*: Exercise significantly different from Rest

HRV		C	A	M	DB
P_{TOT} [ms ² Hz ⁻¹]	R	6351.4 ± 4476.4	22.5 ± 13.8*	7883.2 ± 5965.9 ^{#§}	12.9 ± 4.9*
	E	185.42 ± 77.14	10.10 ± 3.25*	93.6 ± 30.9 ^{*#§}	14.8 ± 4.7*
LF [ms ² Hz ⁻¹]	R	1717.5 ± 1290.6	1.45 ± 1.19*	2711.9 ± 2061.8 ^{#§}	1.07 ± 0.46*
	E	40.6 ± 29.3	1.72 ± 1.38*	41.3 ± 29.3 ^{#§}	1.59 ± 1.49*
HF [ms ² Hz ⁻¹]	R	1441.0 ± 1296.1	0.85 ± 0.55	2552.3 ± 2245.0 ^{#§}	2.57 ± 0.75
	E	10.8 ± 7.8	0.3 ± 0.13*	11.2 ± 9.2 ^{#§}	3.12 ± 1.55
LF/HF	R	1.43 ± 1.01	1.81 ± 0.85 [#]	1.38 ± 0.77	0.50 ± 0.35
	E	4.05 ± 2.03	3.98 ± 2.61 [#]	3.97 ± 1.97 [#]	0.26 ± 0.15*
LFnu [%]	R	46.8 ± 19.3	57.7 ± 28.2 [#]	46.1 ± 14.7	25.9 ± 13.8
	E	69.6 ± 16.5	61.6 ± 22.7 [#]	65.3 ± 21.2 [#]	12.7 ± 8.0*
HFnu [%]	R	51.1 ± 18.3	38.3 ± 26.9	51.1 ± 15.4	62.6 ± 15.6
	E	15.5 ± 8.7	22.6 ± 14.4 [#]	17.41 ± 5.0 [#]	45.5 ± 23.5*
SAP variability		C	A	M	DB
P_{TOT} [ms ² Hz ⁻¹]	R	25.70 ± 11.52	16.99 ± 17.17	26.91 ± 15.50	15.63 ± 8.19
	E	70.83 ± 41.42	28.09 ± 6.77*	29.07 ± 12.24*	17.46 ± 7.00*
LF [ms ² Hz ⁻¹]	R	7.03 ± 3.60	1.55 ± 0.64*	4.96 ± 1.90 [§]	2.09 ± 1.38*
	E	18.68 ± 17.97	10.93 ± 6.15	5.51 ± 1.52	5.8 ± 2.9
HF [ms ² Hz ⁻¹]	R	4.04 ± 3.21	1.20 ± 0.45*	2.57 ± 1.79	1.09 ± 0.67*
	E	5.49 ± 4.2	3.29 ± 1.80	5.48 ± 3.96	2.48 ± 0.93
LF/HF	R	2.27 ± 1.07	1.46 ± 0.70	2.36 ± 1.09	2.32 ± 1.54
	E	2.61 ± 1.39	3.46 ± 2.39	1.87 ± 1.32	2.17 ± 0.67
DAP variability		C	A	M	DB
P_{TOT} [ms ² Hz ⁻¹]	R	9.65 ± 6.06	4.64 ± 3.03	9.01 ± 3.47	5.10 ± 2.32
	E	7.63 ± 2.56	3.92 ± 1.00	5.52 ± 2.56	4.90 ± 3.40
LF [ms ² Hz ⁻¹]	R	3.54 ± 2.57	0.77 ± 0.52*	2.56 ± 1.17	0.97 ± 0.67*
	E	2.70 ± 1.8	1.63 ± 0.48	1.97 ± 0.86	1.11 ± 0.31
HF [ms ² Hz ⁻¹]	R	2.22 ± 2.77	0.40 ± 0.20	1.88 ± 2.10	0.46 ± 0.52
	E	1.66 ± 1.25	0.89 ± 0.52	1.13 ± 0.75	0.92 ± 0.5
LF/HF	R	3.65 ± 1.26	2.03 ± 1.26*	2.40 ± 1.21*	3.03 ± 1.48*
	E	3 ± 2.53	2.05 ± 0.73	1.69 ± 0.98	1.53 ± 0.63

Figure 1



5.3.3. Conclusion

Après l'influence des afférences pulmonaires sur la composante HF du spectre de puissance de la VFC, mise en évidence dans le deuxième article, nous mettons en lumière, avec ce troisième article, une autre contribution, indépendante de la première. En effet, les blocages pharmacologiques ont confirmé notre hypothèse de départ voulant que les influx nerveux sous régulation vagale soient bien les principaux déterminants de la variabilité de fréquence cardiaque. La suppression quasi complète de la VFC lors du blocage vagal et l'absence de modification observée sous blocage sympathique confirment cela. La presque totale suppression similaire observée sous double blocage pousse à croire que ce résultat est uniquement dû à la suppression des modulations parasympathiques.

Les différences observées entre le repos et l'exercice confirment que la chute de VFC observée au début de l'exercice n'est due qu'au retrait de l'influx parasympathique, sans influence sympathique. Malgré la chute des influx vagues, les valeurs en condition contrôle restent plus élevées que lors du blocage complet des afférences nerveuses au cœur durant le double blocage. Ces résultats montrent que le retrait du vague en début d'exercice n'est pas total, laissant une faible, mais toujours existante, part de régulation vagale atteindre le cœur.

En normalisant les valeurs de composante LF et HF de la VFC, on observe des résultats plus hétérogènes au sein du groupe des sept sujets. D'une part, le ratio LF/HF n'est pas significativement modifié par les substances pharmacologiques par rapport à la situation contrôle (à l'exception du double

blocage à l'exercice). Par contre, HFnu étant plus bas à l'exercice lors des situations contrôle et de blocage sympathique, le ratio LF/HF augmente significativement aussi à l'exercice dans ces conditions. Ceci pourrait indiquer que la composante HF est plus sensible au retrait du tonus vagal à l'exercice que la composante LF.

Concernant la pression artérielle, une absence d'effet des substances pharmacologiques est claire au repos. Cela s'explique aisément : vu que la branche vagale affecte uniquement les fonctions cardiovasculaires centrales et que le blocage sympathique est cardio-sélectif (bloquant ainsi les récepteurs adrénergiques β_1 uniquement au niveau du cœur), on ne constate pas d'effet sur la régulation vasculaire. Les différences de variabilité de pression sont intéressantes entre le repos et l'exercice. À l'exercice, la composante LF de la pression artérielle systolique augmente uniquement en condition contrôle et sous atropine. Cela nous indique que cette élévation est probablement due à l'augmentation de la régulation sympathique à l'exercice. La variabilité de la pression artérielle diastolique n'étant pas affectée par les administrations pharmacologiques, les changements observés pour la pression systolique seraient induits par une régulation centrale du sympathique. La composante HF de la pression systolique ne changeant pas, cette dernière semble donc être indépendante de l'activité du système nerveux autonome.

Les résultats obtenus pour le BRS au repos vont dans le sens d'une régulation réalisée par la branche parasympathique du SNA (Airaksinen *et al.*, 1994 ; Wray *et al.*, 2001). La réduction des valeurs observées à l'exercice, uniquement en condition contrôle et sous metoprolol, est volontiers attribuée au retrait du tonus vagal en début d'exercice (Ogoh *et al.*, 2005). En effet, la branche

vagale étant bloquée sous atropine et double blocage, nous ne pouvons voir de réduction à l'exercice dans ces conditions.

5.4. Article IV

Dynamics of the RR-interval versus blood pressure relationship at exercise onset in humans

5.4.1. Introduction

Les articles présentés jusqu'ici traitent de conditions dites stables, avec des mesures réalisées après plusieurs minutes de stabilisation des variables physiologiques. La modification de ces variables durant les phases de transition entre deux états stables apportant aussi des informations utiles à une meilleure compréhension des phénomènes de régulation de variabilité cardiaque et de sensibilité du baroréflexe, réaliser une étude sur le sujet prend tout son sens dans le cadre de ce travail de thèse.

L'exercice implique une évidente augmentation de la pression artérielle. En réaction à cette élévation, les mécanismes de régulation du baroréflexe cherchent à diminuer la fréquence cardiaque afin de réguler et de maintenir une pression artérielle constante. Or, lors de l'exercice, le corps a nécessairement besoin d'une augmentation du débit cardiaque afin de combler les besoins croissants en oxygène. Le corps se retrouve face à un dilemme, qu'il résout grâce à ce qui fut défini comme un processus de réinitialisation du baroréflexe (Heesch and Carey, 1987 ; Potts *et al.*, 1993 ; Rowell *et al.*, 1996) permettant de déplacer l'« operating point », défini comme étant la fréquence cardiaque et la pression artérielle dominantes avant l'introduction d'un stimulus (Kent *et al.*, 1972). La littérature démontre que la commande centrale et les réflexes de pression à l'exercice ont été proposés comme mécanismes permettant d'expliquer ce phénomène de réinitialisation du baroréflexe (Gallagher *et al.*, 2001, 2006 ; McIlveen *et al.*, 2001 ; Mitchell, 2013; Ogoh *et al.*, 2002 ; Querry *et al.*, 2001 ; Raven *et al.*, 1997 ; Smith *et al.*, 2003). Cependant, les relations entre ces deux mécanismes sont inconnues. C'est donc le but de cet article qui, par méthode séquentielle (Bertinieri *et al.*, 1988), une méthode en boucle fermée, a cherché à

analyser le déplacement de cet « operating point » du baroréflexe en début d'exercice et à en expliquer les mécanismes sous-jacents.

L'utilisation de la méthode séquentielle est habituellement réalisée dans des conditions stables (Di Rienzo *et al.* 2008 ; Ferretti *et al.* 2009 ; Iellamo *et al.* 1994, 1997, 2002). Cependant, ces dernières ne sont pas un prérequis et l'application de cette méthode dans des conditions dynamiques telles que la phase de transition en début d'exercice est tout à fait possible. Cette approche est inédite dans ce cadre de recherche particulier.

5.4.2. Manuscrit

Dynamics of the RR-interval versus blood pressure relationship at exercise onset in humans

Aurélien Bringard, Alessandra Adami, Nazzareno Fagoni, Timothée Fontolliet, Frédéric Lador, Christian Moia, Enrico Tam, Guido Ferretti

Accepté dans:

European Journal of Applied Physiology, janvier 2017.

Dynamics of the RR-interval versus blood pressure relationship at exercise onset in humans

Aurélien Bringard^{1,2} · Alessandra Adami^{2,3} · Nazzareno Fagoni⁴ ·
Timothée Fontollet^{1,2} · Frédéric Lador⁵ · Christian Moia^{1,2} · Enrico Tam⁶ ·
Guido Ferretti^{1,2,4} 

Received: 17 November 2016 / Accepted: 7 February 2017
© Springer-Verlag Berlin Heidelberg 2017

Abstract

Purpose The dynamics of the postulated phenomenon of exercise baroreflex resetting is poorly understood, but can be investigated using closed-loop procedures. To shed light on some mechanisms and temporal relationships participating in the resetting process, we studied the time course of the relationship between the R–R interval (RRi) and arterial pressure with a closed-loop approach.

Methods On ten young volunteers at rest and during light exercise in supine and upright position, we continuously determined, on single-beat basis, RRi

(electrocardiography), and arterial pressure (non-invasive finger pressure cuff). From pulse pressure profiles, we determined cardiac output (CO) by Modelflow, computed mean arterial pressure (MAP), and calculated total peripheral resistance (TPR).

Results At exercise start, RRi was lower than in quiet rest. As exercise started, MAP fell to a minimum (MAP_m) of 72.8 ± 9.6 mmHg upright and 73.9 ± 6.2 supine, while RRi dropped. The initial RRi versus MAP relationship was linear, with flatter slope than resting baroreflex sensitivity, in both postures. TPR fell and CO increased. After MAP_m, RRi and MAP varied in opposite direction toward exercise steady state, with further CO increase.

Conclusion These results suggest that, initially, the MAP fall was corrected by a RRi reduction along a baroreflex curve, with lower sensitivity than at rest, but eventually in the same pressure range as at rest. After attainment of MAP_m, a second phase started, where the postulated baroreflex resetting might have occurred. In conclusion, the change in baroreflex sensitivity and the resetting process are distinct phenomena, under different control systems.

Communicated by Massimo Pagani.

✉ Guido Ferretti
Guido.Ferretti@unige.ch

¹ Département d'Anesthésiologie, Pharmacologie et Soins Intensifs, Hôpitaux Universitaires de Genève, 4 rue Gabrielle-Perret-Gentil, CH-1211 Genève 4, Switzerland

² Département des Neurosciences Fondamentales, Université de Genève, 1 rue Michel Servet, CH-1211 Genève 4, Switzerland

³ Division of Respiratory and Critical Care Physiology and Medicine, Rehabilitation Clinical Trials Center, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 W Carson St, Torrance, CA 90502, USA

⁴ Dipartimento di Medicina Molecolare e Traslazionale, Università di Brescia, Viale Europa 11, 25123 Brescia, Italy

⁵ Service de Pneumologie, Programme Hypertension Pulmonaire, Département des Spécialités de Médecine, Hôpitaux Universitaires de Genève, 4 rue Gabrielle-Perret-Gentil, CH-1211 Genève, Switzerland

⁶ Dipartimento di Scienze Neurologiche, Biomediche e del Movimento, Università di Verona, Via Felice Casorati 43, 37131 Verona, Italy

Keywords Mean arterial pressure · Heart rate · Baroreflex resetting · Baroreflex sensitivity · Exercise transients · Peripheral resistance

Abbreviations

BP	Blood pressure
BRS	Baroreflex sensitivity
CO	Cardiac output
DAP	Diastolic arterial pressure
HR	Heart rate
MAP	Mean arterial pressure
MAP _m	Minimum of mean arterial pressure upon exercise onset

RRi	R–R interval
SAP	Systolic arterial pressure
SAP _m	Minimum of systolic arterial pressure upon exercise onset
SV	Stroke volume
TPR	Total peripheral resistance

Introduction

Of the three effector functions of the arterial baroreflex system (cardiac–chronotropic, cardiac–inotropic, and vascular), only the first, and most easily accessible, has widely been studied, whether with open-loop or with closed-loop methods. Open-loop methods implied modifying the carotid distending pressure either mechanically or pharmacologically, and recording the ensuing heart rate (HR) or blood pressure (BP) responses as dependent variables (Eckberg and Sleight 1992). The classical arterial baroreflex curve, which Kent et al. (1972) treated with a logistic model, was derived from open-loop studies.

Kent's model identified several parameters describing the baroreflex curve, including the operating point, defined as the prevailing BP and HR before the introduction of an acute stimulus, and the maximal baroreflex gain, defined as the gain value at the centring point of a baroreflex curve (see Raven et al. 2006 for details). Open-loop studies in animals (Heesch and Carey 1987), then also in humans (DiCarlo and Bishop 2001; Fadel and Raven 2012; Mitchell 2013; Potts et al. 1993; Raven 2008; Raven et al. 2002, 2006; Rowell et al. 1996), showed that the baroreflex operating point can move over a wide range of BP and HR values: at exercise, it is displaced upward and rightward with respect to rest, normally without changes in maximal gain. These results led to the formulation of the *baroreflex resetting* hypothesis (Heesch and Carey 1987; Potts et al. 1993; Rowell et al. 1996). Central command and exercise pressor reflexes were proposed as possible mechanisms behind baroreflex resetting (Gallagher et al. 2001, 2006; McIlveen et al. 2001; Mitchell 2013; Ogoh et al. 2002; Querry et al. 2001; Raven et al. 1997; Smith et al. 2003). These mechanisms are not mutually exclusive.

A study of the kinetics of displacement of the baroreflex curve, or at least of its operating point, upon exercise onset is needed to understand the possible interrelationship between the central command and the exercise pressor reflex hypotheses. This analysis may be done only by applying closed-loop methods, which rely on a continuous beat-by-beat follow-up of the HR and BP changes, thereby assuming the possibility of a counter effect of HR on BP. The most classical closed-loop method, i.e., the sequence method (Bertinieri et al. 1988), was developed after prolonged steady-state recordings, and was generally applied

at steady state (restricting citations to physiology, see Di Rienzo et al. 2008; Ferretti et al. 2009; Iellamo et al. 1994, 1997, 2002; Legramante et al. 1999; Mancina et al. 1997; O'Leary et al. 2003; Steinback et al. 2005; Vallais et al. 2009). Due to simplicity in analysis, SAP instead of MAP was taken as pressure input.

Notwithstanding, steady state is not a prerequisite of the sequence method for the computation of BRS (Bertinieri et al. 1988). Admitting a counter effect of HR on BP implies the possibility of continuous control loops on single-beat basis, so that nothing prevents from using a closed-loop approach in dynamic conditions (unsteady states), as Iellamo et al. (1998) did during incremental exercise (2-min duration steps, sequence method). A closed-loop analysis of baroreflex dynamics in unsteady state was performed also during the reambulation procedure at the end of prolonged bed rest (Adami et al. 2013) and during the initial phase of dry apnoeas at rest and exercise in air (Sivieri et al. 2015) and in oxygen (Fagoni et al. 2015). This approach, however, was never applied to the study of the classical exercise transients yet.

The aim of this study was to analyse the dynamics of displacement of the baroreflex operating point upon exercise onset using a closed-loop approach. We investigated the RRi versus BP relationship during exercise transients in a fully aerobic exercise domain, trying to shed light on some mechanisms and temporal relationships that participate in the postulated baroreflex resetting process, with reference to the central command and the exercise pressor reflex hypotheses. Exercise was performed in upright and supine position, to discriminate the effect of a change in the cardiac filling pressure induced by posture, on the hypothesis that the baroreflex operating point in supine posture is displaced with respect to the upright posture, even at rest (Schwartz et al. 2013).

Methods

Subjects

Ten healthy, non-smoking, young volunteers (2 men and 8 women) participated in the experiments. They were 23.8 ± 5.8 years old, 167 ± 8 cm tall, and weighed 58.5 ± 9.1 kg. The volunteers were instructed to take only light meals on the day of the experiments and to refrain from caffeine and other substances stimulating the autonomic nervous system. They also had to refrain from strenuous exercise the day before the tests. Women were admitted to the study independent of the phase of the menstrual cycle, since it was reported that the menstrual cycle does not influence indexes of baroreflex control of heart activity (Cooke et al. 2002; Minson et al. 2000).

All subjects were preliminarily informed about all procedures and risks associated with the experiments. Signed informed consent was obtained from each volunteer, who was aware of the right of withdrawing from the study at any time without jeopardy. The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the institutional ethical committee.

Measurements

HR was continuously measured by electrocardiography (Elmed ETM 2000, Heiligenhaus, Germany). Continuous monitoring of arterial pulse pressure profile was obtained at the middle phalange of a left-arm finger by means of a non-invasive cuff pressure recorder (Portapres, FMS, Amsterdam, The Netherlands). SAP and diastolic blood pressures (DAP) were identified at each beat. Beat-by-beat MAP was computed as the integral mean of each pressure profile, using the Beatscope® software package (FMS, Amsterdam, The Netherlands).

Single-beat stroke volume (SV) was determined by means of the Modelflow method (Wesseling et al. 1993), applied off-line to the pulse pressure profiles, using the Beatscope® software package. Beat-by-beat cardiac output (CO) was computed as the product of single-beat SV times the corresponding single-beat HR. The data were then corrected for method's inaccuracy, as previously described (Azabji Kenfack et al. 2004; Lador et al. 2006; Tam et al. 2004), using steady-state cardiac output values obtained by means of the open circuit acetylene method (Barker et al. 1999) on each subject, after determination of individual partition coefficients for acetylene (Meyer and Scheid 1980). The mean calibration factors were the same at rest (1.01 ± 0.55) as at exercise (1.04 ± 0.36 , $p=0.79$), so that corrected CO values could be and thus were used for beat-by-beat analysis during exercise transients. (van Lieshout et al. 2003).

All the signals were digitalized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta, CA, USA) and stored on a computer. The acquisition rate was 200 Hz.

Protocol

In both postures, exercise was performed on an electrically braked cycle ergometer (Ergoselect 400, Ergoline GmbH, Bitz, Germany). After performance of the open circuit acetylene CO determination at rest, and after further 3 min of quiet resting recordings, the subject performed a series of three exercises at 50 W, the first lasting 7 min, and the other two 5 min. The steady-state CO values by the open circuit acetylene method were obtained during the first exercise, after the end of the fifth minute of exercise. Subsequent

exercises were separated by a 6-min recovery. The protocol was performed twice, once in upright, and once in supine position, administered in random order. The subjects pedalled at a spontaneously selected frequency comprised between 60 and 70 rpm; they maintained their own selected frequency throughout the study.

Caution was taken to avoid pre-exercise stress. After rest monitoring, participants received the following communication "We are ready: you can start whenever you want." If the participant delayed the exercise onset by more than 10 s after communication, the procedure was interrupted and started again after further 2 min at rest. During off-line data processing, the exact time of exercise start was detected from the pedalling frequency recording.

Data treatment

Individual beat-by-beat values of HR, RRi, SAP, MAP, and CO values from the three repetitions were time aligned, by setting the time of exercise onset as analysis time zero. Then, they were linearly interpolated on a 0.1 s basis (10 Hz) and averaged to obtain a single superimposed time series for each parameter, using Matlab (version 7.9.0, MathWorks, Natick, MA, USA). This analysis was not performed beyond fifth minute of exercise. The individual total peripheral resistance (TPR) was calculated from these averaged time series, by dividing each MAP value by the corresponding CO value, on the assumption that the pressure in the right atrium can be neglected as a determinant of peripheral resistance in healthy individuals (Faisal et al. 2010; Lador et al. 2006).

Rest and exercise values at steady state for each investigated variable were calculated as the mean during the last minute of rest (from -60 to 0 s) and during the last minute of exercise (from 240 to 300 s of exercise), respectively.

At rest and exercise steady states, we also computed BRS by means of the sequence method (Bertinieri et al. 1988), using both SAP and MAP as independent variables. Briefly, sequences of three or more consecutive beats in which SAP (or MAP) and RRi changed in the same direction, either increasing or decreasing, were identified. A phase shift of one beat was introduced between the SAP and the RRi values of each sequence (Bertinieri et al. 1988). Within each individual sequence, the RRi versus MAP (or SAP) relationship was analysed by linear regression, to compute the slope and the corresponding coefficient of determination (r^2). Only slopes showing r^2 values higher than 0.85 were retained (Iellamo et al. 1994, 1997). For each subject, the mean slope of the RRi versus MAP or SAP relationships was then computed and taken as a measure of individual BRS, at rest and at exercise, respectively. Baroreflex sequences were searched during the 2 min of quiet rest (from -120 to -10 s); at exercise,

baroreflex sequences were identified during exercise min 3-to-5. In each subject and condition, the number of analysed sequences ranged between 1 and 17.

Assuming closed-loop condition, the relationship between RRi and either MAP or SAP in the early phase of exercise was constructed. On this relationship, the minimum of MAP (MAP_m) or SAP (SAP_m) was identified. The segment of this relationship between the exercise onset and the attainment of MAP_m or SAP_m was linear and had a positive slope, being characterised by consensual variations of MAP (or SAP) and RRi. The slope of this segment, which consisted of 8-to-13 consecutive beats, was computed by linear regression. The resulting slopes were taken as representative of the spontaneous baroreflex gain in the early phase of exercise, and were compared with the BRS obtained at rest and at exercise steady state by means of the sequence method.

Statistics

Data are given as mean and standard deviation of the values obtained for each variable from the average superimposed files of each subject, to account for inter-individual variability. The cardiovascular values at the time instant of exercise onset ($t=0$ s) and at the instant of MAP_m attainment were compared with the corresponding mean values at rest and at exercise steady state, using two-way ANOVA for repeated measures, for time (four conditions) and posture (upright versus supine), with Tukey *post-hoc* test. The slopes of the linear segment of the RRi versus MAP or SAP relationships at the beginning of exercise were compared with MAP-based or SAP-based BRS values, respectively, at rest and exercise, using two-way ANOVA for repeated measures, for condition (BRS at rest, slope of the linear segment after exercise start, and BRS at exercise steady state) and posture (upright versus supine), either for RRi/MAP or for RRi/SAP relationship, with Tukey *post-hoc* test. The MAP-based BRS values were compared with the SAP-based BRS values at rest and exercise, using three-way ANOVA for repeated measures, for condition (rest and exercise steady state), method (MAP-based versus SAP-based BRS) and posture (upright versus supine), with Tukey *post-hoc* test. The slopes of the linear segment of the RRi versus MAP or SAP relationships at the beginning of exercise were compared using two-way ANOVA for repeated measures, for posture (upright versus supine) and pressure (SAP versus MAP), with Tukey *post-hoc* test. The differences were considered significant if $p < 0.05$.

The linear regression parameters were calculated by the least squares method, using a function implemented under Matlab (version 7.9.0, MathWorks, Natick, MA, USA). Linear regression was performed using a robust weighting algorithm (fitlm Matlab function with robust option)

that uses iteratively reweighted least squares with bisquare weighting function. Since the conventional least-square linear regression models are based on the assumption of a normal distribution of error, the robust weighting algorithm was originally proposed as being poorly influenced by outliers, compared to the conventional least-square linear regression fit. The r^2 of the linear regressions were obtained by the fitlm Matlab function.

Results

The values obtained at steady state are reported in Table 1. HR, SV, and CO were higher at exercise than at rest in all conditions. SV was higher in supine than in upright posture, both at rest and at exercise steady-state. HR was lower in supine than in upright posture at rest, but not at exercise steady-state. As a consequence, resting CO did not differ significantly between postures, despite a tendency to be higher supine than upright; conversely, at exercise, CO turned out to be higher supine than upright. In both postures, steady-state MAP was higher and TPR was lower at exercise than at rest. Resting TPR was lower supine than upright. No differences either in MAP or in TPR were observed between postures at exercise steady-state.

An example of individual RR versus MAP (upper panels) or SAP (lower panel) sequences at rest and exercise steady states in the two postures is shown in Fig. 1, along with the segment representing the mean BRS in each condition. At exercise with respect to rest, the sequences are not only displaced rightward, but also characterised by a lower slope (lower BRS). It is of note that their slope was the same for increasing as for decreasing sequences. The BRS values obtained with the sequence method at rest and exercise steady states, along with the corresponding number of analysed sequences, are reported in Table 2. Both SAP-based and MAP-based BRS values differed significantly between rest and exercise. At rest, a significant difference between postures was also observed; this was not the case at exercise. The BRS at rest differed when computed using MAP instead of SAP in supine, but not in upright posture; at exercise, no difference between MAP-based and SAP-based BRS was observed, whatever the posture. The number of eligible sequences, however, was systematically smaller with MAP.

An example of the time courses of the recorded and calculated variables upon exercise onset is shown in Fig. 2. At the time instant of exercise start ($t=0$ s), in both postures, the single-beat MAP and SAP values were definitely equal to the mean resting steady-state values ($+2.9 \pm 4.7$ mmHg upright, and $+1.7 \pm 3.8$ mmHg supine for MAP; $+2.9 \pm 5.2$ mmHg upright, and $+1.7 \pm 5.3$ mmHg supine for SAP; NS with respect to the mean value at

Table 1 Steady-state values of cardiopulmonary parameters in quiet rest (Rest), at start of exercise (at $t=0$ s), at the time instant of the attainment of the minimum in mean blood pressure (at MAP_m), and at exercise steady-state (Exercise), in upright and supine posture

	Upright				Supine			
	Rest	At $t=0$ s	At MAP_m	Exercise	Rest	At $t=0$ s	At MAP_m	Exercise
HR (min^{-1})	88.6 ± 15.5	94.3 ± 12.7^a	$108.8 \pm 10.9^{a,b}$	$116.1 \pm 17.5^{a,b,c}$	73.3 ± 12.8^d	$80.2 \pm 11.3^{a,b,d}$	$92.4 \pm 10.3^{a,b,d}$	$112.2 \pm 15.0^{a,b,c}$
SV (ml)	57.9 ± 14.0	57.2 ± 12.5	$66.9 \pm 14.5^{a,b}$	$80.8 \pm 15.4^{a,b,c}$	74.9 ± 15.1^d	72.7 ± 14.0^d	77.1 ± 16.6^d	$91.1 \pm 17.4^{a,b,c,d}$
CO (l min^{-1})	5.03 ± 1.14	5.35 ± 1.22	$7.25 \pm 1.67^{a,b}$	$9.17 \pm 1.12^{a,b,c}$	5.40 ± 1.19	5.73 ± 0.95	$7.05 \pm 1.46^{a,b}$	$10.11 \pm 1.89^{a,b,c}$
MAP (mmHg)	86.8 ± 8.5	89.7 ± 12.0	$73.8 \pm 9.5^{a,b}$	93.1 ± 13.1^c	83.2 ± 6.8	84.8 ± 7.3	$73.9 \pm 6.5^{a,b}$	$92.0 \pm 8.9^{a,b,c}$
SAP (mmHg)	110.7 ± 9.0	113.6 ± 11.8	$101.6 \pm 12.1^{a,b}$	$127.0 \pm 13.2^{a,b,c}$	114.1 ± 7.4	115.8 ± 6.0	$107.2 \pm 5.0^{a,b}$	$130.5 \pm 8.9^{a,b,c}$
TPR (mmHg min l^{-1})	18.27 ± 4.29	17.79 ± 4.80	$10.64 \pm 2.00^{a,b}$	$10.34 \pm 1.93^{a,b}$	16.11 ± 3.42	15.23 ± 2.63	$10.87 \pm 2.04^{a,b}$	$9.39 \pm 1.87^{a,b}$

Data are means $\pm$ SD
Since for both postures and all variables, rest data were significantly different from the exercise steady-state in the same posture; for the sake of clarity, the corresponding symbols have not been represented
^aSignificantly different from the value at rest in the same posture
^bSignificantly different from the value at $t=0$ s in the same posture
^cSignificantly different from the value at MAP_m in the same posture
^dSignificantly different from the corresponding value in upright posture

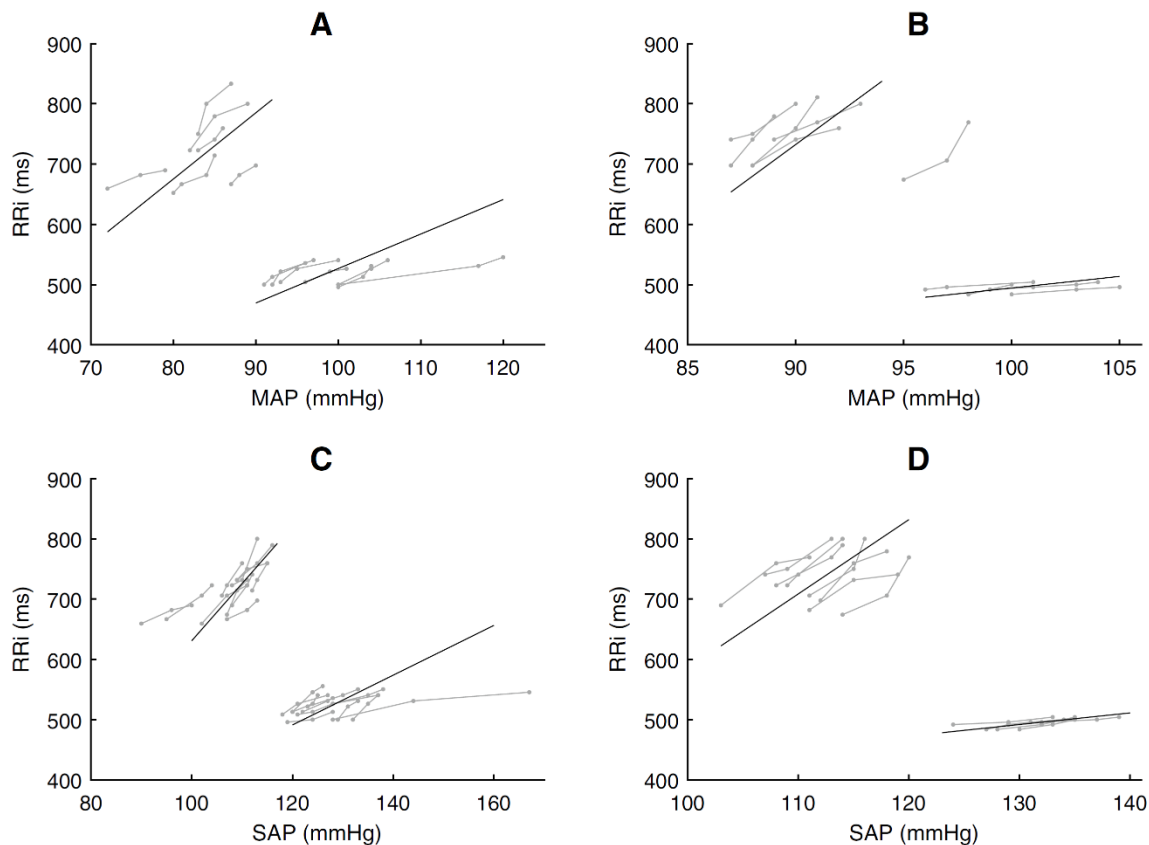


Fig. 1 Graphical representation for one subject of the series of at least three consecutive beats (grey dots connected by grey lines) in which RR-intervals (RRi) and either the mean (MAP) or the systolic (SAP) arterial pressure varied consensually, at rest (upper left region of each panel) and at exercise steady state (lower right region of each panel). Data are presented for RRi–MAP relationship in upright (A) and supine (B) positions, and RRi–SAP relationship in upright (C) and supine (D) positions. Black lines represent the averaged slopes of all linear regressions, calculated over each sequence

Table 2 Steady-state values of cardiopulmonary BRS slopes at rest (BRS_rest) and at exercise steady state (BRS_exercise) with the slope of the linear segment at the beginning of exercise (Initial_slope), in upright and supine postures

	Upright			Supine		
	BRS_rest	Initial_slope	BRS_exercise	BRS_rest	Initial_slope	BRS_exercise
RRi/MAP						
Slope (ms mmHg ⁻¹)	12.42 ± 5.22	3.82 ± 1.25 ^a	4.74 ± 3.11 ^{a,b}	32.13 ± 11.02 ^a	8.94 ± 4.53 ^b	4.85 ± 2.01 ^{a,b}
Number of sequences	9.0 ± 4.7		4.9 ± 4.1	4.4 ± 4.2		3.6 ± 2.2
RRi/SAP						
Slope (ms mmHg ⁻¹)	10.44 ± 7.10	3.80 ± 1.79 ^a	3.47 ± 2.51 ^{a,b}	21.83 ± 11.04 ^a	7.68 ± 5.14 ^b	3.55 ± 1.81 ^{a,b}
Number of sequences	13.5 ± 4.2		6.4 ± 5.8	7.2 ± 4.7		4.4 ± 2.2

Data are means ± SD, $N=10$ for the slopes. The number of sequences represents the number of individual sequences detected and from which the regression was calculated and used for calculation of the individual BRS slope

^aSignificantly different from the value at rest in upright posture

^bSignificantly different from the value at rest in the supine posture

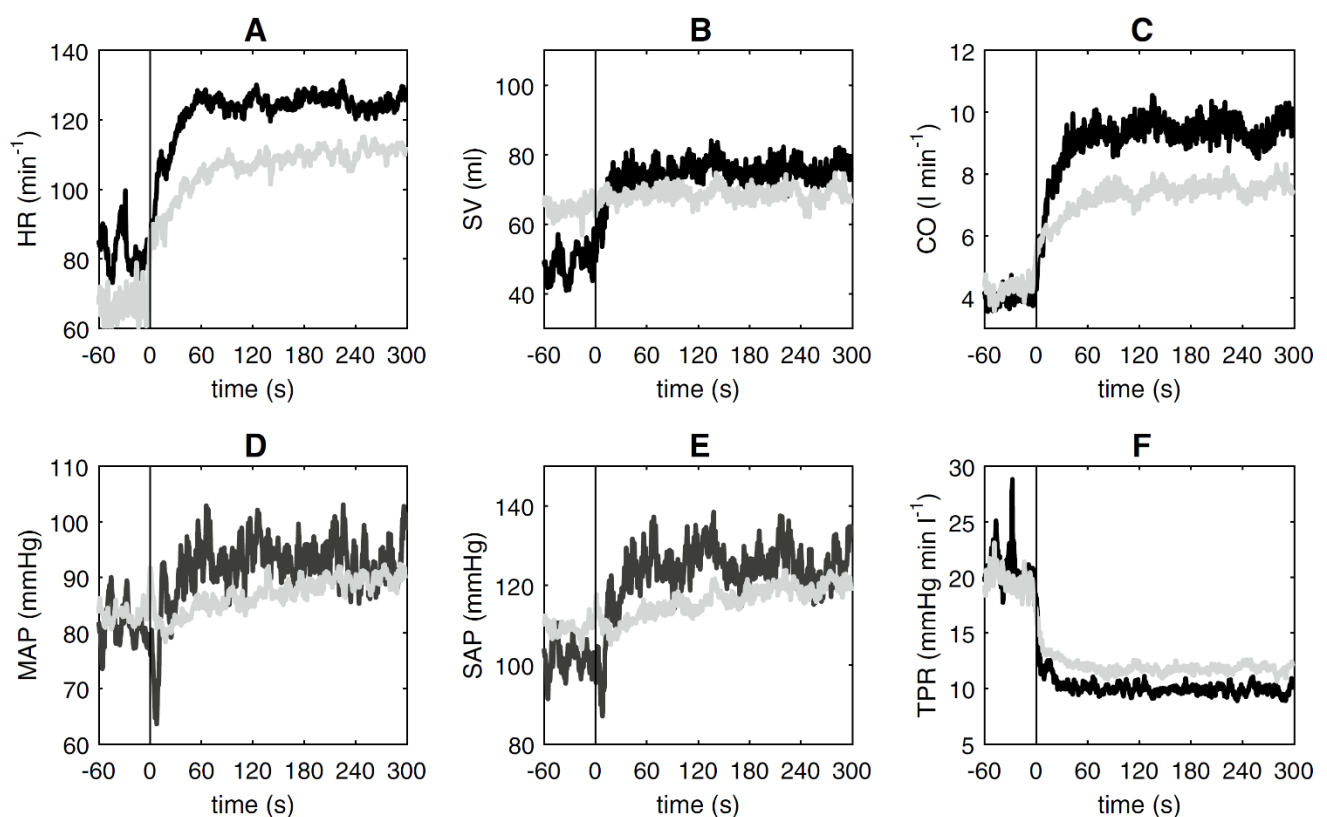


Fig. 2 Representative example of heart rate (HR, **A**), stroke volume (SV, **B**), cardiac output (CO, **C**), mean arterial pressure (MAP, **D**), systolic arterial pressure (SAP, **E**), and total peripheral resistance

(TPR, **F**) responses upon exercise onset, for upright (grey line) and supine (black line) positions. Vertical lines identify the exercise start

rest for both MAP and SAP). In spite of this, the single-beat HR value at exercise start was higher than the corresponding mean value at quiet rest in both postures ($+5.7 \pm 6.7 \text{ min}^{-1}$ upright, $p=0.023$, and $+7.0 \pm 5.8 \text{ min}^{-1}$ supine, $p=0.003$). As exercise started, HR, SV, and CO increased to reach a new steady state within 60-to-120 s.

MAP was characterised by an initial rapid drop, which led to MAP_m within $10.6 \pm 5.6 \text{ s}$ upright and $12.3 \pm 7.3 \text{ s}$ supine (NS). TPR decreased rapidly at exercise onset, toward a new steady-state value. In both postures, MAP_m and SAP_m were significantly lower than the MAP and SAP values at $t=0 \text{ s}$, as was the corresponding TPR value. Subsequently,

MAP increased slowly and progressively toward a new steady state. This increase in MAP was opposite to the corresponding decrease in RRI (i.e., consensual with the corresponding increase in HR). These patterns were followed by all volunteers, whether in supine or in upright posture.

A contour plot showing the evolution of beat-by-beat RRI as a function of beat-by-beat MAP or SAP over the last 10 s of rest and the first minute of exercise is presented in Fig. 3. These relationships are characterised by two distinct phases. The initial segment, comprised between the exercise onset and the attainment of MAP_m or SAP_m , was characterised by a series of consecutive beats in which the RRI and the MAP or SAP varied consensually, as when BRS is computed with the sequence method. The slope of the RRI versus MAP relationship within these segments was equal to $3.82 \pm 1.25 \text{ ms mmHg}^{-1}$ upright and $8.94 \pm 4.53 \text{ ms mmHg}^{-1}$ supine ($p < 0.05$ between postures). The corresponding r^2 was 0.80 ± 0.16 upright and 0.71 ± 0.27 supine. The slopes of the RRI versus MAP relationships obtained upon exercise onset were lower than the BRS obtained at rest ($p < 0.05$), but equal to the exercise BRS, in both postures.

In the first seconds of exercise, the changes in SAP were smaller than the changes in MAP. In the time interval between the exercise start and the attainment of MAP_m , the sequences of consecutive heart beats representing consensual changes in RRI and SAP were shorter than those obtained using MAP instead of SAP. The slopes of the RRI

versus SAP relationship within these segments were equal to $3.80 \pm 1.79 \text{ ms mmHg}^{-1}$ upright and $7.68 \pm 5.14 \text{ ms mmHg}^{-1}$ supine ($p < 0.05$ between postures; NS with respect to the corresponding RRI versus MAP relationship). The r^2 of these relationships were equal to 0.71 ± 0.16 upright and 0.64 ± 0.22 supine. Both slopes were significantly lower than the corresponding SAP-based BRS in the same posture at rest. None of these slopes differed from the corresponding SAP-based BRS at exercise steady state.

After the attainment of MAP_m or SAP_m , RRI kept decreasing (further increase in HR), whereas MAP and SAP suddenly started to increase, and the relationship pointed to the exercise steady-state values along complex patterns. Some differences between postures yet appear, as long as in upright posture, the pattern followed a large loop, whereas in supine posture, the way to steady state was more straightforward. The RRI (and HR) and MAP or SAP values at the first minute of exercise were equal to the corresponding exercise steady-state value upright, but they were lower supine. An analogous contour plot showing the evolution of beat-by-beat MAP as a function of beat-by-beat CO over the last 10 s of rest and the first minute of exercise is presented in Fig. 4. This relationship also showed two distinct phases. In both postures, the portion comprised between the exercise onset and the attainment of MAP_m was negative, implying a decrease of MAP with increasing CO during the first seconds of exercise and a remarkable decrease in TPR. After the attainment of MAP_m , in supine

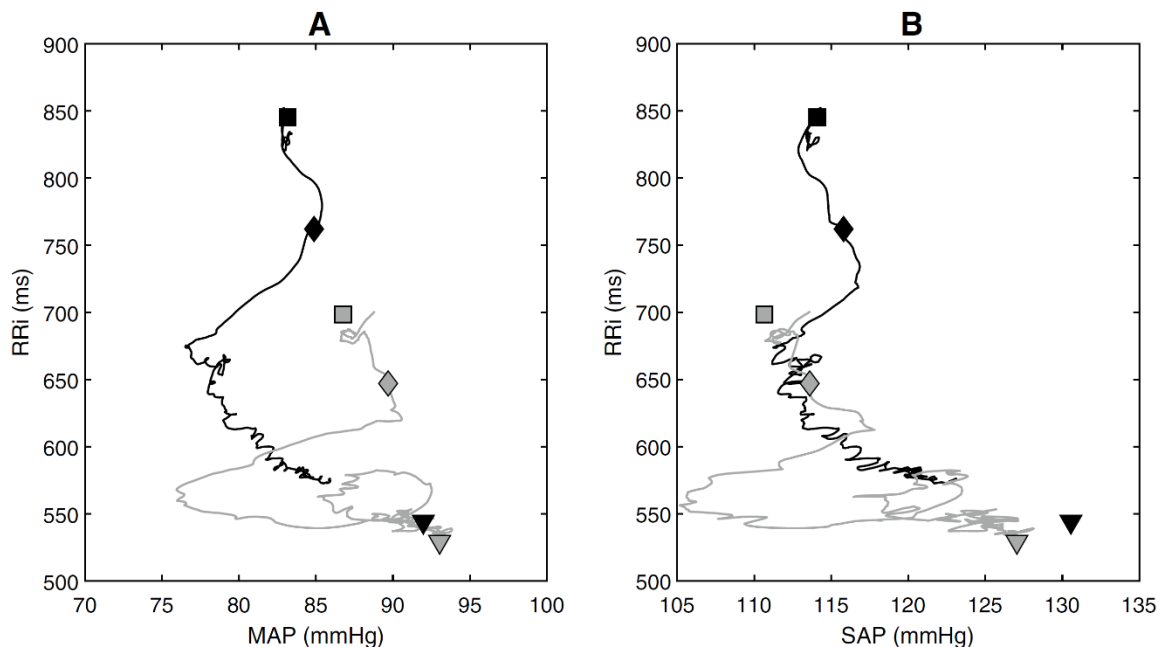


Fig. 3 Contour plots describing the relationship between RR-intervals (RRI) and mean arterial pressure (MAP, **A**), or RRI and systolic arterial pressure (SAP, **B**), during the exercise transient for the upright (grey line) and supine (black line) positions. The continuous

lines represent the group averaged data for the last 10 s of rest and the first min of exercise. Diamonds represent the values at the exercise onset. Squares and triangles represent the mean values during quiet rest and at exercise steady-state, respectively, as from Table 1

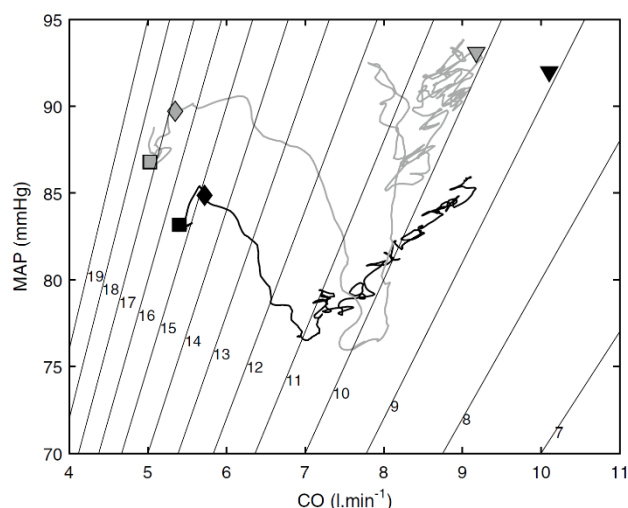


Fig. 4 Contour plot describing the relationship between mean arterial pressure (MAP) and cardiac output (CO) during the exercise transient, for the upright (grey line) and supine (black line) positions. The continuous lines represent the group averaged data for the last 10 s of rest and the first min of exercise. Diamonds represent the combination of MAP and CO values at the exercise onset. Squares and triangles represent the combination of MAP and CO mean values during quiet rest and at exercise steady state, respectively, as from Table 1. The lines are isopleths for TPR, with the corresponding TPR values as labels

posture, CO and MAP increased consensually, pointing to the new steady state: the pattern implied a tendency ($p=0.096$) toward a further decrease in TPR. In upright posture, MAP increased much more than CO, which was already close to its steady state, so that MAP_m and MAP at exercise steady state were on the same TPR isopleth.

Discussion

General considerations

This study reports the first description of the kinetics of displacement of the arterial baroreflex operating point at exercise onset, using a beat-by-beat, non-invasive investigation of the relationship between RRi and BP, in the context of a closed-loop approach to baroreflex analysis. The results indicated that (1) no displacement of arterial baroreflex operating point (baroreflex resetting) was evident at the very exercise start; (2) in the first seconds of exercise, there was a linear positive segment of the RRi versus MAP (or SAP) relationship, which corresponded conceptually—yet including a bigger number of single-beat values—to a sequence of heart beats, as defined by Bertinieri et al. (1988) in resting steady state; (3) the slopes of these segments did not differ from the corresponding BRS at exercise, but significant differences were observed with respect

to rest, suggesting that the change in baroreflex sensitivity occurred since the very exercise start; (4) that linear segment was suddenly interrupted upon the attainment of a minimum value of MAP (MAP_m); (5) the subsequent part of the RRi versus MAP relationship was characterised by an essential progressive decrease in RRi (i.e. increase in HR) associated with a progressive increase in BP, toward the exercise steady state, suggesting that a resetting of the baroreflex operating point, without further changes in baroreflex gain, occurred after the attainment of MAP_m .

The closed-loop approach and the unsteady state

The only a-priori assumption behind the closed-loop approach to the study of arterial baroreflexes, as nicely pointed out by Bertinieri et al. (1988), is that each heart beat has a biunivocal effect on the following beat. In the study by Bertinieri et al. (1988), no maximum limit in the length of baroreflex sequences was imposed; moreover, linear regression provided $r^2 > 0.85$ for all analysed sequences, with r^2 values tending to be lower, the larger was the number of beats within the sequence. This being the case, the closed-loop analysis of arterial baroreflexes has no constraints preventing from using it in unsteady state conditions. The only actual constraint is the presence of a minimum of three consecutive heart beats in each sequence.

The relationship between heart beats and BP during exercise is treated as a relationship between RRi and MAP (or SAP), as it is the only approach allowing a direct comparison with rest and steady-state BRS values. The displacement (resetting) of the baroreflex operating point, downward, and leftward on a RRi versus MAP diagram during the exercise transient is independent of the shape and of the values taken by the parameters depicting a baroreflex function. As a consequence, the resetting process would be equally visible whether it is represented by a RRi versus MAP (or SAP) relationship, or by a HR versus MAP curve: under this respect, these two representations are mirror images of each other (see Iellamo et al. 1998).

Bertinieri et al. (1988) used SAP as pressure input for baroreflex analysis: this allowed identification of a single pressure value, corresponding to the peak pressure value within an arterial pressure cycle, and simultaneous to an R peak in an ECG recording. This certainly has a physiological meaning in a steady-state condition at rest, in which TPR is high and invariant, and thus, the changes in MAP are mostly the result of changes in SAP. However, the baroreceptor stimulation is a continuous phenomenon, which is more precisely represented by MAP than by SAP, since MAP corresponds to the mean baroreceptor stimulation pressure within a heart cycle. Moreover, MAP is more importantly affected than SAP by the changes in TPR occurring at the beginning of an exercise. Notwithstanding,

it is noteworthy that SAP, but not MAP, contains information on dP/dt , which is also sensed by the baroreceptors (Eckberg and Sleight 1992). These considerations explain why, in this study, we analysed baroreflexes in the exercise transients using both SAP and MAP as pressure inputs. It is noteworthy, for the sake of future studies, that, despite their different physiological meaning, similar BRS results were found and similar patterns in the pressure—heartbeat relationship were observed with SAP as with MAP.

Blood pressure and heart rate at exercise start

At the single beat corresponding to exercise start, both SAP and MAP kept the same value as in quiet rest. However, HR was higher (RRi lower) than in quiet rest both supine and upright, being lower in the former than in the latter posture. Since no changes in SV were observed, CO did not differ significantly from quiet rest. As a consequence, TPR, calculated as the ratio between MAP and CO, turned out to be the same as at rest.

This brief description shows that the increase in HR preceded the exercise start, compatibly more with the central command theory (Fisher et al. 2015; Legramante et al. 1999; Raven et al. 1997) than with the exercise pressor reflex theory. Within the former theory, the lack of changes in MAP, CO, and TPR suggests that a selective stimulation of the sinus node is the distal component of the feed-forward mechanism. The lack of changes in SV, however, implied that either the intensity of the hypothesized feed-forward stimulation was not strong enough to determine visible inotropic effects, or the decrease in RRi was a consequence of withdrawal of vagal stimulation of the heart.

The linear segment of the HR versus MAP relationship

The BP fall at the beginning of exercise is considered a consequence of the massive muscle vasodilatation that is attributed to the action of a variety of vasoactive substances liberated following the first contraction (Saltin et al. 1998) and leads to a sudden increase in muscle blood flow (Chin et al. 2010; Clifford 2007; DeLorey et al. 2003; Ferretti et al. 1995) and to a dramatic fall of TPR (Elstad et al. 2009; Faisal et al. 2010; Lador et al. 2006, 2008; Wieling et al. 1996). The concomitant sudden decrease in RRi can be interpreted as a baroreflex attempt at correcting the MAP fall. This explains why, in the early phase of exercise, at least between the exercise start and MAP_m , there was a linear positive segment of the RRi versus MAP relationship. The slope of this segment corresponds conceptually—yet including a bigger number of single-beat values—to the BRS computed from a baroreflex sequence, as defined by Bertinieri et al. (1988) in resting steady state. When SAP was used instead of MAP (see Fig. 3), the initial segment

of the RRi versus pressure relationship included a smaller number of heartbeats than if using MAP, and thus, it was less clearly identifiable. This is because SAP is unaffected by TPR, which acts on MAP only through its effects on DAP. This being so, a baroreflex fall of RRi would correct a fall of MAP rather than of SAP. Notwithstanding, the slope of the analysed segments was the same using SAP as using MAP.

The observation, over a series of consecutive beats, of linear MAP (or SAP) relationships in the early phase of exercise reveals that the point defining the instantaneous combination of MAP (or SAP) values was displaced downward and leftward along a baroreflex curve, the slope of which (sensitivity) was lower than the BRS at rest, but equal to the BRS at exercise steady state. This would suggest that the BRS decrease during exercise appears since the exercise start, compatibly with the central command hypothesis.

We, nevertheless, point out a difference between the slope of the linear segments of the RRi versus MAP relationship at exercise onset and the steady-state BRS: although both provide what we can call a baroreflex sensitivity value, computed as the slope of a linear relationship, the former seems to describe the displacement of a baroreflex operating point along a baroreflex curve, while the latter would describe the spontaneous oscillations of a baroreflex operating point around the prevailing mean values of HR and BP at steady state. Thus, if we define baroreflex resetting as the displacement of the prevailing baroreflex function, with consequent shift of the baroreflex operating point (Gallagher et al. 2006; Raven et al. 2006), we would have to accept that the initial linear segment of the HR versus MAP relationship is not representative of baroreflex resetting, but of an attempt at correcting a fall in MAP by means of an increase in HR along a given baroreflex curve.

Of the negative RRi versus MAP relationship after the attainment of MAP_m

In this study, the fall of MAP continued for about 10 s since the beginning of exercise, until the attainment of MAP_m (~73 mmHg in both postures in this study). The MAP_m values of this study were similar to the threshold MAP values observed during the standing-up procedure at the end of prolonged bed rest (Adami et al. 2013), and to the minimum MAP values observed in the early phase of the cardiovascular response to apnoea (Sivieri et al. 2015). The sudden pattern change in the RRi versus MAP relationship (Fig. 3) after attainment of MAP_m led us to propose that baroreflex resetting, implying a negative RRi versus MAP relationship, started only after the achievement of MAP_m . If, indeed, this is the case, then (1) MAP_m might represent a threshold value triggering exercise baroreflex resetting;

(2) the central command theory may not explain the resetting process, even if it may explain the sudden change in baroreflex gain at the beginning of exercise; (3) the resetting process would not imply further changes in baroreflex gain, as witnessed by the equality of the slope of the RRi versus MAP segments at exercise start and of the BRS at exercise steady state.

The pattern of the relationship between RRi and MAP (or SAP) changed drastically (Fig. 3) after attainment of MAP_m and SAP_m , compatibly with the resetting hypothesis. Nevertheless, the opposite variations of RRi and MAP followed complex wandering pathways, which are hard to disentangle, and thus to represent by means of neat mathematical functions in the absence of an a-priori model. A new steady state was achieved within 1 min upright, but not supine. This indicates, on one side, faster cardiovascular adaptation during exercise in the former than in the latter posture, as already reported (Leyk et al. 1994; Loeppky et al. 1981), and on the other side, that the postulated resetting process may not be complete yet at the end of the first min of exercise (Fig. 3), compatibly with a possible role of increasing sympathetic stimulation of the sinus node during exercise (Fagraeus and Linnarsson 1976; Orizio et al. 1988). This hypothesis could be nicely tested under selective blockade of β 1-adrenergic receptors.

Steady-state data

The HR, SV, and CO data at rest conformed to the previous results in upright and supine postures (Leyk et al. 1994; Loeppky et al. 1981; Nishiyasu et al. 1998; Spaak et al. 2005). Resting BRS showed similar values to those found in previous studies (Kardos et al. 2001) in both postures. The previous studies showed that in the upright versus supine position, there is a reduction of vagal (Cooke et al. 1999) and increase of sympathetic (Burke et al. 1977; Ray et al. 1993) activities at rest. In fact, coherently with previous observations (Schwartz et al. 2013), the BRS supine was greater than the BRS upright, whether for MAP-based BRS, or for SAP-based BRS. Since TPR acts on DAP, and thus on MAP, without affecting SAP, the fact that TPR at rest was higher upright than supine, due to peripheral vasoconstriction in the former posture, may explain the different behaviour of MAP-based and SAP-based BRS.

At exercise steady state, the single-beat RRi and MAP values clustered around new stable mean values, defining the new location of the operating point (Fig. 3). This location is compatible with the predictions of baroreflex open-loop steady-state studies (Raven et al. 2006). SV remained lower upright than supine, although in the former posture, a larger amount of blood, located in the lower limbs, was made available for sudden SV increase upon exercise onset (Lador et al. 2006; Sheriff et al. 1993). These changes in SV

were not fully compensated for by an equivalent increase in HR, so that lower CO values at exercise were obtained upright than supine. The present results at exercise steady state in upright and supine postures are in good agreement with the results of previous studies (Nishiyasu et al. 1998).

Conclusions

In conclusion, our results show that the patterns of the dynamics of baroreflex resetting upon exercise onset are characterised by two distinct phases, independently of the posture. A first phase goes from the beginning of the exercise till the attainment of MAP_m : in this phase, a fall in MAP was corrected by a decrease in RRi along a baroreflex curve, which had lower sensitivity than that at rest but equal to that at exercise steady state, and which was likely operating in the same range of RRi and MAP values as at rest. A second phase was characterised by a negative RRi versus MAP relationship toward the exercise steady state prevailing RRi and MAP values. This second phase started after the attainment of MAP_m .

To sum up, a resetting of the baroreflex operating point and a fall in baroreflex gain both took place in the exercise transient, being temporally dissociated phenomena. The latter was immediate, whereas baroreflex resetting did not begin at exercise start. These results seem incompatible with the two hypotheses of central command and exercise pressor reflexes as possible mechanisms behind baroreflex resetting. Nevertheless, the central command hypothesis may still explain the reductions of baroreflex gain that we observed at exercise start: therefore, it cannot be refuted as a hypothesis at least partially explaining the baroreflex changes in the rest-to-exercise transient.

Acknowledgements This study was supported by the Swiss National Science Foundation Grants 32003B_127620 and 3200B0-114033 (to G. Ferretti). The authors thank Prof. Denis R. Morel for his enthusiastic contribution to this work. He suddenly deceased on August 20, 2013.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest for this work.

References

- Adami A, Pizzinelli P, Bringard A, Capelli C, Malacarne M, Lucini D, Simunič B, Pišot R, Ferretti G (2013) Cardiovascular re-adjustments and baroreflex response during clinical reambulation procedure at the end of 35-day bed rest in humans. *Appl Physiol Nutr Metab* 38:673–680
- Azabji Kenfack M, Lador F, Licker M, Moia C, Tam E, Capelli C, Morel D, Ferretti G (2004) Cardiac output by Modelflow method

- from intra-arterial and fingertip pulse pressure profiles. *Clin Sci Lond* 106:365–369
- Barker RC, Hopkins SR, Kellogg N, Olfert IM, Brutsaert TD, Gavin TP, Entin PL, Rice AJ, Wagner PD (1999) Measurement of cardiac output during exercise by open-circuit acetylene uptake. *J Appl Physiol* 87:1506–1512
- Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancina G (1988) Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol Heart Circ Physiol* 254:H377–H383
- Burke D, Sundlöf G, Wallin G (1977) Postural effects on muscle nerve sympathetic activity in man. *J Physiol* 272:399–414
- Chin LMK, Heigenhauser GJF, Paterson DH, Kowalchuk JM (2010) Pulmonary O₂ uptake and leg blood flow kinetics during moderate exercise are slowed by hyperventilation-induced hypocapnic alkalosis. *J Appl Physiol* 108:1641–1650
- Clifford PS (2007) Skeletal muscle vasodilatation at the onset of exercise. *J Physiol* 583:825–833
- Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KU, Eckberg DL (1999) Human responses to upright tilt: a window on central autonomic integration. *J Physiol* 517:617–628
- Cooke WH, Ludwig DA, Hogg PS, Eckberg DL, Convertino VA (2002) Does the menstrual cycle influence the sensitivity of vagally mediated baroreflexes? *Clin Sci Lond* 102:639–644
- DeLorey DS, Kowalchuk JM, Paterson DH (2003) Relationship between pulmonary O₂ uptake kinetics and muscle deoxygenation during moderate-intensity exercise. *J Appl Physiol* 95:113–120
- Di Rienzo M, Castiglioni P, Iellamo F, Volterrani M, Pagani M, Mancina G, Karemaker JM, Parati G (2008) Dynamic adaptation of cardiac baroreflex sensitivity to prolonged exposure to microgravity: data from a 16-day spaceflight. *J Appl Physiol* 105:1569–1575
- DiCarlo SE, Bishop VS (2001) Central baroreflex resetting as a means of increasing and decreasing sympathetic outflow and arterial pressure. *Ann N Y Acad Sci* 940:324–337
- Eckberg DL, Sleight P (1992) *Human Baroreflexes in Health and Disease*. Oxford, Clarendon Press
- Elstad M, Nådland IH, Toska K, Walløe L (2009) Stroke volume decreases during mild dynamic and static exercise in supine humans. *Acta Physiol Oxf* 195:289–300
- Fadel PJ, Raven PB (2012) Human investigations into the arterial and cardiopulmonary baroreflexes during exercise. *Exp Physiol* 97:39–50
- Fagoni N, Sivieri A, Antonutto G, Moia C, Taboni A, Bringard A, Ferretti G (2015) Cardiovascular responses to dry resting apnoeas in elite divers while breathing pure oxygen. *Respir Physiol Neurobiol* 219:1–8
- Fagraeus L, Linnarsson D (1976) Autonomic origin of heart rate fluctuations at the onset of muscular exercise. *J Appl Physiol* 40:679–682
- Faisal A, Beavers KR, Hughson RL (2010) O₂ uptake and blood pressure regulation at the onset of exercise: interaction of circadian rhythm and priming exercise. *Am J Physiol Heart Circ Physiol* 299:H1832–H1842
- Ferretti G, Binzoni T, Hulo N, Kayser B, Thomet JM, Cerretelli P (1995) Kinetics of oxygen consumption during maximal exercise at different muscle temperatures. *Respir Physiol* 102:261–268
- Ferretti G, Iellamo F, Pizzinelli P, Kenfack MA, Lador F, Lucini D, Porta A, Narkiewicz K, Pagani M (2009) Prolonged head down bed rest-induced inactivity impairs tonic autonomic regulation while sparing oscillatory cardiovascular rhythms in healthy humans. *J Hypertens* 27:551–561
- Fisher JP, Young CN, Fadel PJ (2015) Autonomic adjustments to exercise in humans. *Compr Physiol* 5:475–512
- Gallagher KM, Fadel PJ, Strømstad M, Ide K, Smith SA, Querry RG, Raven PB, Secher NH (2001) Effects of exercise pressor reflex activation on carotid baroreflex function during exercise in humans. *J Physiol* 533:871–880
- Gallagher KM, Fadel PJ, Smith SA, Strømstad M, Ide K, Secher NH, Raven PB (2006) The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in humans. *Exp Physiol* 91:79–87
- Heesch CM, Carey LA (1987) Acute resetting of arterial baroreflexes in hypertensive rats. *Am J Physiol Heart Circ Physiol* 253:H974–H979
- Iellamo F, Hughson RL, Castrucci F, Legramante JM, Raimondi G, Peruzzi G, Tallarida G (1994) Evaluation of spontaneous baroreflex modulation of sinus node during isometric exercise in healthy humans. *Am J Physiol Heart Circ Physiol* 267:H994–H1001
- Iellamo F, Legramante JM, Raimondi G, Peruzzi G (1997) Baroreflex control of sinus node during dynamic exercise in humans: effects of central command and muscle reflexes. *Am J Physiol Heart Circ Physiol* 272:H1157–H1164
- Iellamo F, Massaro M, Legramante JM, Raimondi G, Peruzzi G, Galante A (1998) Spontaneous baroreflex modulation of heart rate during incremental exercise in humans. *FASEB J* 12:A692 (abstract)
- Iellamo F, Legramante JM, Pigozzi F, Spataro A, Norbiato G, Lucini D, Pagani M (2002) Conversion from vagal to sympathetic predominance with strenuous training in high-performance world class athletes. *Circulation* 105:2719–2724
- Kardos A, Watterich G, de Menezes R, Csanady M, Casadei B, Rudas L (2001) Determinants of spontaneous baroreflex sensitivity in a healthy working population. *Hypertens* 37:911–916
- Kent BB, Drane JW, Blumenstein B, Manning JW (1972) A mathematical model to assess changes in the baroreceptor reflex. *Cardiology* 57:295–310
- Lador F, Azabji Kenfack M, Moia C, Cautero M, Morel DR, Capelli C, Ferretti G (2006) Simultaneous determination of the kinetics of cardiac output, systemic O₂ delivery, and lung O₂ uptake at exercise onset in men. *Am J Physiol Regul Integr Comp Physiol* 290:R1071–R1079
- Lador F, Tam E, Azabji Kenfack M, Cautero M, Moia C, Morel DR, Capelli C, Ferretti G (2008) Phase I dynamics of cardiac output, systemic O₂ delivery, and lung O₂ uptake at exercise onset in men in acute normobaric hypoxia. *Am J Physiol Regul Integr Comp Physiol* 295:R624–R632
- Legramante JM, Raimondi G, Massaro M, Cassarino S, Peruzzi G, Iellamo F (1999) Investigating feed-forward neural regulation of circulation from analysis of spontaneous arterial pressure and heart rate fluctuations. *Circulation* 99:1760–1766
- Leyk D, Essfeld D, Hoffmann U, Wunderlich HG, Baum K, Stegemann J (1994) Postural effect on cardiac output, oxygen uptake and lactate during cycle exercise of varying intensity. *Eur J Appl Physiol* 68:30–35
- Loeppky JA, Greene ER, Hoekenga DE, Caprihan A, Luft UC (1981) Beat-by-beat stroke volume assessment by pulsed Doppler in upright and supine exercise. *J Appl Physiol* 50:1173–1182
- Mancina G, Groppelli A, Di Rienzo M, Castiglioni P, Parati G (1997) Smoking impairs baroreflex sensitivity in humans. *Am J Physiol Heart Circ Physiol* 273:H1555–H1560
- McIlveen SA, Hayes SG, Kaufman MP (2001) Both central command and exercise pressor reflex reset carotid sinus baroreflex. *Am J Physiol Heart Circ Physiol* 280:H1454–H1463
- Meyer M, Scheid P (1980) Solubility of acetylene in human blood determined by mass spectrometry. *J Appl Physiol* 48:1035–1037
- Minson CT, Halliwill JR, Young TM, Joyner MJ (2000) Influence of the menstrual cycle on sympathetic activity, baroreflex

- sensitivity, and vascular transduction in young women. *Circulation* 101:862–868
- Mitchell JH (2013) Neural circulatory control during exercise: early insights. *Exp Physiol* 98:867–878
- Nishiyasu T, Nagashima K, Nadel ER, Mack GW (1998) Effects of posture on cardiovascular responses to lower body positive pressure at rest and during dynamic exercise. *J Appl Physiol* 85:160–167
- O’Leary DD, Kimmerly DS, Cechetto AD, Shoemaker JK (2003) Differential effect of head-up tilt on cardiovagal and sympathetic baroreflex sensitivity in humans. *Exp Physiol* 88:769–774
- Ogoh S, Wasmund WL, Keller DM, O-Yurvati A, Gallagher KM, Mitchell JH, Raven PB (2002) Role of central command in carotid baroreflex resetting in humans during static exercise. *J Physiol* 543:349–364
- Orizio C, Perini R, Comandè A, Castellano M, Beschi M, Veicsteinas A (1988) Plasma catecholamines and heart rate at the beginning of muscular exercise in man. *Eur J Appl Physiol* 57:644–651
- Potts JT, Shi XR, Raven PB (1993) Carotid baroreflex responsiveness during dynamic exercise in humans. *Am J Physiol Heart Circ Physiol* 265:H1928–H1938
- Querry RG, Smith SA, Strömstad M, Ide K, Raven PB, Secher NH (2001) Neural blockade during exercise augments central command’s contribution to carotid baroreflex resetting. *Am J Physiol Heart Circ Physiol* 280:H1635–H1644
- Raven PB (2008) Recent advances in baroreflex control of blood pressure during exercise in humans: an overview. *Med Sci Sports Exerc* 40:2033–2036
- Raven PB, Potts JT, Shi X (1997) Baroreflex regulation of blood pressure during dynamic exercise. *Exerc Sport Sci Rev* 25:365–389
- Raven PB, Fadel PJ, Smith SA (2002) The influence of central command on baroreflex resetting during exercise. *Exerc Sport Sci Rev* 30:39–44
- Raven PB, Fadel PJ, Ogoh S (2006) Arterial baroreflex resetting during exercise: a current perspective. *Exp Physiol* 91:37–49
- Ray CA, Rea RF, Clary MP, Mark AL (1993) Muscle sympathetic nerve responses to dynamic one-legged exercise: effect of body posture. *Am J Physiol Heart Circ Physiol* 264:H1–H7
- Rowell LB, O’Leary DS, Kellogg DL (1996) Integration of Cardiovascular Control Systems in Dynamic Exercise. In: *Handbook of physiology. Exercise: regulation integration of multiple systems*. American Physiological Society, Bethesda, MD, pp 770–838
- Saltin B, Rådegran G, Koskolou MD, Roach RC (1998) Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol Scand* 162:421–436
- Schwartz CE, Medow MS, Messer Z, Stewart JM (2013) Spontaneous fluctuation indices of the cardiovagal baroreflex accurately measure the baroreflex sensitivity at the operating point during upright tilt. *Am J Physiol Regul Integr Comp Physiol* 304:R1107–R1113
- Sheriff DD, Rowell LB, Secher AM (1993) Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *Am J Physiol Heart Circ Physiol* 265:H1227–H1234
- Sivieri A, Fagoni N, Bringard A, Capogrosso M, Perini R, Ferretti G (2015) A beat-by-beat analysis of cardiovascular responses to dry resting and exercise apnoeas in elite divers. *Eur J Appl Physiol* 115:119–128
- Smith SA, Querry RG, Fadel PJ, Gallagher KM, Strömstad M, Ide K, Raven PB, Secher NH (2003) Partial blockade of skeletal muscle somatosensory afferents attenuates baroreflex resetting during exercise in humans. *J Physiol* 551:1013–1021
- Spaak J, Montmerle S, Sundblad P, Linnarsson D (2005) Long-term bed rest-induced reductions in stroke volume during rest and exercise: cardiac dysfunction vs. volume depletion. *J Appl Physiol* 98:648–654
- Steinback CD, O’Leary DD, Bakker J, Cechetto AD, Ladak HM, Shoemaker JK (2005) Carotid distensibility, baroreflex sensitivity, and orthostatic stress. *J Appl Physiol* 99:64–70
- Tam E, Azabji Kenfack M, Cautero M, Lador F, Antonutto G, di Prampero PE, Ferretti G, Capelli C (2004) Correction of cardiac output obtained by Modelflow from finger pulse pressure profiles with a respiratory method in humans. *Clin Sci Lond* 106:371–376
- Vallais F, Baselli G, Lucini D, Pagani M, Porta A (2009) Spontaneous baroreflex sensitivity estimates during graded bicycle exercise: a comparative study. *Physiol Meas* 30:201–213
- van Lieshout JJ, Toska K, van Lieshout EJ, Eriksen M, Walloe L, Wesseling KH (2003) Beat-to-beat noninvasive stroke volume from arterial pressure and Doppler ultrasound. *Eur J Appl Physiol* 90:131–137
- Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ (1993) Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol* 74:2566–2573
- Wieling W, Harms MP, ten Harkel AD, van Lieshout JJ, Sprangers RL (1996) Circulatory response evoked by a 3 s bout of dynamic leg exercise in humans. *J Physiol* 494:601–611

5.4.3. Conclusion

Au vu des résultats obtenus, on observe à la fois une chute du gain du baroréflexe en tout début d'exercice, et un mécanisme de « resetting » du baroréflexe arrivant plus tardivement, après le changement de BRS. Ces résultats semblent incompatibles avec les hypothèses évoquées de mécanismes de régulation de la réinitialisation du baroréflexe par la commande centrale et par les réflexes provenant de propriocepteurs des muscles activés durant l'exercice. Cependant, il se peut que la commande centrale influence la réduction du BRS en début d'exercice, ce qui expliquerait partiellement les changements du baroréflexe entre repos et exercice.

Afin de compléter ces résultats, il serait intéressant par la suite de connaître les influences respectives de chacune des branches du SNA sur les mécanismes de resetting et de changement de gain du baroréflexe. Pour ce faire, l'élaboration d'un protocole de recherche utilisant, par exemple, les blocages pharmacologiques, tels que ceux décrits dans la troisième étude de cette thèse, pourrait apporter de nouvelles informations permettant de comprendre plus précisément des phénomènes de régulation cardiovasculaire en début d'exercice.

6. Conclusions et perspectives

6.1 Conclusion générale

La variabilité de la fréquence cardiaque et celle de la pression artérielle constitue un vaste sujet d'étude possédant de multiples applications diagnostiques et thérapeutiques en médecine et dans les sciences biologiques. Elle est utilisée depuis des décennies pour tenter de cerner qualitativement et semi-quantitativement, de manière indirecte et non-vulnérante, les activités régulatrices du SNA sur le cœur, la vasomotricité artérielle et une part de l'activité ventilatoire. De nombreuses procédures expérimentales offrent des cadres codifiés d'analyse et de quantification de ces régulations, par exemple en effectuant des manœuvres de respiration ample, des manœuvres de Valsalva, en imposant des modifications posturales, ou des tests de tolérance orthostatique (tilt). L'immersion, à neutralité thermique ou en eau froide, les tests de stress mental, l'application de pression négative autour des membres inférieurs ou encore l'activité physique sont autant de situations d'expérimentations particulières au cours desquelles on peut recueillir des informations sur l'état normal ou altéré des régulations des fonctions cardiaque ou vasculaires par le SNA (Freeman, 2006).

De nombreux protocoles permettent de quantifier et analyser cette activité régulatrice du SNA, que ce soit lors de respirations profondes, de manœuvres de Valsalva, avec des modifications posturales, ou encore des tests de tolérance orthostatique (tilt). L'immersion en eau froide, les tests de stress mental, la dépression au niveau des jambes, ou encore l'exercice sont autant de situations d'expérimentations particulières apportant des informations utiles à la compréhension des mécanismes de régulations du système cardiovasculaire par le SNA (Freeman, 2006). Cette thèse de doctorat, au travers de quatre articles,

a essayé d'apporter un éclairage supplémentaire dans le domaine. L'objectif de ce travail a été d'analyser la VFC, la variabilité de la pression artérielle et le BRS dans diverses conditions d'études afin d'apporter des informations sur la modulation et la régulation de ces phénomènes physiologiques par le système neurovégétatif.

Ces travaux ont menés à des conclusions que nous pouvons résumer de la manière suivante :

1. En condition d'hypergravité, il semblerait que, tout comme durant l'exercice, seule la branche sympathique du SNA soit active. De plus, la composante LF de la variabilité de fréquence cardiaque diminue, alors que celle de la variabilité de pression artérielle augmente, suggérant une dissociation des deux systèmes.
2. Malgré un contexte impliquant une augmentation de la régulation vasculaire, le BRS diminue en hypergravité, mettant en lumière la complexité des mécanismes de régulation de pression.
3. L'étude sur les transplantés pulmonaires complets a mis en évidence le rôle régulateur que jouent les efférences pulmonaires sur la VFC, et ce, principalement sur la composante HF du spectre de puissance.
4. Le baroréflexe montre une sensibilité réduite, même si la dénervation pulmonaire ne semble pas affecter la variabilité de la pression artérielle. Ce dernier point démontre, une fois encore, l'indépendance des mécanismes de contrôle des paramètres cardiovasculaires centraux et périphériques.
5. D'un point de vue clinique, les valeurs des indices de VFC et de BRS chutant suite à la transplantation, les patients s'exposent de manière plus importante aux risques de mortalité liés à de nombreux troubles cardiovasculaires. L'activité

physique régulière améliorant ces indices, il est primordial de ré-entraîner ces patients adéquatement.

6. Les blocages pharmacologiques des branches du SNA montrent que les afférences parasympathiques au cœur sont les principaux déterminants de la VFC, le rôle de la branche sympathique étant, quant à lui, moins important. À l'exercice, la chute du tonus parasympathique n'est pas totale, laissant une faible part de régulation vagale. Les changements concomitants de BRS et de la variabilité de la pression artérielle suggèrent que les baroréflexes pourraient moduler la composante LF du spectre de variabilité de la tension artérielle.

7. L'analyse en boucle ouverte du baroréflexe lors de la phase de transition repos-exercice démontre l'indépendance des deux phénomènes distincts que sont le processus de réinitialisation et le changement de sensibilité du baroréflexe. Ce dernier apparaît dès les premières secondes d'exercice ce qui n'est pas le cas lors du processus de réinitialisation. Ces mécanismes sont donc bien indépendants l'un de l'autre.

6.2 Perspectives

Chacune des études présentées dans cette thèse amène, en condition particulière, un éclairage sur la régulation du système cardiovasculaire par les branches vagale et sympathique du SNA. Certains résultats nous amènent naturellement à nous poser de nouvelles questions. Ainsi, les résultats obtenus chez les transplantés pulmonaires nous poussent, comme prévu, à observer et à quantifier les effets d'un ré-entraînement spécifique sur les indices de variabilité cardiaque et sur le BRS, facteurs associés à l'incidence d'événements

cardiovasculaires létaux. Certains aspects observés pourraient être investigués davantage en croisant les types d'analyses utilisés. L'analyse du baroréflexe durant la phase de transition repos-exercice pourrait ainsi être approfondie en utilisant les blocages pharmacologiques afin de mieux comprendre les rôles distincts de chacune des branches du SNA.

7. Références bibliographiques

1. Adami A, Pizzinelli P, Bringard A, Capelli C, Malacarne M, Lucini D, et al. Cardiovascular re-adjustments and baroreflex response during clinical reambulation procedure at the end of 35-day bed rest in humans. *Appl Physiol Nutr Metab*. 2013 Jun;38(6):673–80.
2. Airaksinen KE, Niemelä MJ, Huikuri HV. Effect of beta-blockade on baroreflex sensitivity and cardiovascular autonomic function tests in patients with coronary artery disease. *Eur Heart J*. 1994 Nov;15(11):1482–5.
3. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science*. 1981 Jul 10;213(4504):220–2.
4. Akselrod S. Spectral analysis of fluctuations in cardiovascular parameters: a quantitative tool for the investigation of autonomic control. *Trends Pharmacol Sci*. 1988 Jan;9(1):6–9.
5. Aoki K, Stephens DP, Johnson JM. Diurnal variation in cutaneous vasodilator and vasoconstrictor systems during heat stress. *Am J Physiol Regul Integr Comp Physiol*. 2001 Aug;281(2):R591-595.
6. Arai Y, Saul JP, Albrecht P, Hartley LH, Lilly LS, Cohen RJ, et al. Modulation of cardiac autonomic activity during and immediately after exercise. *Am J Physiol*. 1989 Jan;256(1 Pt 2):H132-141.
7. Arbeille P, Kerbeci P, Mattar L, Shoemaker JK, Hughson R. Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. *Am J Physiol Heart Circ Physiol*. 2008 Nov;295(5):H1846-1854.
8. Assoumou H-GN, Bertholon F, Barthélémy J-C, Pichot V, Celle S, Gosse P, et al. Alteration of baroreflex sensitivity in the elderly: the relationship with metabolic syndrome components. *Int J Cardiol*. 2012 Mar 8;155(2):333–5.
9. Åstrand P-O. *Textbook of Work Physiology: Physiological Bases of Exercise*. Human Kinetics; 2003. 664 p.
10. Aubert AE, Seps B, Beckers F. Heart rate variability in athletes. *Sports Med*. 2003;33(12):889–919.
11. Azabji Kenfack M, Lador F, Licker M, Moia C, Tam E, Capelli C, et al. Cardiac output by Modelflow method from intra-arterial and fingertip pulse pressure profiles. *Clin Sci*. 2004 Apr;106(4):365–9.
- 12.

Barbe P, Galitzky J, Thalamas C, Langin D, Lafontan M, Senard JM, et al. Increase in epinephrine-induced responsiveness during microgravity simulated by head-down bed rest in humans. *J Appl Physiol*. 1999 Nov;87(5):1614–20.

13.

Barker RC, Hopkins SR, Kellogg N, Olfert IM, Brutsaert TD, Gavin TP, et al. Measurement of cardiac output during exercise by open-circuit acetylene uptake. *J Appl Physiol*. 1999 Oct;87(4):1506–12.

14.

Barrett CJ, Navakatikyan MA, Malpas SC. Long-term control of renal blood flow: what is the role of the renal nerves? *Am J Physiol Regul Integr Comp Physiol*. 2001 May;280(5):R1534-1545.

15.

Beckers F, Verheyden B, Ramaekers D, Swynghedauw B, Aubert AE. Effects of autonomic blockade on non-linear cardiovascular variability indices in rats. *Clin Exp Pharmacol Physiol*. 2006 Jun;33(5–6):431–9.

16.

Benarroch EE. The arterial baroreflex: functional organization and involvement in neurologic disease. *Neurology*. 2008 Nov 18;71(21):1733–8.

17.

Berakis A, Williams TJ, Naughton MT, Martin JH, Muhlmann M, Krum H. Altered sympathetic and parasympathetic activity in lung transplantation patients at rest and following autonomic perturbation. *Chest*. 2002 Oct;122(4):1192–9.

18.

Berntson GG, Bigger JT, Eckberg DL, Grossman P, Kaufmann PG, Malik M, et al. Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology*. 1997 Nov;34(6):623–48.

19.

Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancina G. Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol*. 1988 Feb;254(2 Pt 2):H377-383.

20.

Bigger JT, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation*. 1992 Jan;85(1):164–71.

21.

Billman GE, Schwartz PJ, Stone HL. Baroreceptor reflex control of heart rate: a predictor of sudden cardiac death. *Circulation*. 1982 Oct;66(4):874–80.

22.

Billman GE. Cardiac autonomic neural remodeling and susceptibility to sudden cardiac death: effect of endurance exercise training. *Am J Physiol Heart Circ Physiol*. 2009 Oct;297(4):H1171-1193.

23.

Billman GE. Heart rate variability - a historical perspective. *Front Physiol*. 2011;2:86.

24.

Billman GE. The effect of heart rate on the heart rate variability response to autonomic interventions. *Front Physiol.* 2013;4:222.

25.

Bjurstedt H, Rosenhamer G, Tydén G. Acceleration stress and effects of propranolol on cardiovascular responses. *Acta Physiol Scand.* 1974 Feb;90(2):491–500.

26.

Bjurstedt H, Rosenhamer G, Wigertz O. High-G environment and responses to graded exercise. *J Appl Physiol.* 1968 Dec;25(6):713–9.

27.

Blumberg MS, Koot TG, Kirby RF. Neural and hormonal control of arterial pressure during cold exposure in unanesthetized week-old rats. *Am J Physiol Regul Integr Comp Physiol.* 2001 Nov;281(5):R1514-1521.

28.

Bonjour J, Bringard A, Antonutto G, Capelli C, Linnarsson D, Pendergast DR, et al. Effects of acceleration in the Gz axis on human cardiopulmonary responses to exercise. *Eur J Appl Physiol.* 2011 Dec;111(12):2907–17.

29.

Bonjour J, Capelli C, Antonutto G, Calza S, Tam E, Linnarsson D, et al. Determinants of oxygen consumption during exercise on cycle ergometer: The effects of gravity acceleration. *Respiratory Physiology & Neurobiology.* 2010 Apr 30;171(2):128–34.

30.

Braga ANG, Da Silva Lemos M, Da Silva JR, Fontes WRP, Augusto Souza Dos Santos R. Effects of angiotensins on day-night fluctuations and stress-induced changes in blood pressure. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 2002 Jun 1;282(6):R1663–71.

31.

Buijs RM, Swaab DF, editors. *Autonomic nervous system.* Edinburgh ; London ; New York ; Oxford ; Philadelphia ; St. Louis ; Sydney ; Toronto: Elsevier; 2013. 417 p. (Handbook of clinical neurology).

32.

Burke D, Sundlöf G, Wallin G. Postural effects on muscle nerve sympathetic activity in man. *J Physiol (Lond).* 1977 Nov;272(2):399–414.

33.

Burke SN, Barnes CA. Neural plasticity in the ageing brain. *Nature Reviews Neuroscience.* 2006 Jan;7(1):30–40.

34.

Burton RR, Smith AH. *Adaptation to Acceleration Environments.* In: *Comprehensive Physiology.* John Wiley & Sons, Inc.; 2011

35.

Castiglioni P, Parati G, Di Rienzo M, Carabalona R, Cividjian A, Quintin L. Scale exponents of blood pressure and heart rate during autonomic blockade as assessed by detrended fluctuation analysis: Cardiovascular scale exponents and autonomic blockade. *The Journal of Physiology.* 2011 Jan 15;589(2):355–69.

36.
Castleberry AW, Martin JT, Osho AA, Hartwig MG, Hashmi ZA, Zanotti G, et al. Coronary revascularization in lung transplant recipients with concomitant coronary artery disease. *Am J Transplant*. 2013 Nov;13(11):2978–88.
37.
Challapalli S, Kadish AH, Horvath G, Goldberger JJ. Differential effects of parasympathetic blockade and parasympathetic withdrawal on heart rate variability. *J Cardiovasc Electrophysiol*. 1999 Sep;10(9):1192–9.
38.
Chamberlain DA, Turner P, Sneddon JM. Effects of atropine on heart rate in healthy man. *The Lancet*. 1967 Jul;290(7505):12–5.
39.
Chen S-W, Chen H-C, Chan H-L. A real-time QRS detection method based on moving-averaging incorporating with wavelet denoising. *Computer Methods and Programs in Biomedicine*. 2006 Jun;82(3):187–95.
40.
Cheung MN. Detection of and Recovery from Errors in Cardiac Interbeat Intervals. *Psychophysiology*. 1981 May;18(3):341–6.
41.
Chin LMK, Heigenhauser GJF, Paterson DH, Kowalchuk JM. Pulmonary O₂ uptake and leg blood flow kinetics during moderate exercise are slowed by hyperventilation-induced hypocapnic alkalosis. *J Appl Physiol*. 2010 Jun;108(6):1641–50.
42.
Christensen NJ, Heer M, Ivanova K, Norsk P. Sympathetic nervous activity decreases during head-down bed rest but not during microgravity. *J Appl Physiol*. 2005 Oct;99(4):1552–7.
43.
Clifford PS. Skeletal muscle vasodilatation at the onset of exercise. *J Physiol (Lond)*. 2007 Sep 15;583(Pt 3):825–33.
44.
Cogliati C, Colombo S, Ruscone TG, Gruosso D, Porta A, Montano N, et al. Acute beta-blockade increases muscle sympathetic activity and modifies its frequency distribution. *Circulation*. 2004 Nov 2;110(18):2786–91.
45.
Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KU, Eckberg DL. Human responses to upright tilt: a window on central autonomic integration. *J Physiol (Lond)*. 1999 Jun 1;517 (Pt 2):617–28.
46.
Cooke WH, Ludwig DA, Hogg PS, Eckberg DL, Convertino VA. Does the menstrual cycle influence the sensitivity of vagally mediated baroreflexes? *Clin Sci*. 2002 Jun;102(6):639–44.
- 47.

Costes F, Roche F, Pichot V, Vergnon JM, Garet M, Barthelemy JC. Influence of exercise training on cardiac baroreflex sensitivity in patients with COPD. *Eur Respir J*. 2004 Mar;23(3):396–401.

48.

Crandall CG. Heat Stress and Baroreflex Regulation of Blood Pressure. *Med Sci Sports Exerc*. 2008 Dec;40(12):2063.

49.

Crawford MH, Bernstein SJ, Deedwania PC, DiMarco JP, Ferrick KJ, Garson A, et al. ACC/AHA Guidelines for Ambulatory Electrocardiography. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the Guidelines for Ambulatory Electrocardiography). Developed in collaboration with the North American Society for Pacing and Electrophysiology. *J Am Coll Cardiol*. 1999 Sep;34(3):912–48.

50.

Dalla Pozza R, Kleinmann A, Bechtold S, Netz H. Hypertension in heart and heart-lung transplanted children: does impaired baroreceptor function play a role? *Transplantation*. 2006 Jan 15;81(1):71–5.

51.

Daugirdas JT. Pathophysiology of dialysis hypotension: an update. *Am J Kidney Dis*. 2001 Oct;38(4 Suppl 4):S11-17.

52.

Dauphinaud V, Kossovsky MP, Gueyffier F, Pichot V, Gosse P, Roche F, et al. Impaired baroreflex sensitivity and the risks of new-onset ambulatory hypertension, in an elderly population-based study. *Int J Cardiol*. 2013 Oct 9;168(4):4010–4.

53.

DeLorey DS, Kowalchuk JM, Paterson DH. Relationship between pulmonary O₂ uptake kinetics and muscle deoxygenation during moderate-intensity exercise. *J Appl Physiol*. 2003 Jul;95(1):113–20.

54.

DiCarlo SE, Bishop VS. Central baroreflex resetting as a means of increasing and decreasing sympathetic outflow and arterial pressure. *Ann N Y Acad Sci*. 2001 Jun;940:324–37.

55.

Di Rienzo M, Castiglioni P, Iellamo F, Volterrani M, Pagani M, Mancina G, et al. Dynamic adaptation of cardiac baroreflex sensitivity to prolonged exposure to microgravity: data from a 16-day spaceflight. *J Appl Physiol*. 2008 Nov;105(5):1569–75.

56.

Dougherty CM, Burr RL. Comparison of heart rate variability in survivors and nonsurvivors of sudden cardiac arrest. *Am J Cardiol*. 1992 Aug 15;70(4):441–8.

57.

Drouin E, Gournay V, Calamel J, Mouzard A, Rozé JC. Assessment of spontaneous baroreflex sensitivity in neonates. *Arch Dis Child Fetal Neonatal Ed*. 1997 Mar;76(2):F108-112.

58.
Dwain L. Eckberg, Peter Sleight. Human Baroreflexes in Health and Disease. Front Cover. Clarendon Press, 1992 - Baroreceptors. In.
59.
Eckberg DL, Neurolab Autonomic Nervous System Team. Bursting into space: alterations of sympathetic control by space travel. *Acta Physiol Scand*. 2003 Mar;177(3):299–311.
60.
Eckberg DL. Sympathovagal Balance : A Critical Appraisal. *Circulation*. 1997 Nov 4;96(9):3224–32.
61.
Eckberg DL, Sleight P. Human Baroreflexes in Health and Disease. Clarendon Press; 1992. 596 p.
62.
Edgell H, Zuj KA, Greaves DK, Shoemaker JK, Custaud M-A, Kerbeci P, et al. WISE-2005: adrenergic responses of women following 56-days, 6 degrees head-down bed rest with or without exercise countermeasures. *Am J Physiol Regul Integr Comp Physiol*. 2007 Dec;293(6):R2343-2352.
63.
Elstad M, Nådland IH, Toska K, Walløe L. Stroke volume decreases during mild dynamic and static exercise in supine humans. *Acta Physiol (Oxf)*. 2009 Feb;195(2):289–300.
64.
Ertl AC, Diedrich A, Biaggioni I, Levine BD, Robertson RM, Cox JF, et al. Human muscle sympathetic nerve activity and plasma noradrenaline kinetics in space. *J Physiol (Lond)*. 2002 Jan 1;538(Pt 1):321–9.
65.
Fadel PJ, Raven PB. Human investigations into the arterial and cardiopulmonary baroreflexes during exercise. *Exp Physiol*. 2012 Jan;97(1):39–50.
66.
Fagard RH, Pardaens K, Staessen JA, Thijs L. Power spectral analysis of heart rate variability by autoregressive modelling and fast Fourier transform: a comparative study. *Acta Cardiol*. 1998;53(4):211–8.
67.
Fagoni N, Sivieri A, Antonutto G, Moia C, Taboni A, Bringard A, et al. Cardiovascular responses to dry resting apnoeas in elite divers while breathing pure oxygen. *Respir Physiol Neurobiol*. 2015 Dec;219:1–8.
68.
Fagraeus L, Linnarsson D. Autonomic origin of heart rate fluctuations at the onset of muscular exercise. *J Appl Physiol*. 1976 May;40(5):679–82.
69.
Faisal A, Beavers KR, Hughson RL. O₂ uptake and blood pressure regulation at the onset of exercise: interaction of circadian rhythm and priming exercise. *Am J Physiol Heart Circ Physiol*. 2010 Dec;299(6):H1832-1842.
- 70.

Ferretti G, Binzoni T, Hulo N, Kayser B, Thomet JM, Cerretelli P. Kinetics of oxygen consumption during maximal exercise at different muscle temperatures. *Respir Physiol*. 1995 Dec;102(2–3):261–8.

71.

Ferretti G, Iellamo F, Pizzinelli P, Kenfack MA, Lador F, Lucini D, et al. Prolonged head down bed rest-induced inactivity impairs tonic autonomic regulation while sparing oscillatory cardiovascular rhythms in healthy humans. *J Hypertens*. 2009 Mar;27(3):551–61.

72.

Ferretti G, Licker MJ, Anchisi S, Moia C, Susta D, Morel DR. The effects of beta1-adrenergic blockade on cardiovascular oxygen flow in normoxic and hypoxic humans at exercise. *Eur J Appl Physiol*. 2005 Oct;95(2–3):250–9.

73.

Fisher JP, Young CN, Fadel PJ. Autonomic adjustments to exercise in humans. *Compr Physiol*. 2015 Apr;5(2):475–512.

74.

Florian JP, Simmons EE, Chon KH, Faes L, Shykoff BE. Cardiovascular and autonomic responses to physiological stressors before and after six hours of water immersion. *J Appl Physiol*. 2013 Nov;115(9):1275–89.

75.

Fontollet T, Pichot V, Antonutto G, Bonjour J, Capelli C, Tam E, et al. Effects of gravitational acceleration on cardiovascular autonomic control in resting humans. *Eur J Appl Physiol*. 2015 Jul;115(7):1417–27.

76.

Formes KJ, Wray DW, O-Yurvati AH, Weiss MS, Shi X. Sympathetic cardiac influence and arterial blood pressure instability. *Auton Neurosci*. 2005 Mar 31;118(1–2):116–24.

77.

Fortrat JO, Sigaud D, Hughson RL, Maillet A, Yamamoto Y, Gharib C. Effect of prolonged head-down bed rest on complex cardiovascular dynamics. *Auton Neurosci*. 2001 Jan 14;86(3):192–201.

78.

Freeman R. Assessment of cardiovascular autonomic function. *Clinical Neurophysiology*. 2006 Apr;117(4):716–30.

79.

Furlan R, Guzzetti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, et al. Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. *Circulation*. 1990 Feb;81(2):537–47.

80.

Gallagher KM, Fadel PJ, Smith SA, Strømstad M, Ide K, Secher NH, et al. The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in humans. *Exp Physiol*. 2006 Jan;91(1):79–87.

81.

Gallagher KM, Fadel PJ, Strømstad M, Ide K, Smith SA, Querry RG, et al. Effects of exercise pressor reflex activation on carotid baroreflex function during exercise in humans. *J Physiol (Lond)*. 2001 Jun 15;533(Pt 3):871–80.

82.

Girardis, Linnarsson, Moia, Pendergast, Ferretti. Oxygen cost of dynamic leg exercise on a cycle ergometer: effects of gravity acceleration. *Acta Physiologica Scandinavica*. 1999;166(3):239–246.

83.

Goldberger JJ, Le FK, Lahiri M, Kannankeril PJ, Ng J, Kadish AH. Assessment of parasympathetic reactivation after exercise. *Am J Physiol Heart Circ Physiol*. 2006 Jun;290(6):H2446-2452.

84.

Goldberger JJ, Challapalli S, Tung R, Parker MA, Kadish AH. Relationship of heart rate variability to parasympathetic effect. *Circulation*. 2001 Apr 17;103(15):1977–83.

85.

Goldstein DS, Benth O, Park M-Y, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes: Low-frequency power of heart rate variability. *Experimental Physiology*. 2011 Dec;96(12):1255–61.

86.

Guyenet PG, Stornetta RL, Bayliss DA. Central respiratory chemoreception. *The Journal of Comparative Neurology*. 2010 May 20;518(19):3883–906.

87.

Hales S. Foundations of anesthesiology. An account of some hydraulic and hydrostatical experiments made on the blood and blood-vessels of animals. 1710 [classical article]. *J Clin Monit Comput*. 2000 Jan;16(1):45–7.

88.

Hartwich D, Dear WE, Waterfall JL, Fisher JP. Effect of muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity during exercise in humans. *J Physiol (Lond)*. 2011 Dec 15;589(Pt 24):6157–71.

89.

Hawkins MN, Barnes Q, Purkayastha S, Eubank W, Ogoh S, Raven PB. The effects of aerobic fitness and beta1-adrenergic receptor blockade on cardiac work during dynamic exercise. *J Appl Physiol*. 2009 Feb;106(2):486–93.

90.

Heathers JAJ. Everything Hertz: methodological issues in short-term frequency-domain HRV. *Front Physiol*. 2014;5:177.

91.

Heesch CM, Carey LA. Acute resetting of arterial baroreflexes in hypertensive rats. *Am J Physiol*. 1987 Oct;253(4 Pt 2):H974-979.

92.

Hirayanagi K, Iwase S, Kamiya A, Sasaki T, Mano T, Yajima K. Functional changes in autonomic nervous system and baroreceptor reflex induced by 14

days of 6 degrees head-down bed rest. *Eur J Appl Physiol.* 2004 Jun;92(1–2):160–7.

93.

Houle MS, Billman GE. Low-frequency component of the heart rate variability spectrum: a poor marker of sympathetic activity. *Am J Physiol.* 1999 Jan;276(1 Pt 2):H215-223.

94.

Hruban RH, Beschorner WE, Hutchins GM. Lymphocytic bronchitis and lung allograft rejection. *Transplantation.* 1990 Oct;50(4):723.

95.

Hua LPT, Brown CA, Hains SJM, Godwin M, Parlow JL. Effects of low-intensity exercise conditioning on blood pressure, heart rate, and autonomic modulation of heart rate in men and women with hypertension. *Biol Res Nurs.* 2009 Oct;11(2):129–43.

96.

Hughson RL, Maillet A, Gauquelin G, Arbeille P, Yamamoto Y, Gharib C. Investigation of hormonal effects during 10-h head-down tilt on heart rate and blood pressure variability. *J Appl Physiol.* 1995 Feb;78(2):583–96.

97.

Hughson RL, Yamamoto Y, McCullough RE, Sutton JR, Reeves JT. Sympathetic and parasympathetic indicators of heart rate control at altitude studied by spectral analysis. *J Appl Physiol.* 1994 Dec;77(6):2537–42.

98.

Iber C, Simon P, Skatrud JB, Mahowald MW, Dempsey JA. The Breuer-Hering reflex in humans. Effects of pulmonary denervation and hypocapnia. *Am J Respir Crit Care Med.* 1995 Jul;152(1):217–24.

99.

Iellamo F, Hughson RL, Castrucci F, Legramante JM, Raimondi G, Peruzzi G, et al. Evaluation of spontaneous baroreflex modulation of sinus node during isometric exercise in healthy humans. *Am J Physiol.* 1994 Sep;267(3 Pt 2):H994-1001.

100.

Iellamo F, Legramante JM, Raimondi G, Peruzzi G. Baroreflex control of sinus node during dynamic exercise in humans: effects of central command and muscle reflexes. *Am J Physiol.* 1997 Mar;272(3 Pt 2):H1157-1164.

101.

Iellamo F, Massaro M, Legramante JM, Raimondi G, Peruzzi G, Galante, A. A. Spontaneous baroreflex modulation of heart rate during incremental exercise in humans. *FASEB.* 1998 Mar 20;J 12(5):A692.

102.

Iellamo F, Legramante JM, Pigozzi F, Spataro A, Norbiato G, Lucini D, et al. Conversion from vagal to sympathetic predominance with strenuous training in high-performance world class athletes. *Circulation.* 2002 Jun 11;105(23):2719–24.

103.

Inagaki T, Sonobe T, Poole DC, Kano Y. Progressive arteriolar vasoconstriction and fatigue during tetanic contractions of rat skeletal muscle are inhibited by α -receptor blockade. *J Physiol Sci*. 2011 May;61(3):181–9.

104.

Joyner MJ, Green DJ. Exercise protects the cardiovascular system: effects beyond traditional risk factors. *J Physiol (Lond)*. 2009 Dec 1;587(Pt 23):5551–8.

105.

Kamath MV, Fallen EL. Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. *Crit Rev Biomed Eng*. 1993;21(3):245–311.

106.

Kamath MV, Fallen EL. Correction of the heart rate variability signal for ectopics and missing beats. In: *Heart Rate Variability*. Futura Publishing Company. M. Malik and A. Camm; 1995. p. 75–85.

107.

Kamimori GH, Bellar D, Fein HG, Smallridge RC. Hormonal and cardiovascular response to low-intensity exercise with atropine administration. *Mil Med*. 2009 Mar;174(3):253–8.

108.

Kamiya A, Michikami D, Iwase S, Hayano J, Kawada T, Sugimachi M, et al. Alpha-adrenergic vascular responsiveness to sympathetic nerve activity is intact after head-down bed rest in humans. *Am J Physiol Regul Integr Comp Physiol*. 2004 Jan;286(1):R151-157.

109.

Kamiya A, Iwase S, Kitazawa H, Mano T. Muscle sympathetic nerve activity (MSNA) after 120 days of 6 degrees head-down bed rest (HDBR). *Environ Med*. 1999 Dec;43(2):150–2.

110.

Kardos A, Watterich G, Menezes R de, Csanády M, Casadei B, Rudas L. Determinants of Spontaneous Baroreflex Sensitivity in a Healthy Working Population. *Hypertension*. 2001 Mar 1;37(3):911–6.

111.

Kasiske BL. Risk factors for accelerated atherosclerosis in renal transplant recipients. *Am J Med*. 1988 Jun;84(6):985–92.

112.

Kempsell AT, Fieber LA. Age-related deficits in synaptic plasticity rescued by activating PKA or PKC in sensory neurons of *Aplysia californica*. *Frontiers in Aging Neuroscience* [Internet]. 2015 Sep 3 [cited 2016 Sep 21];7. Available from: <http://journal.frontiersin.org/Article/10.3389/fnagi.2015.00173/abstract>

113.

Kent BB, Drane JW, Blumenstein B, Manning JW. A mathematical model to assess changes in the baroreceptor reflex. *Cardiology*. 1972;57(5):295–310.

114.

Kleiger RE, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol*. 1987 Feb 1;59(4):256–62.

115.

Kleiger RE, Stein PK, Bigger JT. Heart rate variability: measurement and clinical utility. *Ann Noninvasive Electrocardiol*. 2005 Jan;10(1):88–101.

116.

Krediet RT, Dunning AJ. Baroreflex sensitivity in hypertension during beta-adrenergic blockade. *Br Heart J*. 1979 Jan;41(1):106–10.

117.

Kudaiberdieva G, Görennek B, Timuralp B. Heart rate variability as a predictor of sudden cardiac death. *Anadolu Kardiyol Derg*. 2007 Jul;7 Suppl 1:68–70.

118.

Kumar A, Rani A, Tchigranova O, Lee W-H, Foster TC. Influence of late-life exposure to environmental enrichment or exercise on hippocampal function and CA1 senescent physiology. *Neurobiol Aging*. 2012 Apr;33(4):828.e1-17.

119.

Lador F, Azabji Kenfack M, Moia C, Cautero M, Morel DR, Capelli C, et al. Simultaneous determination of the kinetics of cardiac output, systemic O₂ delivery, and lung O₂ uptake at exercise onset in men. *Am J Physiol Regul Integr Comp Physiol*. 2006 Apr;290(4):R1071-1079.

120.

Lador F, Tam E, Azabji Kenfack M, Cautero M, Moia C, Morel DR, et al. Phase I dynamics of cardiac output, systemic O₂ delivery, and lung O₂ uptake at exercise onset in men in acute normobaric hypoxia. *Am J Physiol Regul Integr Comp Physiol*. 2008 Aug;295(2):R624-632.

121.

Lahiri MK, Kannankeril PJ, Goldberger JJ. Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. *J Am Coll Cardiol*. 2008 May 6;51(18):1725–33.

122.

Laitinen T, Hartikainen J, Vanninen E, Niskanen L, Geelen G, Länsimies E. Age and gender dependency of baroreflex sensitivity in healthy subjects. *J Appl Physiol*. 1998 Feb 1;84(2):576–83.

123.

Lanfranchi PA, Somers VK. Arterial baroreflex function and cardiovascular variability: interactions and implications. *Am J Physiol Regul Integr Comp Physiol*. 2002 Oct;283(4):R815-826.

124.

La Rovere MT, Bersano C, Gnemmi M, Specchia G, Schwartz PJ. Exercise-induced increase in baroreflex sensitivity predicts improved prognosis after myocardial infarction. *Circulation*. 2002 Aug 20;106(8):945–9.

125.

La Rovere MT, Pinna GD, Maestri R, Mortara A, Capomolla S, Febo O, et al. Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. *Circulation*. 2003 Feb 4;107(4):565–70.

126.

La Rovere MT, Pinna GD, Raczak G. Baroreflex Sensitivity: Measurement and Clinical Implications. *Annals of Noninvasive Electrocardiology*. 2008;13(2):191–207.

127.

La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, et al. Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: implications for clinical trials. *Circulation*. 2001 Apr 24;103(16):2072–7.

128.

Latson TW. Heart rate variability and anesthesiology: reasons for cautious optimism. *J Cardiothorac Vasc Anesth*. 1992 Dec;6(6):647–50.

129.

Laude D, Elghozi J-L, Girard A, Bellard E, Bouhaddi M, Castiglioni P, et al. Comparison of various techniques used to estimate spontaneous baroreflex sensitivity (the EuroBaVar study). *Am J Physiol Regul Integr Comp Physiol*. 2004 Jan;286(1):R226-231.

130.

Legramante JM, Raimondi G, Massaro M, Cassarino S, Peruzzi G, Iellamo F. Investigating feed-forward neural regulation of circulation from analysis of spontaneous arterial pressure and heart rate fluctuations. *Circulation*. 1999 Apr 6;99(13):1760–6.

131.

Levy WC, Cerqueira MD, Harp GD, Johannessen KA, Abrass IB, Schwartz RS, et al. Effect of endurance exercise training on heart rate variability at rest in healthy young and older men. *Am J Cardiol*. 1998 Nov 15;82(10):1236–41.

132.

Leyk D, Essfeld D, Hoffmann U, Wunderlich HG, Baum K, Stegemann J. Postural effect on cardiac output, oxygen uptake and lactate during cycle exercise of varying intensity. *Eur J Appl Physiol Occup Physiol*. 1994;68(1):30–5.

133.

Linnarsson D, Rosenhamer G. Exercise and arterial pressure during simulated increase of gravity. *Acta Physiol Scand*. 1968 Oct;74(1):50–7.

134.

Loeppky JA, Greene ER, Hoekenga DE, Caprihan A, Luft UC. Beat-by-beat stroke volume assessment by pulsed Doppler in upright and supine exercise. *J Appl Physiol Respir Environ Exerc Physiol*. 1981 Jun;50(6):1173–82.

135.

Lombardi F, Malliani A, Pagani M, Cerutti S. Heart rate variability and its sympatho-vagal modulation. *Cardiovasc Res*. 1996 Aug;32(2):208–16.

136.

Lombardi F, Stein PK. Origin of heart rate variability and turbulence: an appraisal of autonomic modulation of cardiovascular function. *Front Physiol.* 2011;2:95.

137.

Madden CJ, Sved AF. Rostral ventrolateral medulla C1 neurons and cardiovascular regulation. *Cell Mol Neurobiol.* 2003 Oct;23(4–5):739–49.

138.

Malik M. Standard Measurement of Heart Rate Variability. In: Malik M, Camm AJ, editors. *Dynamic Electrocardiography* [Internet]. Oxford, UK: Blackwell Publishing; 2004 [cited 2017 Mar 3]. p. 13–21. Available from: <http://doi.wiley.com/10.1002/9780470987483.ch2>

139.

Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation.* 1991 Aug;84(2):482–92.

140.

Malliani A, Pagani M, Lombardi F. Power spectral analysis of heart rate variability and baroreflex gain. *Clin Sci.* 1995 Nov;89(5):555–6.

141.

Malliani A, Pagani M, Montano N, Mela GS. Sympathovagal balance: a reappraisal. *Circulation.* 1998 Dec 8;98(23):2640–3.

142.

Mancia G, Groppelli A, Di Rienzo M, Castiglioni P, Parati G. Smoking impairs baroreflex sensitivity in humans. *Am J Physiol.* 1997 Sep;273(3 Pt 2):H1555–1560.

143.

Marieb E, Hoehn K. *Anatomie et physiologie humaines*. Pearson Education France; 2014. 1506 p.

144.

McIlveen SA, Hayes SG, Kaufman MP. Both central command and exercise pressor reflex reset carotid sinus baroreflex. *Am J Physiol Heart Circ Physiol.* 2001 Apr;280(4):H1454–1463.

145.

Mendelowitz D. Advances in Parasympathetic Control of Heart Rate and Cardiac Function. *News Physiol Sci.* 1999 Aug;14:155–61.

146.

Meyer M, Scheid P. Solubility of acetylene in human blood determined by mass spectrometry. *J Appl Physiol Respir Environ Exerc Physiol.* 1980 Jun;48(6):1035–7.

147.

Minson CT, Halliwill JR, Young TM, Joyner MJ. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation.* 2000 Feb 29;101(8):862–8.

148.

Miranda Dantas E, Lima Sant’Anna M, Varejão Andreão R, Pereira Gonçalves C, Aguiar Morra E, Perim Baldo M, et al. Spectral analysis of heart rate variability

with the autoregressive method: What model order to choose? *Computers in Biology and Medicine*. 2012 Feb;42(2):164–70.

149.

Mitchell JH. Neural circulatory control during exercise: early insights. *Exp Physiol*. 2013 Apr;98(4):867–78.

150.

Montano N, Cogliati C, Porta A, Pagani M, Malliani A, Narkiewicz K, et al. Central vagotonic effects of atropine modulate spectral oscillations of sympathetic nerve activity. *Circulation*. 1998 Oct 6;98(14):1394–9.

151.

Morikami Y, Yasue H, Okumura K, Horio Y, Fujii H, Matsuyama K. Effects of phentolamine and atropine on angina pectoris induced by handgrip test in patients with variant angina. *Am J Cardiol*. 1988 Jan 1;61(1):71–6.

152.

Morlet J, Arens G, Fourgeau E, Glard D. Wave propagation and sampling theory—Part I: Complex signal and scattering in multilayered media. *GEOPHYSICS*. 1982 Feb;47(2):203–21.

153.

Mullen TJ, Appel ML, Mukkamala R, Mathias JM, Cohen RJ. System identification of closed-loop cardiovascular control: effects of posture and autonomic blockade. *Am J Physiol*. 1997 Jan;272(1 Pt 2):H448-461.

154.

Nakata A, Takata S, Yuasa T, Shimakura A, Maruyama M, Nagai H, et al. Spectral analysis of heart rate, arterial pressure, and muscle sympathetic nerve activity in normal humans. *Am J Physiol*. 1998 Apr;274(4 Pt 2):H1211-1217.

155.

Ng J, Sundaram S, Kadish AH, Goldberger JJ. Autonomic effects on the spectral analysis of heart rate variability after exercise. *Am J Physiol Heart Circ Physiol*. 2009 Oct;297(4):H1421-1428.

156.

Ninomiya I, Nisimaru N, Irisawa H. Sympathetic nerve activity to the spleen, kidney, and heart in response to baroreceptor input. *Am J Physiol*. 1971 Nov;221(5):1346–51.

157.

Nishiyasu T, Nagashima K, Nadel ER, Mack GW. Effects of posture on cardiovascular responses to lower body positive pressure at rest and during dynamic exercise. *J Appl Physiol*. 1998 Jul;85(1):160–7.

158.

Notarius CF, Atchison DJ, Rongen GA, Floras JS. Effect of adenosine receptor blockade with caffeine on sympathetic response to handgrip exercise in heart failure. *Am J Physiol Heart Circ Physiol*. 2001 Sep;281(3):H1312-1318.

159.

Ogoh S, Wasmund WL, Keller DM, O-Yurvati A, Gallagher KM, Mitchell JH, et al. Role of central command in carotid baroreflex resetting in humans during static exercise. *J Physiol (Lond)*. 2002 Aug 15;543(Pt 1):349–64.

160.

Ogoh S, Fisher JP, Dawson EA, White MJ, Secher NH, Raven PB. Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans: Arterial baroreflex function in exercise. *The Journal of Physiology*. 2005 Jul;566(2):599–611.

161.

O'Leary DD, Kimmerly DS, Cechetto AD, Shoemaker JK. Differential effect of head-up tilt on cardiovagal and sympathetic baroreflex sensitivity in humans. *Exp Physiol*. 2003 Nov;88(6):769–74.

162.

Orizio C, Perini R, Comandè A, Castellano M, Beschi M, Veicsteinas A. Plasma catecholamines and heart rate at the beginning of muscular exercise in man. *Eur J Appl Physiol Occup Physiol*. 1988;57(5):644–51.

163.

Ormezzano O, Cracowski J-L, Quesada J-L, Pierre H, Mallion J-M, Baguet J-P. EVALuation of the prognostic value of BARoreflex sensitivity in hypertensive patients: the EVABAR study. *J Hypertens*. 2008 Jul;26(7):1373–8.

164.

Overton JM, Williams TD, Chambers JB, Rashotte ME. Cardiovascular and metabolic responses to fasting and thermoneutrality are conserved in obese Zucker rats. *Am J Physiol Regul Integr Comp Physiol*. 2001 Apr;280(4):R1007–1015.

165.

Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res*. 1986 Aug;59(2):178–93.

166.

Pagani M, Malliani A. Interpreting oscillations of muscle sympathetic nerve activity and heart rate variability. *J Hypertens*. 2000 Dec;18(12):1709–19.

167.

Pagani M, Montano N, Porta A, Malliani A, Abboud FM, Birkett C, et al. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation*. 1997 Mar 18;95(6):1441–8.

168.

Pagani M, Somers V, Furlan R, Dell'Orto S, Conway J, Baselli G, et al. Changes in autonomic regulation induced by physical training in mild hypertension. *Hypertension*. 1988 Dec;12(6):600–10.

169.

Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Groppelli A, et al. Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension*. 1988 Aug;12(2):214–22.

170.

Parati G, Di Rienzo M, Mancia G. Dynamic modulation of baroreflex sensitivity in health and disease. *Ann N Y Acad Sci*. 2001 Jun;940:469–87.

171.
Parati G, Mancia G, Di Rienzo M, Castiglioni P. Point:Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology*. 2006 Apr 6;101(2):676–82.
172.
Parati G, Ulian L, Santucci C, Omboni S, Mancia G. Blood pressure variability, cardiovascular risk and antihypertensive treatment. *J Hypertens Suppl*. 1995 Dec;13(4):S27-34.
173.
Pendergast DR, Olszowka A, Farhi LE. Cardiovascular and Pulmonary Responses to Increased Acceleration Forces During Rest and Exercise. *Aviation, Space, and Environmental Medicine*. 2012 May 1;83(5):488–95.
174.
Perini R, Orizio C, Baselli G, Cerutti S, Veicsteinas A. The influence of exercise intensity on the power spectrum of heart rate variability. *Eur J Appl Physiol Occup Physiol*. 1990;61(1–2):143–8.
175.
Perini R, Veicsteinas A. Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. *Eur J Appl Physiol*. 2003 Oct;90(3–4):317–25.
176.
Persson PB, Stauss H, Chung O, Wittmann U, Unger T. Spectrum analysis of sympathetic nerve activity and blood pressure in conscious rats. *Am J Physiol*. 1992 Nov;263(5 Pt 2):H1348-1355.
177.
Persson PB. Modulation of cardiovascular control mechanisms and their interaction. *Physiol Rev*. 1996 Jan;76(1):193–244.
178.
Piazza S, Furlan R, Dell’Orto S, Porta A, Lombardi F, Pagani M, et al. Mechanical effects of respiration and stepping on systolic arterial pressure variability during treadmill exercise. *J Hypertens*. 1995 Dec;13(12 Pt 2):1643–7.
179.
Piccirillo G, Cacciafesta M, Viola E, Santagada E, Nocco M, Lionetti M, et al. Influence of aging on cardiac baroreflex sensitivity determined non-invasively by power spectral analysis. *Clin Sci*. 2001 Mar;100(3):267–74.
180.
Pichot V, Gaspoz JM, Molliex S, Antoniadis A, Busso T, Roche F, et al. Wavelet transform to quantify heart rate variability and to assess its instantaneous changes. *J Appl Physiol*. 1999 Mar;86(3):1081–91.
181.
Pichot V, Roche F, Celle S, Barthélémy J-C, Chouchou F. HRVanalysis: A Free Software for Analyzing Cardiac Autonomic Activity. *Front Physiol*. 2016;7:557.
- 182.

Pickering TG, Gribbin B, Petersen ES, Cunningham DJ, Sleight P. Effects of autonomic blockade on the baroreflex in man at rest and during exercise. *Circ Res*. 1972 Feb;30(2):177–85.

183.

Pipilis A, Flather M, Ormerod O, Sleight P. Heart rate variability in acute myocardial infarction and its association with infarct site and clinical course. *Am J Cardiol*. 1991 May 15;67(13):1137–9.

184.

Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, et al. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol*. 1985 Jan;248(1 Pt 2):H151-153.

185.

Ponikowski P, Anker SD, Chua TP, Szelemej R, Piepoli M, Adamopoulos S, et al. Depressed heart rate variability as an independent predictor of death in chronic congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1997 Jun 15;79(12):1645–50.

186.

Porter GA, Rivkees SA. Ontogeny of humoral heart rate regulation in the embryonic mouse. *Am J Physiol Regul Integr Comp Physiol*. 2001 Aug;281(2):R401-407.

187.

Potts JT, Shi XR, Raven PB. Carotid baroreflex responsiveness during dynamic exercise in humans. *Am J Physiol*. 1993 Dec;265(6 Pt 2):H1928-1938.

188.

Pozza RD, Kleinmann A, Bechtold S, Fuchs A, Netz H. Reinnervation after heart transplantation in children: results of short-time heart rate variability testing. *Pediatr Transplant*. 2006 Jun;10(4):429–33.

189.

Querry RG, Smith SA, Strømstad M, Ide K, Raven PB, Secher NH. Neural blockade during exercise augments central command's contribution to carotid baroreflex resetting. *Am J Physiol Heart Circ Physiol*. 2001 Apr;280(4):H1635-1644.

190.

Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. *Med Biol Eng Comput*. 2006 Dec;44(12):1031–51.

191.

Raven PB, Potts JT, Shi X. Baroreflex regulation of blood pressure during dynamic exercise. *Exerc Sport Sci Rev*. 1997;25:365–89.

192.

Raven PB, Fadel PJ, Ogoh S. Arterial baroreflex resetting during exercise: a current perspective. *Exp Physiol*. 2006 Jan;91(1):37–49.

193.

Raven PB, Fadel PJ, Smith SA. The influence of central command on baroreflex resetting during exercise. *Exerc Sport Sci Rev*. 2002 Jan;30(1):39–44.

194.

Raven PB. Recent advances in baroreflex control of blood pressure during exercise in humans: an overview. *Med Sci Sports Exerc.* 2008 Dec;40(12):2033–6.

195.

Ray CA, Rea RF, Clary MP, Mark AL. Muscle sympathetic nerve responses to dynamic one-legged exercise: effect of body posture. *Am J Physiol.* 1993 Jan;264(1 Pt 2):H1-7.

196.

Reed RM, Eberlein M, Girgis RE, Hashmi S, Iacono A, Jones S, et al. Coronary artery disease is under-diagnosed and under-treated in advanced lung disease. *Am J Med.* 2012 Dec;125(12):1228.e13-1228.e22.

197.

Remington JW, Wood EH. Formation of peripheral pulse contour in man. *J Appl Physiol.* 1956 Nov;9(3):433–42.

198.

Rimoldi O, Furlan R, Pagani MR, Piazza S, Guazzi M, Pagani M, et al. Analysis of neural mechanisms accompanying different intensities of dynamic exercise. *Chest.* 1992 May;101(5 Suppl):226S–230S.

199.

Robinson BF, Epstein SE, Beiser GD, Braunwald E. Control of heart rate by the autonomic nervous system. Studies in man on the interrelation between baroreceptor mechanisms and exercise. *Circ Res.* 1966 Aug;19(2):400–11.

200.

Robinson TG, Dawson SL, Eames PJ, Panerai RB, Potter JF. Cardiac baroreceptor sensitivity predicts long-term outcome after acute ischemic stroke. *Stroke.* 2003 Mar;34(3):705–12.

201.

Rohdin M, Linnarsson D. Differential changes of lung diffusing capacity and tissue volume in hypergravity. *J Appl Physiol.* 2002 Sep;93(3):931–5.

202.

Rosenhamer G. Influence of increased gravitational stress on the adaptation of cardiovascular and pulmonary function to exercise. *Acta Physiol Scand Suppl.* 1967;276:1–61.

203.

Rosenstock E, Cassuto Y, Zmora E. Heart rate variability in the neonate and infant: analytical methods, physiological and clinical observations. *Acta Pædiatrica.* 1999;88(5):477–482.

204.

Rowell LB, O’Leary DS, Kellogg DL. Integration of Cardiovascular Control Systems in Dynamic Exercise. In: *Handbook of physiology Exercise: regulation integration of multiple systems.* Bethesda, MD: American Physiological Society; 1996. p. 770–838.

205.

Rowell R. *Human Cardiovascular Control.* New York, NY; Oxford.: Oxford University Press;

206.
Saltin B, Rådegran G, Koskolou MD, Roach RC. Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol Scand*. 1998 Mar;162(3):421–36.
207.
Sandercock GRH, Brodie DA. The Role of Heart Rate Variability in Prognosis for Different Modes of Death in Chronic Heart Failure. *Pacing and Clinical Electrophysiology*. 2006 Aug 1;29(8):892–904.
208.
Saul JP, Rea RF, Eckberg DL, Berger RD, Cohen RJ. Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. *Am J Physiol*. 1990 Mar;258(3 Pt 2):H713-721.
209.
Saul JP, Arai Y, Berger RD, Lilly LS, Colucci WS, Cohen RJ. Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis. *The American Journal of Cardiology*. 1988 Jun;61(15):1292–9.
210.
Schaeffers HJ, Waxman MB, Patterson GA, Frost AE, Maurer J, Cooper JD. Cardiac innervation after double lung transplantation. Toronto Lung Transplant Group. *J Thorac Cardiovasc Surg*. 1990 Jan;99(1):22–9.
211.
Schneider S, Guardiera S, Kleinert J, Steinbacher A, Abel T, Carnahan H, et al. Centrifugal acceleration to 3Gz is related to increased release of stress hormones and decreased mood in men and women. *Stress*. 2008 Sep;11(5):339–47.
212.
Schwartz CE, Medow MS, Messer Z, Stewart JM. Spontaneous fluctuation indices of the cardiovagal baroreflex accurately measure the baroreflex sensitivity at the operating point during upright tilt. *Am J Physiol Regul Integr Comp Physiol*. 2013 Jun 15;304(12):R1107-1113.
213.
Schwartz PJ, Zaza A, Pala M, Locati E, Beria G, Zanchetti A. Baroreflex sensitivity and its evolution during the first year after myocardial infarction. *J Am Coll Cardiol*. 1988 Sep;12(3):629–36.
214.
Seals DR, Suwarno NO, Joyner MJ, Iber C, Copeland JG, Dempsey JA. Respiratory modulation of muscle sympathetic nerve activity in intact and lung denervated humans. *Circ Res*. 1993 Feb;72(2):440–54.
215.
Sheriff DD, Rowell LB, Scher AM. Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *Am J Physiol*. 1993 Oct;265(4 Pt 2):H1227-1234.
216.
Sigaud D, Fortrat JO, Allevard AM, Maillet A, Cottet-Emard JM, Vouillarmet A, et al. Changes in the sympathetic nervous system induced by 42 days of head-down bed rest. *Am J Physiol*. 1998 Jun;274(6 Pt 2):H1875-1884.

217.

Singh N, Mironov D, Armstrong PW, Ross AM, Langer A. Heart rate variability assessment early after acute myocardial infarction. Pathophysiological and prognostic correlates. GUSTO ECG Substudy Investigators. Global Utilization of Streptokinase and TPA for Occluded Arteries. *Circulation*. 1996 Apr 1;93(7):1388–95.

218.

Sivieri A, Fagoni N, Bringard A, Capogrosso M, Perini R, Ferretti G. A beat-by-beat analysis of cardiovascular responses to dry resting and exercise apnoeas in elite divers. *Eur J Appl Physiol*. 2015 Jan;115(1):119–28.

219.

Skrapari I, Tentolouris N, Katsilambros N. Baroreflex function: determinants in healthy subjects and disturbances in diabetes, obesity and metabolic syndrome. *Curr Diabetes Rev*. 2006 Aug;2(3):329–38.

220.

Smith SA, Query RG, Fadel PJ, Gallagher KM, Strømstad M, Ide K, et al. Partial blockade of skeletal muscle somatosensory afferents attenuates baroreflex resetting during exercise in humans. *J Physiol (Lond)*. 2003 Sep 15;551(Pt 3):1013–21.

221.

Smyth HS, Sleight P, Pickering GW. Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ Res*. 1969 Jan;24(1):109–21.

222.

Spaak J, Montmerle S, Sundblad P, Linnarsson D. Long-term bed rest-induced reductions in stroke volume during rest and exercise: cardiac dysfunction vs. volume depletion. *J Appl Physiol*. 2005 Feb;98(2):648–54.

223.

Stauss HM. Heart rate variability. *Am J Physiol Regul Integr Comp Physiol*. 2003 Nov;285(5):R927–931.

224.

Steinback CD, O’Leary DD, Bakker J, Cechetto AD, Ladak HM, Shoemaker JK. Carotid distensibility, baroreflex sensitivity, and orthostatic stress. *J Appl Physiol*. 2005 Jul;99(1):64–70.

225.

Strempel S, Schroeder C, Hemmersbach R, Boese A, Tank J, Diedrich A, et al. Norepinephrine transporter inhibition alters the hemodynamic response to hypergravitation. *J Appl Physiol*. 2008 Mar;104(3):756–60.

226.

Taha BH, Simon PM, Dempsey JA, Skatrud JB, Iber C. Respiratory sinus arrhythmia in humans: an obligatory role for vagal feedback from the lungs. *J Appl Physiol*. 1995 Feb;78(2):638–45.

227.

Tam E, Azabji Kenfack M, Cautero M, Lador F, Antonutto G, di Prampero PE, et al. Correction of cardiac output obtained by Modelflow from finger pulse pressure profiles with a respiratory method in humans. *Clin Sci*. 2004 Apr;106(4):371–6.

228.

Tanaka K, Nishimura N, Sato M, Kanikowska D, Shimizu Y, Inukai Y, et al. Arterial pressure oscillation and muscle sympathetic nerve activity after 20 days of head-down bed rest. *Auton Neurosci*. 2013 Oct;177(2):266–70.

229.

Tank J, Baevski RM, Fender A, Baevski AR, Graves KF, Ploewka K, et al. Reference values of indices of spontaneous baroreceptor reflex sensitivity. *Am J Hypertens*. 2000 Mar;13(3):268–75.

230.

Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of Measurement, physiological interpretation, and clinical use. *Circulation*. 1996;(93):1043–65.

231.

Taylor JA, Carr DL, Myers CW, Eckberg DL. Mechanisms underlying very-low-frequency RR-interval oscillations in humans. *Circulation*. 1998 Aug 11;98(6):547–55.

232.

Traon AP, Sigaud D, Vasseur P, Maillet A, Fortrat JO, Hughson RL, et al. Cardiovascular responses to orthostatic tests after a 42-day head-down bed-rest. *Eur J Appl Physiol Occup Physiol*. 1998;77(1–2):50–9.

233.

Tuininga YS, van Veldhuisen DJ, Brouwer J, Haaksma J, Crijns HJ, Man in't Veld AJ, et al. Heart rate variability in left ventricular dysfunction and heart failure: effects and implications of drug treatment. *Br Heart J*. 1994 Dec;72(6):509–13.

234.

Vallais F, Baselli G, Lucini D, Pagani M, Porta A. Spontaneous baroreflex sensitivity estimates during graded bicycle exercise: a comparative study. *Physiol Meas*. 2009 Feb;30(2):201–13.

235.

Van de Louw A. Etude des interactions cardiorespiratoires chez le patient sous ventilation mécanique: contribution respective des effets mécaniques et nerveux dans la genèse de la variabilité à court terme des paramètres cardiovasculaires. Thèse de l'Université d'Evry Val d'Essonne; 2010.

236.

van Lieshout JJ, Toska K, van Lieshout EJ, Eriksen M, Walløe L, Wesseling KH. Beat-to-beat noninvasive stroke volume from arterial pressure and Doppler ultrasound. *Eur J Appl Physiol*. 2003 Sep;90(1–2):131–7.

237.

Verlinde D, Beckers F, Ramaekers D, Aubert AE. Wavelet decomposition analysis of heart rate variability in aerobic athletes. *Auton Neurosci*. 2001 Jul 20;90(1–2):138–41.

238.

Victor RG, Secher NH, Lyson T, Mitchell JH. Central Command Increases Muscle Sympathetic Nerve Activity During Intense Intermittent Isometric Exercise in Humans. *Circulation Research*. 1995 Jan 1;76(1):127–31.

239.
Viola E. La Variabilité Cardiaque au Cours des Cycles de Sommeil chez l'Homme: un Test d'Evaluation de l'Activité du Système Nerveux Autonome. 2004.
240.
Warren JH, Jaffe RS, Wraa CE, Stebbins CL. Effect of autonomic blockade on power spectrum of heart rate variability during exercise. *Am J Physiol*. 1997 Aug;273(2 Pt 2):R495-502.
241.
Wehrwein EA, Joyner MJ. Autonomic Nervous System: Chapter 8. Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. Elsevier Inc. Chapters; 2013. 30 p.
242.
Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*. 1993 May;74(5):2566–73.
243.
Westerhof BE, Gisolf J, Karemaker JM, Wesseling KH, Secher NH, van Lieshout JJ. Time course analysis of baroreflex sensitivity during postural stress. *Am J Physiol Heart Circ Physiol*. 2006 Dec;291(6):H2864-2874.
244.
White DW, Raven PB. Autonomic neural control of heart rate during dynamic exercise: revisited. *J Physiol (Lond)*. 2014 Jun 15;592(12):2491–500.
245.
Wickerson L, Mathur S, Brooks D. Exercise training after lung transplantation: A systematic review. *The Journal of Heart and Lung Transplantation*. 2010 May;29(5):497–503.
246.
Wieling W, Harms MP, ten Harkel AD, van Lieshout JJ, Sprangers RL. Circulatory response evoked by a 3 s bout of dynamic leg exercise in humans. *J Physiol (Lond)*. 1996 Jul 15;494 (Pt 2):601–11.
247.
Winer BJ, Brown DR, Michels KM. Statistical Principles in Experimental Design. McGraw-Hill; 1991. 1080 p.
248.
Wolf MM, Varigos GA, Hunt D, Sloman JG. Sinus arrhythmia in acute myocardial infarction. *Med J Aust*. 1978 Jul 15;2(2):52–3.
249.
Wong S. Segmentation de l'intervalle RT et description par analyse factorielle de la variabilité de la fréquence cardiaque et de la repolarisation ventriculaire [Internet]. Thèse de l'Université de Rennes 1; 2004.
250.
Wray DW, Formes KJ, Weiss MS, O-Yurvati AH, Raven PB, Zhang R, et al. Vagal cardiac function and arterial blood pressure stability. *Am J Physiol Heart Circ Physiol*. 2001 Nov;281(5):H1870-1880.

251.

Zuanetti G, Neilson JMM, Latini R, Santoro E, Maggioni AP, Ewing DJ. Prognostic Significance of Heart Rate Variability in Post Myocardial Infarction Patients in the Fibrinolytic Era: The GISSI-2 Results. *Circulation*. 1996 Aug 1;94(3):432–6.

