NONINVASIVE METHODS IN CARDIOLOGY 2022

Edited by: **Cornélissen G., Siegelová J., Dobšák P.**

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Contents

Lifetime Achievement Award:
Prof. MUDr. Jarmila Siegelova, DrSc.

Petr Dobšák

Dept. of Physiotherapy and Rehabilitation, Dept. of Sports Medicine and Rehabilitation, Faculty of Medicine, Masaryk University, St. Anna's Teaching Hospital, Brno

In the first week of January this year, Prof. MUDr. Jarmila Siegelova, DrSc. celebrated her 80th anniversary, former Head of the Department of Functional Diagnostics and Rehabilitation at St. Anne's University Hospital, Faculty of Medicine of Masaryk University in Brno and the Department of Physiotherapy and Rehabilitation of the Faculty of Medicine of Masaryk University (LF MU). We and all those who have or had the honor to work closely with her in the past still appreciate her untiring work commitment, her willingness to help wherever it is needed and the indomitable optimism she is known for. Since her graduation in 1965, she has had an extremely long and sometimes tortuous professional journey, but one that has enabled her to reach a top level of scientific research and teaching. As an assistant professor at the Department of Physiology of the Medical Faculty of Masaryk University (since 1965) she carried out the first scientific work on experimental animals, where she chose methodologically demanding experiments of stimulation and sensing of action potentials of nerve fibres in the framework of research on splanchnic nerves in the regulation of respiration. Later on, she returned to the topic of respiratory regulation many times, but this time in the context of clinical studies in healthy and sick subjects. She also defended her doctoral thesis on this topic in 1990 and habilitated in 1991. She became a member of the International Society of Pathophysiology of Respiration and repeatedly lectured at international scientific conferences. Her doctoral dissertation (defended in 1990) concerned the neural regulation of respiration in healthy humans and in some selected pathological conditions. For many years she has been (and still is) devoted to the problems of pathogenesis and treatment of essential hypertension, issues of chronobiology of blood pressure and heart rate. It is in this field that she has achieved her greatest lifetime scientific success. She holds the world primacy in the discovery of the weekly rhythm in circulatory parameters, which is the result of natural laws and not the result of social evolution.

Professor Siegelova was the first in the world to show that the seven-day variation in blood pressure and heart rate in newborns is synchronized with birth and is independent of the days of the week. This means that the week is programmed in the human organism and the social evolution in different cultures to make the seventh day a holiday is probably determined by this

biologically determined phenomenon. Her observations, made in babies born at the University Hospital in Brno, were then independently confirmed by studies conducted in newborns in Minnesota and in La Coruna, Spain. The main focus of her work is still the study of circulatory rhythms in hypertonic patients. Prof. Siegelová is one of the pioneers of 24-hour blood pressure monitoring in hypertensive patients in the Czech Republic. Already in 1993 she published (as the main author) a study in which she compared different methods of evaluation of 24-hour blood pressure recording in treated and untreated hypertensives. As a long-time member of the Chronobiology Centre in Minnesota (USA), she is the main author or co-author of numerous studies dealing with multi-hour continuous blood pressure monitoring. Here, too, are a number of unique research results with world firsts. From 1996 to 2007 she headed the Department of Physical Medicine and Rehabilitation at St. Anne's Hospital in Brno and until 2012 also the Department of Physiotherapy and Rehabilitation at the Faculty of Medicine of the Medical University of Brno. At this point, it should be emphasized that she has made a fundamental and decisive contribution to the establishment and development of the non-medical field of Physiotherapy at the Faculty of Medicine of the Medical University, both bachelor's and postgraduate studies in Czech and English. From the very beginning of her scientific career, she understood very well the importance of international cooperation. This allowed her to apply her undeniable professional potential, knowledge of foreign languages and excellent organizational skills. She has become a member of many important international societies (such as the International Society of Hypertension, the European Respiratory Society, the Société de Physiologie, the New York Academy of Sciences, etc. She continues to successfully develop collaborations with the Chronobiology Center of the University of Minnesota (USA), University of Graz (Austria), Hôpital Lariboisière Paris (France), Tohoku University of Sendai (Japan), etc. Almost every day he is engaged in teaching work at the clinic and department and still lectures to medical and non-medical students in Czech and English. Prof. Siegelová is the author of more than 400 original scientific publications with extensive citation record (426 in Web of Science, 1385 in Scopus and 459 in other databases). Prof. Siegelová has always been and still is a passionate and ruthless critic of injustice or injustice. She has never appropriated other people's knowledge; on the contrary, she continues to dispense selflessly and with initiative from an incredibly vast well of her own ideas. She never pretends to be omniscient and values and respects the opinions or comments of her colleagues. The professor has a very positive relationship with art and nature. These non-work activities have become an integral part of her optimism in life, which is reflected in her relationship with members of her work team, patients and students. Dear Professor, the team of the Department of Physical Medicine and Rehabilitation and the team of the Department of Physiotherapy and Rehabilitation wish you good health and lots of creative activity in the years to come.

In the course of this year, on the occasion of her life jubilee, Ms. Professor Jarmila Siegelova was awarded by two important Czech institutions as a recognition of her professional biomedical

contributions over the whole of career. In June of 2022, Professor Siegelova was awarded by HONORARY DIPLOMA by the Society of Rehabilitation and Physical Medicine of the Czech Medical Society of Jan Evangelist Purkinje.
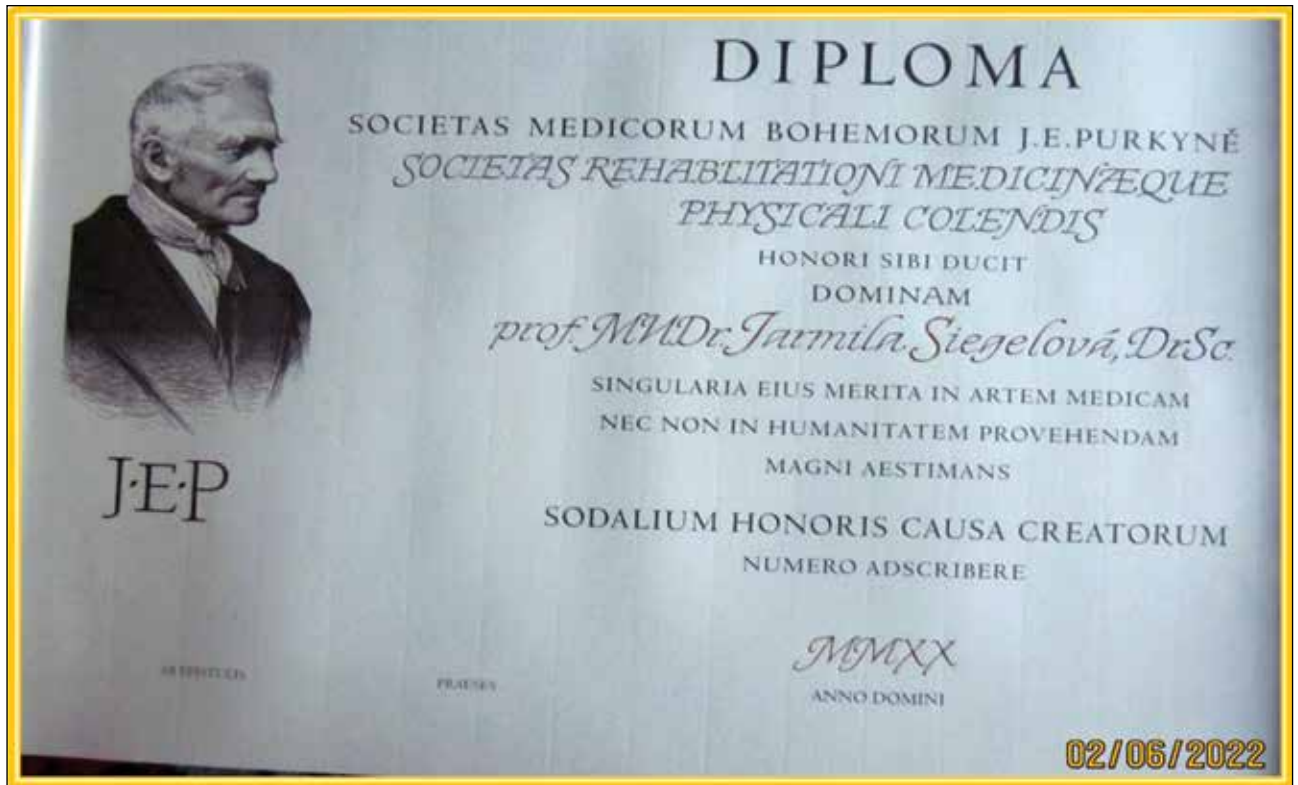
Figure 1: *HONORARY DIPLOMA by the Society of Rehabilitation and Physical Medicine of the Czech Medical Society of Jan Evangelist Purkinje*

Figure 2: *More recently, in October of this year, Professor SIEGELOVA was also was appointed professor emeritus of the Masaryk University in Brno.*

These awards express the respect and prestige that Professor Jarmila Siegelova had earned through a lifetime of work in the field of science, research and education.

Figure 3

Honoring Professor Jarmila Siegelova on the Occasion of her 80th Birthday

Germaine Cornelissen, Mary Sampson, Linda Sackett-Lundeen, A Chase Turner, Larry A Beaty

Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN 5455, USA

It was April 9, 1990 when I first met Professor Jarmila Siegelova at an International Symposium on Hypertension organized by the late Professor Pavel Prykril in Brno, Czechoslovakia. This meeting saw the start of a long-term close cooperation between Masaryk University and the Halberg Chronobiology Center at the Minneapolis campus of the University of Minnesota. At the reception following the lectures, while participants were meddling, Jarmila and I sat down in a quiet corner of the room to design a protocol to test the circadian stage-dependence of low-dose aspirin on platelet aggregation. Results were first published in a letter to JAMA [1].

Many more joint publications followed, as Professor Jarmila Siegelova, Professor Bohumil Fiser, and Dr. Jiri Dusek came to Minnesota to cooperate with us on several other projects, and Professor Franz Halberg, Dr. Othild Schwartzkopff and I went to Brno to participate at scientific meetings, many of them organized by Jarmila. In later years, our participation has been carried out remotely, taking advantage of technological advances in communication. In 2002, the late Prof. MUDr. Bohumil Fiser, CSc., then Czech Minister of Health and Board Member of WHO shared many aspects of Jarmila's professional life, and commented on her achievements in great detail [2].

Jarmila's work throughout her distinguished career focused on important topics, introducing a chronobiologic aspect in most of her studies. Only selected works are highlighted below. Prof. Siegelova showed with us that the effect of low-dose aspirin on blood pressure was also circadian stage-dependent [3]. She investigated any circadian-stage dependence of the baroreflex in normotensive individuals and in hypertensive patients [4, 5]. Several joint studies revolved around the regulation of breathing, the topic of Jarmila's doctoral thesis. She first mapped the circadian variation of respiratory variables, coordinated by the vegetative nervous system, and their relation to cardiovascular variables [6]. She then documented that the circadian rhythm of blood pressure was reduced in patients with sleep apnea [7] and that CPAP treatment for one year only slightly increased the circadian amplitude of systolic blood pressure [8].

When ambulatory blood pressure monitors first became available, Jarmila studied the effect of several anti-hypertensive medications on the circadian blood pressure rhythm. She showed that the ACE inhibitor Enalapril decreased the MESOR as well as the circadian amplitude of

blood pressure of patients with essential hypertension and patients with secondary hypertension with glomerulonephritis chronica [9]. She further showed that different anti-hypertensive medications had different effects on blood pressure variability, including its circadian amplitude [10].

Prof. Siegelova and her Brno team joined our international project on the BIOsphere and the COSmos (BIOCOS), originally known as the Womb-to-Tomb study, which recommended ambulatory monitoring for a minimum of 7 days due to the large day-to-day variability in blood pressure [11, 12]. The consensus meeting was first held in Brno, where it was signed and ratified [12]. Within the scope of BIOCOS, Jarmila made numerous contributions, documenting the need to monitor blood pressure for longer than 24 hours due to the large day-to-day variability in circadian rhythm characteristics [13], mapping changes in circadian rhythm characteristics as a function of age in clinical health and in patients [14-16], and estimating arterial stiffness from ABPM records [17-19]. Jarmila also showed that illumination of the bedroom at night increases systolic blood pressure by about 11 mmHg [20]. Much interest is now devoted to circadian disruption and the bidirectional relationship it has with ill health.

When early transverse results suggested that blood pressure may undergo a prominent about weekly variation during the first week of life [21], it was important to validate the finding by longitudinal monitoring over spans longer than 7 days. Jarmila's studies of premature babies provided the needed confirmation [22]. She further showed that the weekly variation was synchronized to the time of birth rather than by the social schedule [23]. Later work on Minnesotan premature twins suggested that the circaseptan variation might be partly genetically anchored [24].

The above constitutes just a sample of a long list of contributions made by Professor Siegelova. We congratulate her on her well-deserved life achievement award and wish her many more years of productive scientific accomplishments.

Ad multos annos!

Professor Jarmila Siegelova

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EM. UNIV.-Prof. DR. MED. UNIV. Thomas Kenner, D.h.c. mult. 29.9.1932 – 22.12.2018

Jarmila Siegelova

Dept. of Physiotherapy and Rehabilitation, Dept. of Sports Medicine and Rehabilitation, Faculty of Medicine, Masaryk University, St. Anna's Teaching Hospital, Brno

Prof. Dr. Thomas Kenner, M.D., Dr. h.c. mult.
Dr. h. c. Universität Jena, 1990
Dr. h. c., Semmelweis University Budapest, 1998
Dr. h. c., Masaryk University Brno, 2000
Head, Dept. of Physiology, Karl-Franzens-Universität Austria, 1972-1997
Rektor (president) Karl-Franzens-Universität, Austria, 1989-1991
Dean of Medical School, Karl-Franzens-Universität, Austria, 1991-1997

Prof. Dr. Thomas Kenner, M.D., Dr. h.c. mult. will be remembered at the occasion of 90th years of birth as an exceptional expert in physiology of cardiovascular system medicine, professor emeritus of Karl-Franzens-Universität Austria.

The personality of Prof. Kenner was earlier described Noninvasive Methods of Cardiology 2015, 2017 and 2019.

Prof. Thomas Kenner was exceptional physiologist who focused primarily on physiology of cardiovascular system, covering diverse areas such as aerodynamic properties of arteries, chronobiology of cardiovascular system, pathophysiology and incidence of sudden infant death syndrome, physiology and monitoring of physiological functions in space.

Prof. Thomas Kenner cooperation with Faculty of Medicine, Masaryk University, Brno, Czech Republic started in 1991. Prof. Thomas Kenner was known in our University as a scientist from the publication about the dynamic of arterial pulses from the year 1968. We met personally for the first time in Prague in 1991 on International Physiological Congress, he was also accompanied by his wife Brigitte Kenner. Then he went to Masaryk University and at the meeting we signed an agreement of cooperation and since this time we were meeting every year once or twice in Brno, where we organized every year one Symposium about Chronobiology at Faculty of Medicine and one Symposium during Medical Trade Fair in Brno.

Figure 1: *On the left Prof. Eduard Schmidt, Prof. Franz Halberg, Prof. Thomas, Kenner, Rector Prof. RNDr. Jiri Zlatuska, Prof. Jarmila Siegelova, Prof. Libor Pac, Dr. Honoris Causa, Celebration, Masaryk University, Brno 2000*

We have had a great luck to cooperate with Prof. Thomas Kenner from nineties in the last century, he visited Brno every year two or three times, presented every time one or two lectures and disussed with me, late Prof. Bohumil Fiser, CSc., Dr. Jiri Dusek, CSc., Prof. Petr Dobsak, CSc., late Prof. Jan Penaz, CSc., late Profesor Zdenek Placheta, DrSc., late Prof. Pavel Braveny, CSc., Masaryk University and our other excellent scientist from abroad late Prof. Dr. Franz Halberg, D.h.c, father of chronobiology, Prof. Dr. Germaine Cornelissen, University Minnesota, head of Halberg Chronobiology Center, USA, late Prof. Dr. Jean Paul Martineaud, Medical Faculty, University Paris, France, Prof. Jean Eric Wolf, University Dijon, France, Dr. Jean Christoph Eicher, University Dijon France and other cooperating visitors from Japan Prof. Masario Kohzuki University Sendai and Prof. Kohji Shirai, Toho University, Chiba.

Figure 2: *On the left Brigitte Kenner, Prof. Thomas Kenner, Prof. Dieter Platzer, University of Graz, Austria, MUDr. Jiri Dusek, Prof. Jarmila Siegelova, Masaryk University, on the screen Prof. Germaine Cornelissen, University of Minnesota, USA, in Noninvasive methods 2015 in Brno*

The presentations were published in Scripta Medica, Masaryk University Brno (included in SCOPUS database), in books and in Noninvasive Methods of Cardiology 1996, 1999, 2002,

2003, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016 and it is possible to find then on Masaryk University, CZ web sides https://www.med.muni.cz/noninvasive-methods-in-cardiology.

In honor of excellent scientific work of Professor Thomas Kenner, new head of Dept. of Physiology, Medical University of Graz, Assoc. Prof. Dr. Nandu Goswami, secretary Austrian Physiological Society organized Quadrilateral Physiology Symposium 2019 with the international participation prom Austria, Slovakia, Slovenia, Croatia, also with us from Brno "Vascular Physiology, Physiological Techniques and Medical Education" in Medical University of Graz on 21st of June 2019.

We thank Prof. Kenner very much for his friendship, collaboration, enthusiasm, and for pushing ahead the frontiers of knowledge in medicine and we will continue his scientific work in the medicine.

Brno, October 2022   Prof. MUDr. Jarmila Siegelova, DrSc.

Effect of Telmisartan on Cardiovascular Markers in Cardiac Patients: In Honor of Pavel Prikryl

Germaine Cornelissen[1] and Jarmila Siegelova[2]

[1]*Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA*
[2]*Masaryk University, Brno, Czech Republic*

Correspondence:

Germaine Cornelissen
Halberg Chronobiology Center
University of Minnesota,
420 Delaware St. S.E.
MMC8609
Minneapolis, MN 55455, USA
Tel.: +1 612 624 6976 FAX +1 612 624 9989
E-mail: corne001@umn.edu
Website: https://halbergchronobiologycenter.umn.edu/halberg-chronobiology-center

Dedication:

The data presented herein are those of the late Professor Pavel Prikryl who designed the study and collected the data. We had plans to present the results in a joint publication. Unfortunately, the publication could not be completed before his passing. His data and our analysis thereof are here summarized in his honor.

Abstract

The action of an angiotensin receptor blocker (ARB) and of an angiotensin-converting enzyme (ACE) inhibitor alone or in combination on a number of cardiovascular biomarkers is evaluated in 18 outpatients with essential hypertension, using a double-blind, randomized, crossover design. Study stages lasted 7 days, separated by a 2-day washout. Treatment was administered daily, upon awakening. During each stage (placebo, ARB treatment, ACE inhibitor treatment, combined treatment), blood pressure, heart rate, cardiac output, ejection fraction and brain natriuretic peptide (BNP) as well as angiotensin II, plasma renin activity and bradykinin were measured four times a day, at 6-hour intervals. The least squares fit of a 24-hour cosine curve to each individual 7-day record assessed the circadian variation, further summarized across all 18 patients by population-mean cosinor. Parameter tests assessed treatment effect and any difference in the efficacy of the two kinds of medication. Results indicate that BNP and other cardiovascular variables are circadian periodic. Treatment with Telmisartan (ARB) and Lisinopril (ACE inhibitor) have positive effects on cardiovascular health. BNP is greatly decreased with both treatments. Combined treatment with both drugs achieve even larger effects than with any one drug used alone. Whether treatment effects can be further optimized by timing remains to be determined, whether in terms of important cardiac markers and/or adverse cardiovascular outcomes.

Introduction

In current practice, the diagnosis and management of high blood pressure (BP) still relies primarily on repeated single measurements by health professionals, despite overwhelming evidence that BP is characterized by a prominent circadian variation [1]. Prospective studies in treated and untreated hypertensive patients and in the general population have shown that, even after adjusting for known risk factors, BP correlates with the incidence of cardiovascular events [2]. As such, BP is widely used as a marker of cardiovascular health.

There is increased interest in other biomarkers of hypertension and related cardiovascular diseases for predicting and preventing morbidity and mortality associated with these diseases [3]. Hypertension is known to promote vascular smooth muscle cell remodelling [4], endothelial cell dysfunction and atherosclerosis [5]. Clinical biomarkers used in clinical practice that are associated with cardiovascular events include C-reactive protein (CRP), cardiac troponins I and T (cTnI and cTnT), D-dimer, and B-type natriuretic peptides (BNP and NT-proBNP) [6-8].

- CRP is elevated in inflammatory conditions, such as atherosclerosis, and elevated CRP concentrations correlate with future cardiovascular risks [9]. CRP is also positively associated with BP variability [10] and with pulse pressure [11].

- cTnI and cTnT are biomarkers useful in diagnosing acute MI and in stratifying risks in acute coronary syndrome [3].

- D-dimer is a biomarker of thrombosis, cardiovascular mortality, acute aortic dissection, and ischemic heart disease [3].

- BNP and NT-proBNP are biomarkers used to diagnose heart failure [12].

BNP is a 32-amino acid protein that is released into the bloodstream in response to increased ventricular overload. It is made inside the pumping chambers of the heart when pressure builds up from heart failure [13]. Because of its excellent specificity, BNP has been widely utilized as a cardiac biomarker in the diagnosis of heart failure. BNP testing can also assist in the monitoring of the clinical effectiveness of treatment and the estimation of disease progression.

Herein, we present data from the late Professor Pavel Prikryl who designed a study aimed at assessing the efficacy of Telmisartan and Lisinopril used as single or combined therapy, as gauged by changes in the circadian variation of BP and other biomarkers.

Materials and Methods

The study, conducted in June-July 2002 involved 18 outpatients with essential hypertension (WHO II or III). Some patients were diagnosed with heart failure of an ischemic etiology (NYHA IV) or with stable angina pectoris (CCS class II or III), Table 1. The study used a double-blind, randomized, crossover design. It had four stages, each lasting 7 days. Consecutive stages were separated by a 2-day washout period. Patients received a Placebo (P) during the first stage, the angiotensin receptor blocker Telmisartan (80 mg/day) (A) during the second stage, the angiotensin-converting enzyme inhibitor Lisinopril (10 mg/day) (E) during the third stage, and the A-E combination (C) during the fourth stage. The study started after 7 days of adjustment to study conditions and a 7-day reference stage. Treatment was administered in the morning, after awakening.

Table 1. Patient characteristics

No.	Age	Gender	Diagnosis	Therapy	NYHA	CCS
1	55	M	EH II., AP	BB	I.	I.
2	62	M	EH III., HF	Di, Diu, BB	IV.	
3	50	F	EH II.	BB	I.	
4	48	M	EH III., HF	Di, Diu, BB	IV.	
5	52	F	EH II., AP	BB, Ni	I.	II.
6	58	M	EH III., HF, AP	BB, Ni, Di, Diu	IV.	II.
7	44	F	EH II.	BB	I.	
8	57	M	EH III., HF	Di, Diu, BB	IV.	
9	50	m	EH II.	BB	I.	
10	49	F	EH II., AP	BB, Ni	I.	II.
11	65	M	EH III., HF	Di, Diu, BB	IV.	
12	51	F	EH II.	BB	I.	
13	46	M	EH II., AP	BB, Ni	I.	II.
14	60	F	EH III., HF	Di, Diu, BB	IV.	
15	42	M	EH II.	BB		
16	58	F	EH III., HF	Di, Diu, BB	IV.	
17	50	M	EH II.	BB, Diu	I.	
18	66	M	EH II., AP	BB, Ni	I.	II.

EH: Essential Hypertension (WHO classification); AP: Angina Pectoris; HF: Heart Failure;
Di: Digoxin; Diu: Diuretics; Ni: Nitroglycerin; BB: Beta-blockers;
NYHA: New York Heart Association; CCS: Canadian Cardiovascular Society Classification.

The following variables assessed the response to treatment: Heart rate (HR) was measured by electrocardiography. Systolic (S) and diastolic (D) BP were measured oscillometrcally, using an OMRON BP monitor. The ejection fraction (EF) was assessed by echocardiography [14]. Angiotensin II (ATII) was determined by radioimmunoassay [15], as was plasma renin activity (PRA) [16]. Bradykinin (BK) was determined by colorimetry. Plasma brain natriuretic peptide (BNP) was determined by a method of Murdoch et al. [17]. Natriuretic peptide concentrations may be useful for monitoring effects of ACE inhibitors [18]. Cardiac output (CO) was calculated according to Hatle and Angelsen [19].

These variables were measured four times a day, at 06:00, 12:00, 18:00 and 24:00 for 7 days during each of the four study stages.

A 24-hour cosine curve was fitted to each individual record by cosinor, yielding estimates of the MESOR (M, a rhythm-adjusted mean), 24-hour amplitude (A) and acrophase (ϕ), measures of the extent and timing of predictable change within a day [20, 21]. The acrophase is the phase of the predicted maximum in relation to a reference time. It is expressed in (negative) degrees, with 360° equated to 24 hours and 0° set to local midnight [20, 21]. Individual estimates of

circadian characteristics were summarized by population-mean cosinor [20, 21], and compared among study stages by parameter tests [21, 22].

Results

Figure 1 illustrates the time course of SBP and DBP in each of the four study stages. The circadian variation, which is readily apparent to the naked eye, is validated by population-mean cosinor in each stage of the study (P<0.001). A sharp decrease in SBP and DBP on treatment can also be discerned. The efficacy of treatment is validated by parameter tests. As expected, the combined treatment achieves a larger decrease in the MESOR of both SBP and DBP (P<0.001). Telmisartan lowers the MESOR of SBP (P<0.001) and DBP (P=0.014) more than Lisinopril and does not decrease the circadian amplitude of SBP or DBP, while Lisinopril does (P<0.001).

Figure 1: *Time course of SBP and DBP during each study stage.*
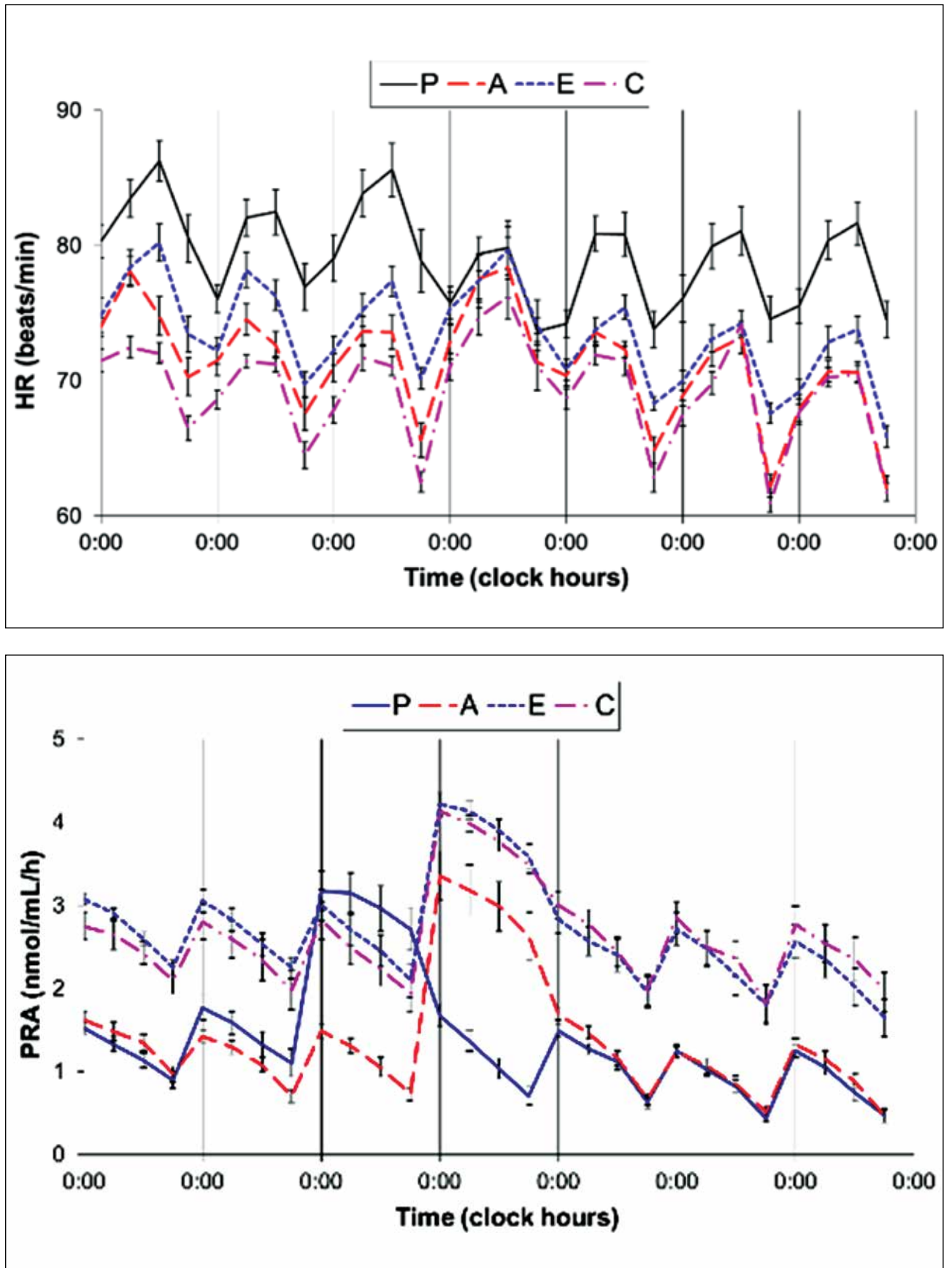*P: Placebo; A: Telmisartan; E: Lisinopril; C: A-E Combined treatment*

Figure 2: *Time course of HR and CO during each study stage.*
P: Placebo; A: Telmisartan; E: Lisinopril; C: A-E Combined treatment

Figure 2 displays the time course of HR and CO in each of the four study stages. The circadian variation in both variables is statistically significant in each stage, as documented by population-mean cosinor (P<0.001). While HR decreases on treatment, CO increases greatly. On placebo, HR averages 79.2 beats/min and CO is 4.0 L/min. By comparison, on combined treatment, HR only averages 69.4 beats/min and CO is 6.1 L/min. The circadian amplitude of CO is also increased from 0.14 ± 0.05 to 0.36 ± 0.06 L/min (P<0.001). HR is slightly lower on Telmisartan than on Lisinopril (71.3 vs. 73.6 beats/min, P=0.029) and its circadian amplitude is slightly larger (4.2 vs. 3.7 beats/min, P=0.080). No difference in CO is observed between the two monotherapies, but the circadian amplitude of CO is larger on Telmisartan than on Lisinopril (0.32 vs. 0.16, P<0.001).

Figure 3: *Time course of ATII, PRA and BK during each study stage.*
P: Placebo; A: Telmisartan; E: Lisinopril; C: A-E Combined treatment

Figure 3 shows the time course of ATII, PRA and BK in each of the four study stages. All three variables are characterized by a circadian rhythm, documented by population-mean cosinor during each study stage (P<0.001). Lisinopril, but not Telmisartan is associated with a decrease in ATII, and an increase in PRA and BK (P<0.001). ATII averages 29.4, 27.9, and 13.4

pg/mL on placebo, Telmisartan, and Lisinopril, respectively. PRA averages 1.39, 1.40, and 2.68 nmol/mL/h on placebo, Telmisartan and Lisinopril, respectively. Likewise, BK averages 47.4, 44.5, and 136.1 pg/mL on placebo, Telmisartan and Lisinopril, respectively. The circadian amplitude of ATII is decreased (from 4.5 to 2.6 pg/mL) and that of BK is slightly increased (from 6.7 to 8.0 pg/mL) on Lisinopril as compared to placebo. The effects of the cmbined treatment reflect the effect of Lisinopril on these three variables.
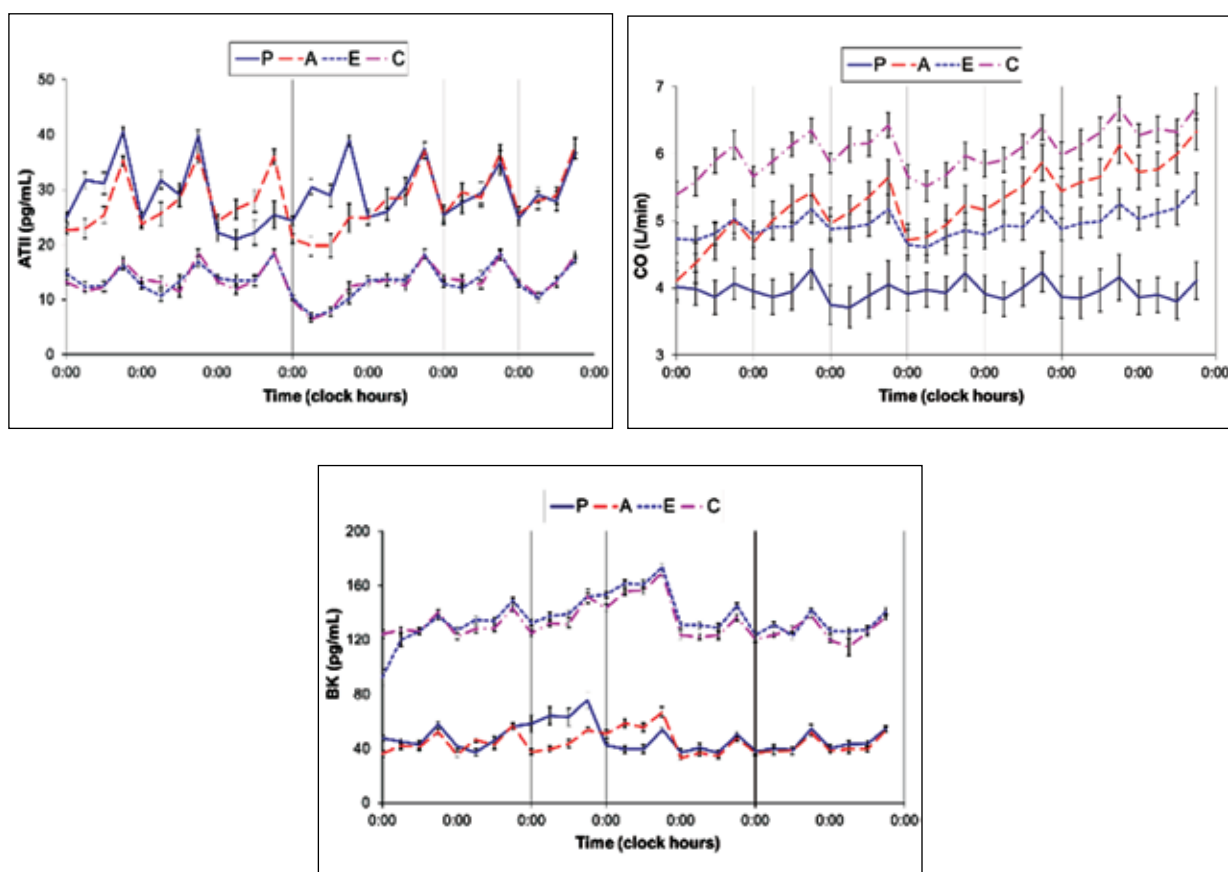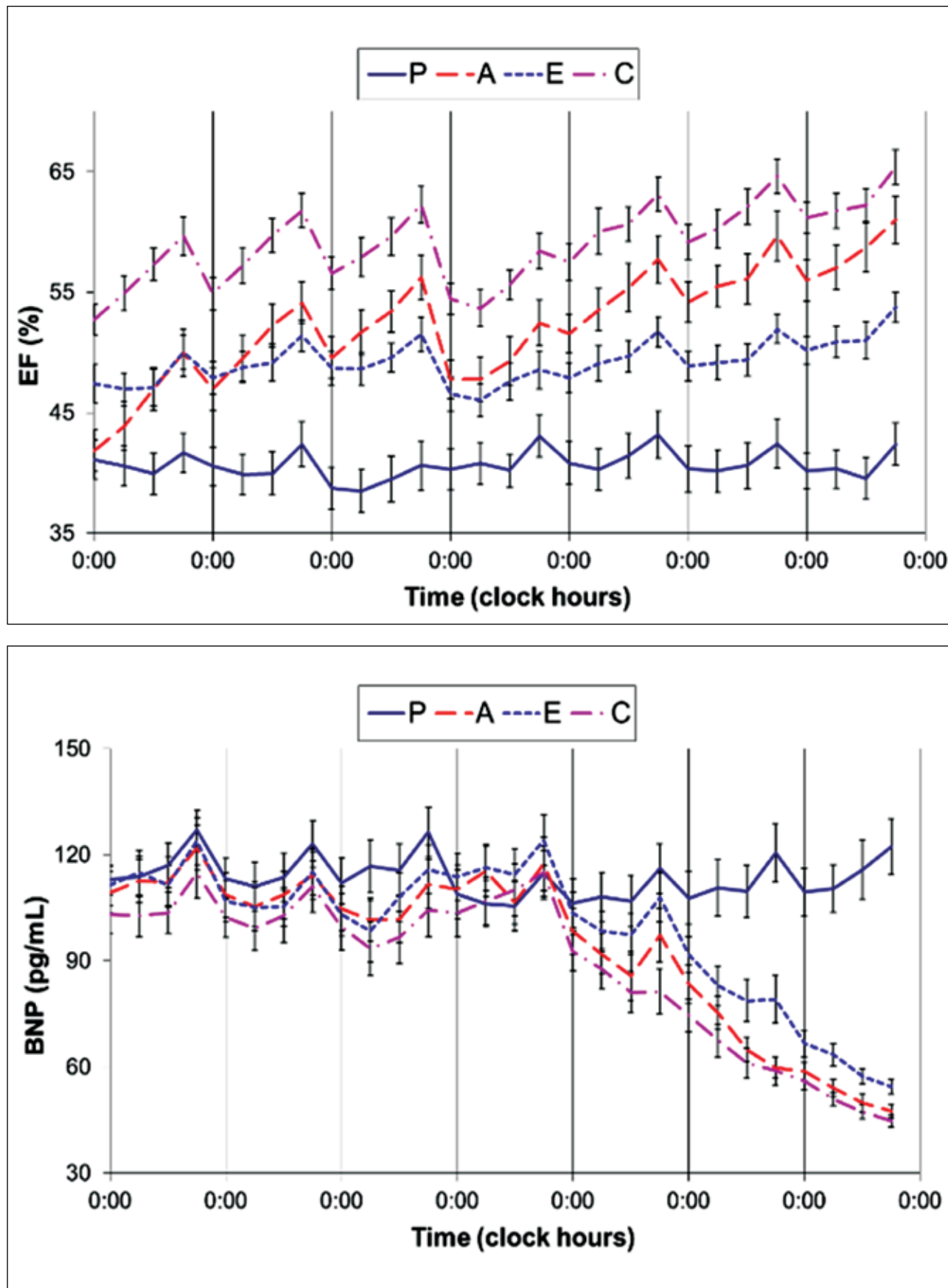
Figure 4: *Time course of EF and BNP during each study stage.*
P: Placebo; A: Telmisartan; E: Lisinopril; C: A-E Combined treatment

Figure 4 illustrates the time course of EF and BNP in each of the four study stages. Overall, a circadian rhythm is documented by population-mean cosinor for EF (P<0.001) and BNP (P<0.005) in each study stage. While EF averages 40.7% on placebo, it is increased to 52.5%, 49.3%, and 59.1% on Telmisartan, Lisinopril, and combines treatment, respectively (P<0.001). Telmisartan also increases the circadian amplitude of EF from 1.1% to 2.9%.
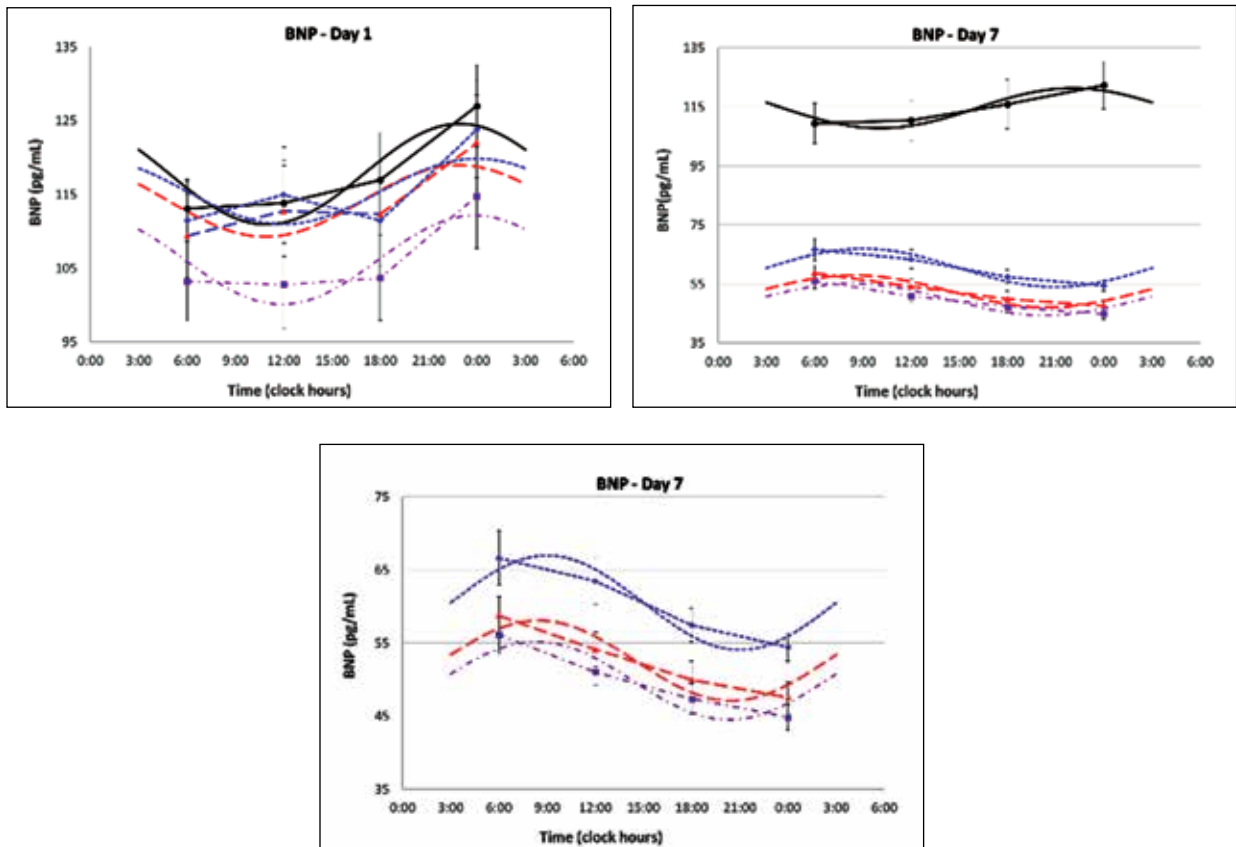
Figure 5: *Circadian variation in BNP at start (Day 1) and end (Day 7) of each study stage. P: Placebo (black); A: Telmisartan (red); E: Lisinopril (blue); C: A-E Combined Rx (purple)*

As apparent from Figure 4, BNP deserves additional analysis in view of the delay in the effect of treatment, starting only about 4 days on a given regimen. Figure 5 illustrates the circadian variation in BNP on the first and last day of each study stage. On the first day of treatment (Figure 5, left), only a small decrease in BNP is seen on treatment, which is most visible for the combined Telmisartan-Lisinopril treatment. At the end of 7 days on a given treatment, a clear decrease in BNP is seen on treatment as compared to placebo (Figure 5, middle). Telmisartan has a greater effect on BNP than Lisinopril (Figure 5, right).

Discussion and Conclusion

Both Telmisartan and Lisinopril exert statistically significant and clinically relevant effects on blood pressure, heart rate, cardiac output, ejection fraction and brain natriuretic peptide. While the effect of treatment can immediately be seen for most of these biomarkers, it takes about 4 days to become apparent in the case of BNP. Treatment with an angiotensin receptor blocker or an angiotensin-converting enzyme inhibitor can also affect the circadian variation in these biomarkers, and these effects can differ between the two treatment kinds. It is thus important to account for the circadian variation of these variables in the absence of treatment when deciding on a drug regimen. In some cases, it may be important to increase the circadian amplitude of a given variable, whereas in other cases, the opposite may be true, as discussed elsewhere in relation to blood pressure and heart rate variability [23, 24].

Natriuretic peptides have an important role in regulating the circulation. They act on blood vessels, causing them to dilate, or widen. They also work on the kidneys, causing them to excrete more salt and water. In addition, the natriuretic peptides reduce the production of various hormones that narrow blood vessels, boost the heart rate, or affect fluid retention; examples include adrenaline, angiotensin, and aldosterone [25]. The net effect of natriuretic peptides is to promote urine excretion, relax blood vessels, lower blood pressure, and reduce the heart's workload. They are part of the body's natural defense mechanisms designed to protect the heart from stress. And they surge into action when they are needed most, when the heart itself is under siege [26].

In this study, treatment was administered upon awakening. Whether treatment effects can be further optimized by timing remains to be determined, whether in terms of important cardiac markers and/or adverse cardiovascular outcomes. Circadian stage-dependent effects of treatment have already been documented earlier in cooperation with Professor Pavel Prikryl in the case of Telmisatran and low-dose aspirin [27, 28]. The importance of accounting for the chronodiagnosis as a guide to treatment timing, considering that the optimal treatment time may differ from one patient to another has also been illustrated [29, 30].

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Cuffless Blood Pressure Monitoring Devices for Chronobiologic Applications

Germaine Cornelissen[1,2], Jarmila Siegelova[3], Kuniaki Otsuka[1,4], Denis Gubin[5,6], Ellis Nolley[2], Christopher Adams[2], Linda Sackett-Lundeen[1], A Chase Turner[1,2], Larry A Beaty[1,2]

[1]*Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA*
[2]*IEEE Twin Cities Phoenix Project Group*
[3]*Masaryk University, Brno, Czech Republic*
[4]*Tokyo Women's Medical University, Tokyo, Japan*
[5]*Laboratory for Chronobiology and Chronomedicine, Research Institute of Biomedicine and Biomedical Technologies, Medical University, Tyumen, Russia*
[6]*Tyumen Cardiology Research Center, Tomsk National Research Medical Center, Russian Academy of Science, Tomsk, Russia*

Correspondence:

Germaine Cornelissen
Halberg Chronobiology Center
University of Minnesota,
420 Delaware St. S.E.
MMC8609
Minneapolis, MN 55455, USA
Tel.: +1 612 624 6976 FAX +1 612 624 9989
E-mail: corne001@umn.edu
Website: https://halbergchronobiologycenter.umn.edu/halberg-chronobiology-center

Abstract

Herein, we comment on the status of cuffless blood pressure (BP) devices recently reviewed by the European Society of Hypertension (ESH). Our own experience in the field supports the ESH's statement that current cuffless devices cannot be recommended for the diagnosis and

management of hypertension. As pointed out by the ESH, it will be important for cuffless BP devices to be fully and correctly validated, not over populations with widely different average BP values, but for each individual citizen, since ultimately, diagnosis and treatment decisions will need to be made for each individual patient. We highlight the distinction that should be made between validation for truly ambulatory BP monitoring and home BP monitoring. We add to the list of technologies considered by the ESH the monitoring of bioimpedance as a surrogate for BP, using flexible electronics. We also show some results we obtained after modifying existing commercial off-the-shelf home-market wrist-cuff-based monitors. We conclude by stressing the importance of analytical methods for a chronobiologic analysis and interpretation of data collected by BP monitors.

Introduction

The European Society of Hypertension (ESH) recently reviewed the status of cuffless blood pressure (BP) devices and issued a statement regarding BP monitoring and cardiovascular variability [1]. The ESH recognizes that technologies underlying cuffless BP devices have considerable potential to improve the awareness, treatment, and management of hypertension, but recent guidelines by the ESH do not recommend cuffless devices for the diagnosis and management of hypertension.

Our earlier publication [2] commented on recommendations specifically made in relation to the use of ambulatory BP monitoring (ABPM) in the most recent guidelines by the American College of Cardiology (ACC) and the American Heart Association (AHA) [3] and those by the European Society of Cardiology (ESC) and the ESH [4]. Noteworthy was the emphasis these guidelines placed on home BP monitoring (HBPM) for the detection of white-coat hypertension and masked hypertension, two conditions long known to underlie the limitation of clinic BP measurements [2].

While recognizing the merits of HBPM for its ability to track changes in BP longitudinally, concerns remain as to its ability to assess circadian characteristics of BP accurately. Concerns remain also that the guidelines only consider ABPM for 24 hours, which is not sufficient in view of the large day-to-day variability in the circadian rhythm characteristics of BP [2]. Our own studies led to our recommendation to obtain 7-day/24-hour ABPM records at the outset in order to identify vascular variability disorders (VVDs) [5]. VVDs are abnormal patterns of BP and/or heart rate (HR) variability, such as deviations from 90% prediction limits for the MESOR, circadian amplitude and/or acrophase of BP, derived from clinically healthy peers of the same gender and a similar age group.

Ideally, one should be able to measure BP around the clock for several days, if not longitudinally, as part of health surveillance. Our desire is to monitor BP "for long-term use on a massive scale to obtain measures of health and encourage the development of diagnostic, prevention and treatment techniques". This prospect prompted the formation of the IEEE Twin Cities Phoenix Project Group, which mission is to "develop an ABPM that is inexpensive, unobtrusive, easy-to-use and collects a week of BP measurements every half hour day and night", based on an open-source business model [6]. The non-invasive, cuffless, automatic monitoring of BP turned out to be very difficult, as illustrated in the ESH review of cuffless devices [1]. Herein, we review the needs and hurdles in assessing BP variability (BPV) from a chronobiologic perspective. We illustrate some new developments in the monitoring of BP with cuffless devices, and discuss any role they may play in chronobiologic research.

Home versus Ambulatory BP Monitoring for longitudinal around-the-clock monitoring

For HBPM there are now different monitors available commercially using either an arm cuff or a wrist cuff. These monitors necessitate the user to initiate a measurement manually. BP measurements are typically obtained in a seated position, after resting for a few minutes. The need for the user to activate a measurement thus limits measurements to be obtained when the user is awake. In order to obtain measurements during sleep, one thus needs to wake up, thereby influencing the measurement, or having somebody else activate the monitor, which can also be problematic. Cuffless BP devices would hence be particularly useful if they could take measurements automatically at regular intervals day and night.

Not all cuffless BP devices considered by the ESH are suitable as ABPM devices, however. Obtaining undisturbed night-time BP measurements is critical to reliably assess the circadian BP variation. Night-time BP measurements are also thought to be important in their own right to predict cardiovascular disease risk [7]. In Japan, participants with masked nocturnal hypertension were found to be at high risk of future cardiovascular (CVD) events [8].

For a reliable screening, diagnosis, and assessment of response to treatment, it is also critical to obtain measurements over several days. The Japanese study of masked nocturnal hypertension monitored participants' daytime and nocturnal BP for 14 consecutive day [8]. The need to follow BP variation for longer than 24 hours stems from the large day-to-day variability in circadian characteristics of BP, which can be observed in hypertension as well as in normotension [2]. Being able to follow BP changes around the clock on a long-term basis prompted interest in cuffless BP devices.

ESH assessment

The ESH statement [1] about cuffless BP devices mentions that "fundamental questions regarding their accuracy, performance, and implementation need to be carefully addressed before they can be recommended for clinical use". While reviewing the literature on the topic in 2017, we also concluded that the testing of cuffless devices was not particularly standardized and did not involve a single organization. We found 2009 to be a turning point in wrist monitor quality. There is a need for testing to be standardized beyond current guidelines, notably if cuffless devices will eventually be considered to serve as ABPM and not just as HBPM.

In their publication [1], the ESH distinguishes between devices requiring user cuff calibration and those not requiring user cuff calibration. The ESH also reviews different technologies underlying the various cuffless BP devices.

Technologies requiring user cuff calibration can be considered to track rather than measure BP since they assess changes from the initial calibration. They include:

- Pulse transit time: time delay between proximal and distal arterial waveforms, serving as a surrogate for the time delay between ECG and finger photoplethysmography (PPG) waveforms;

- Pulse wave analysis: BP-related features extracted from a beat-to-beat arterial waveform;

- Facial video processing: pulse waveform features extracted from the facial skin in a video stream (PPG-based).

Technologies not requiring user cuff calibration include:

- Oscillometric finger pressing: a sensor-unit measures the variable-amplitude PPG waveform and the applied pressure, thus working like a conventional brachial cuff-based monitor;

- Ultrasound: for instance, the cross-sectional area and blood velocity of the carotid artery can be measured to compute pulse pressure;

- Volume control: a finger-worn ring uses a servo-controlled actuator that continually applies external pressure to the finger to clamp the average of PPG-measured blood volume over a cardiac cycle to its unloaded level to measure mean BP.

As mentioned above, not all these technologies support ambulatory monitoring; some only support manual measurements.

The ESF makes the important comment that validation procedures should be carefully undertaken. Too often, validation is performed by linear regression of BP data obtained with the tested device and a trusted device (used as reference) from a population of individuals

with widely different BP values. The resulting good correlation can be misleading when the same procedure applied to single individuals shows a lack of correlation or even contradictory positive and negative correlations on an individual basis. Unless validation procedures become standardized, spurious over-optimistic results will continue to be published. For instance, in one study [9], the good correlation shown between readings from a cuffless BP device and the reference standard covers four clearly apparent clusters likely representing four different populations. Focusing on any one of these clusters, the correlation between the two measurement approaches seems to be lost. In another study [10], lack of fit is readily seen by the naked eye from the linear regression of BP readings between a cuffless BP device and a reference standard, strongly suggesting the relationship to be nonlinear. Correlation analyses based on populations with greatly different average BP values are best replaced with methods focusing on each individual, such as statistics on BP differences between paired measurements obtained from the tested and reference devices, as advocated earlier [11].

Provided that calibrations are made with each change in posture, reliable BP measurements can be obtained with certain cuffless BP devices. This was the case of the CareTaker, a tonometer-based device developed by Dr. Gerdt, which we had the opportunity to familiarize ourselves with a few years ago. It used Bluetooth technology for automatic data transfer to a computer. BP was derived from the beat-to-beat waveform analyzed by pulse decomposition [12].

Bioimpedance as surrogate to measure blood pressure?

Another emerging technology to measure BP without a cuff consists of graphene temporary tattoos [13]. The sensor uses a temporary tattoo made of graphene, which is protected by an ultrathin polymer film. It works by measuring bioimpedance, essentially the tissue's resistance to an alternating electrical current, as blood pulses through the artery under the tattoo. Since a machine-learning algorithm converts bioimpedance to BP, training on individual users is required to extract BP from subtle features of the shape of the impedance curve over the pulse cycle. When a blood pulse passes through an artery, the overall tissue impedance drops. Higher BP correlates with faster propagation of blood pulses. By measuring bioimpedance at two sites on the same artery, pulse transit time then can also be used to assess BP. In one example, the BP sensor used six graphene patches lined up over the radial artery, on the side of the wrist nearest the thumb, and an additional six cover the ulnar artery on the other side. In each set, the electrodes on each end inject an imperceptibly tiny electric current into the wrist. The other four are split into two pairs, each of which measures the induced potential difference, which is proportional to the impedance [13].

Results from our IEEE Twin Cities Phoenix Project

Apart from pulse wave velocity (PWV) and pulse wave analysis (PWA), our experiments with non-arm-cuff technologies to measure BP considered another interesting avenue, consisting of improvements of commercial off-the-shelf (COTS) home-market cuff-based monitors [14]. As part of a feasibility project, circuits for a TI MSP430 microcontroller timer and alarm were added to COTS wrist-cuff monitors such as the OMRON HEM-670IT and the A&D UB- 511USB, without affecting the monitor's performance, Figure 1. Monitors are not reverse-engineered. The microcontroller is wired in parallel with the pushbutton switch via open-collector connection and speaker of the monitor, and it is powered from the monitor's batteries. No other part of the monitor circuit is modified. A&D also supplied us with monitors programmed to automatically take readings (add-on microcontroller timer was not used in this case).

Timer control circuit   **Timer and Buzzer control circuit**
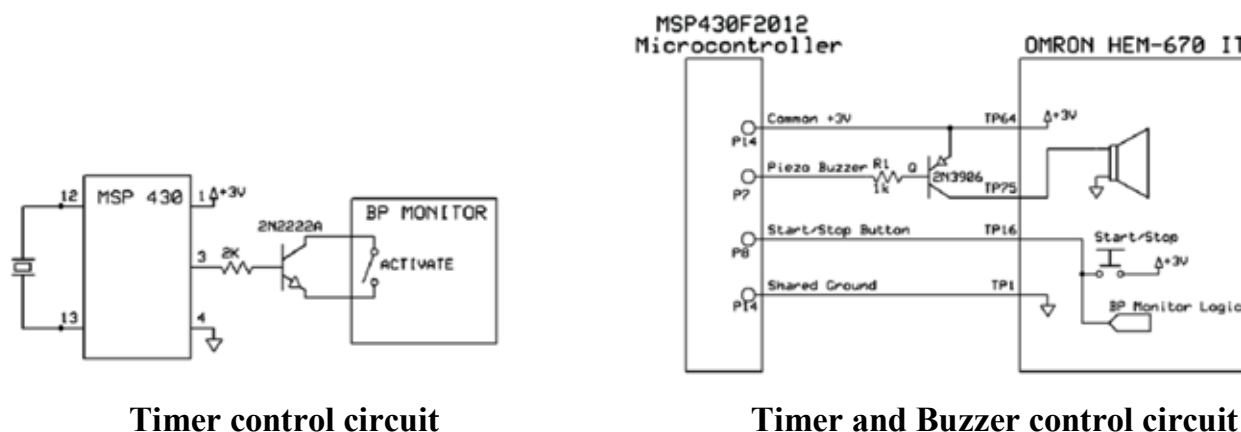
Figure 1: *Circuits for a TI MSP430 microcontroller timer and alarm added to COTS wrist-cuff monitors.*

This proof-of-concept project indicated that the design modification of existing HBPMs for chronobiologically-interpreted ABPM (C-ABPM) led to the feasibility of an inexpensive solution (at a cost below $100). Figure 2 illustrates data obtained from such a modified HBPM.
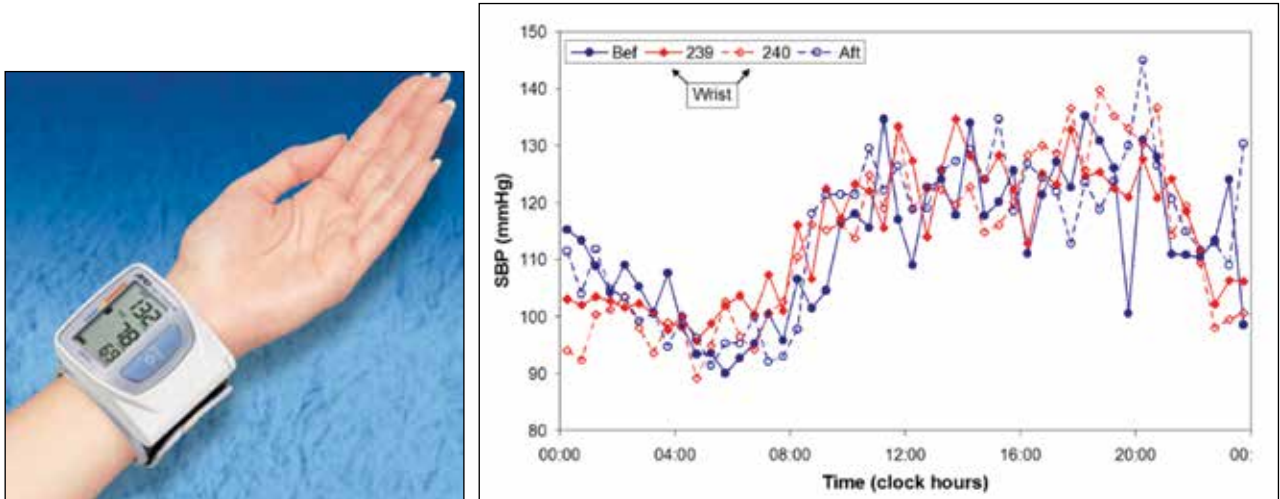
Figure 2:. *Systolic (S) BP data from a clinically healthy 59-year old woman obtained with a modified A&D wrist monitor tested versus the A&D TM-2430 ABPM.*

The protocol used to obtain the data shown in Figure 2 consisted of wearing both the wrist-cuff HBPM and the arm-cuff ABPM on the same non-dominant arm. BP measurements with the wrist HBPM bracketed the ABPM readings obtained after a 2-minute delay. Every 30 minutes, two HBPM measurements were thus obtained with each ABPM measurement, one before and one after the ABPM reading. Parameter tests [15] found no difference in circadian rhythm characteristics between the two HBPM profiles obtained 4 minutes apart (P>0.7). Circadian rhythm characteristics did not differ either between two weeklong ABPM records (#239 and #240 shown in Figure 2) bracketing the HBPM profile (P≥0.2). A comparison of circadian rhythm characteristics between the HBPM record and one of the two ABPM records showed no difference in circadian amplitude (P>0.6) or acrophase (P≥0.2). There was only a small difference in MESOR of about 3 mmHg (P=0.012) due to the fact that the wrist monitor was not invariably kept at heart level when a measurement was taken. Similar results apply to diastolic (D) BP.

While design modifications of existing HBPMs for C-ABPM already provide a documented affordable solution, manufacturers may only target home monitoring. Technical issues remain, such as battery power and making sure that the wrist monitor is correctly positioned when measurements are being taken. Data analysis and chronobiologic interpretation have not (yet?) been a major part of the debate. We only found a few examples of attempts made to productize cuffless BP monitors with inexpensive ABPM capability. They will need FDA approval before becoming commercially available.

Concluding remarks

Cuffless BP devices are attractive to affordably and comfortably obtain repeated BP measurements, but few (if any) have been validated and their accuracy is questionable. Not all cuffless BP devices considered by the ESH yield automated BP measurements needed for chronobiological applications. Wrist cuff devices have been validated for manual use only since their accuracy depends on keeping the monitor at heart level. Further research in this area may be promising. Automated measurements during day and night over several days are important, but so are analytical methods used for their analysis and interpretation, as discussed elsewhere [16].

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Thrombosis Risk During Hypokinesia, in Space and in Disease States

Nandu Goswami [1,2]

[1]*Gravitational Physiology and Medicine Research Unit, Division of Physiology, Otto Löwi Research Center of Vascular Biology, Inflammation, and Immunity, Medical University of Graz, Austria*
[2]*College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates*

Abstract

The presence of gravity on Earth has impacted the evolution of our physiological systems. Ever since our ancestors started standing upright, they have had to compensate for the the gravity-induced blood pooling – and the increased hydrostatic pressure - in the lower limbs during upright standing. Humans have , however, developed excellent mechanisms to ensure that blood is returned to the heart during upright standing and blood pressure is maintained.

During spaceflight, however, all these evolutionary processes have to adjust. The lack of gravity in space does not lead to pooling of blood in the lower limbs but rather to a headward shift of the blood. Depending on the spaceflight duration, compensatory responses occur which lead to a resetting of the physiological systems to a new level, which is not at 1g but rather at microgravity level. In other words, as long as we are in space our physiological systems adapt to the new microgravity set point.

Due to the limited number of astronauts traveling to microgravity, bedrest studies of varying duration are routinely used to understand the physiological deconditional processes that arise in different physiological systems. During bedrest, healthy young volunteers are confined to strict bedrest for periods of up to 60 days! This presentation then points out that older persons spend most of their times, especially during hospitalization, lying in bed. Since many of the older persons already have signs and symptoms associated with aging related frailty, and are not as fit as the astronauts when they assume bedrest confinement (either due to chronic diseases or while awaiting surgical interventions), the physiological deconditioning during bedrest parallels – or is even worse than - what is seen during spaceflight.

Despite human presence in space of over 50 years, incidences of blood clots or thrombo-embolic events during spaceflight or upon return to Earth have not been largely reported.

However, all that changed in January 2020, with a case report of thromboembolism in space in the prestigious *New England Journal of Medicine*. The European Space Agency (ESA) swung into action and created a topical team to address the issue of thrombo-embolism in space and in ground based model. Dr Goswami is the co-ordinator of the ESA topical team. This talk provides an overview of the current literature, the current knowledge gaps in this area as well as the different groups that were formed to tackle this issue in an organized way.

Overall, it appears that there are gaps in almost every aspect related to thromboembolism in space, including in the basic physiological knowledge on human blood coagulation during spaceflight to operational and technical gaps for processes and equipment needed to diagnose and optimally manage this newly identified medical challenge. Similarly, how interventions that address spaceflight induced physiological deconditioning could potentially influence the coagulatory state are poorly studied.

As most of the coagulatory events are related to changes in endothelial health, this presentation then discusses how the knowledge applied from spaceflight was used to provide a rationale to understand COVID-19 associated high risk of thromo-emboli generation. This presentation then concludes by stating that this is a classical example of how life in space can help life on Earth.

Keywords

Gravity, Spaceflight, Aging, COVID-19, Bedrest confinement, Countermeasures, Endothelial health.

Blood Pressure Measurement in 30 Years of Noninvasive Methods of Cardiology in Masaryk University Brno, Czech Republic: Measurement of Blood Pressure with Cuff and Cuffless Blood Pressure Measurement in 2022 According to European Society of Hypertension

[1] **Jarmila Siegelova**, [3] **Germaine Cornelissen**, [1,2] **Alena Havelkova**, [1] **Jiri Dusek**, [1,2] **Leona Dunklerova**, [1] **Michal Pohanka**, [1,2] **Petr Dobsak**

[1]*Dept. of Physiotherapy and Rehabilitation, Faculty of Medicine, Masaryk University, St. Anna's Teaching Hospital, Brno,*
[2]*Dept. of Sports Medicine and Rehabilitation, Faculty of Medicine, Masaryk University, St. Anna's Teaching Hospital, Brno,*
[3]*University of Minnesota, USA*

The paper "Cuffless blood pressure measuring devices: review and statement by the European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability and blood pressure measurement with cuff" was published in J Hypertension in 2022 (1).

30 years history of Noninvasive Methods of Cardiology in Brno wos composed from different international scientific meetings, workshops and congresses, which are published as abstracts and some of them as a publication, all in English language and the scientific findings are quoted all over the world. One of the very important cardiovascular parameters in the studies was blood pressure measurement in human being in health and diseases, blood pressure control in healthy subjects and hypertension and its comparison with other cardiovascular parameters.

In the year 2007 professor Thomas Kenner from Graz published "Minimal requirements for diagnostic blood pressure" in Noninvasive Methods in Cardiology (2). He described some historical data about blood pressure measurement techniques (3,4).

Figure 1: *Prof. Dr. Thomas Kenner, M.D., Dr. h.c. mult. in Brno Noninvasive Methods in Cardiology.*

Professor Kenner in the paper described in 1896 Sciupione Riva Rocci published the most widely used cuff technique for blood pressure measurement. One problem in the application of both cuff or tonometer has to do with the criterion of readings of pressure values. The most interesting once mentioned are: feeling the pulsation, hyperemia and reading of the finger, Korotkow sounds (1905) and the oscillatory criterion (von Reklinghausen,1906) (5,6,7,8).

In the end of 1980th we analyzed in Masaryk University the essential hypertension in measurement of blood pressure at rest and during exercise and started with ambulatory blood pressure monitoring. At that time we started scientific cooperation with Chronobiology center in University of Minnesota, USA.

In Czech Republic we presented the first paper by authors Siegelova J., Fiser B., Dusek J., Semrad B., Halberg F., Cornelissen G. 24-h monitoring of blood pressure in patients with essential hypertension, Vnitr. Lek 1993; 39: 183-190.

Blood pressure measurement using the cuff with ambulatory blood pressure monitoring, was used in many studies of chronobiology of blood pressure by Professor Franz Halberg who together with Professor Germaine Cornelissen and co-workers all over the word including us describe the rhythm in blood pressure, circadian oscillation and the variability in all rhythms in dependence on time and the variability of blood pressure (9-23). Between the years 1990-2008 the Brno team consisting of Prof. J. Siegelova, Dr. J. Dusek and Prof. B. Fiser collected 73 888 sets of blood pressure and ambulatory blood pressure monitoring and all the data were

analyzed in Brno by 24-hours means and also immediately by prof. Cornelissen in Minnesota using Halberg cosinor analysis of blood pressure and heart rate. The daily data exchange and analysis continues until now. Prof. Franz Halberg, prof. Germaine Cornelissen together with us presented Vascular Variability Disorders as Brno Concensus in 2008 (24-28).

Blood pressure (BP) and heart rate (HR) vary greatly, from one individual to another and from moment to moment in any longitudinal record. Variability in BP and HR can be accounted for by genetics, epigenetics, and in response to a variety of stimuli. Reference values in health provide guidelines to distinguish between usual and abnormal variability in BP and/or HR, in terms of deviant circadian characteristics and/or excess/deficit relative to time-specified limits of acceptability. This investigation examines the day-to-day variability in circadian rhythm characteristics determined from analyses of 7-day/24-hour records obtained by ambulatory BP monitoring (ABPM) in Brno, Czech Republic together with Halberg Chronobiology Center in University of Minnesota. A novelty pressor effect is quantified by comparing circadian parameters in consecutive days of monitoring. The results interpreted in terms of clinical implications indicate the need to monitor BP around the clock for longer than 24 hours, preferably for 7 days at the outset, in keeping with recommendations from the 2008 consensus meeting held in Brno. The Brno consensus, under the leadership of prof. Franz Halberg and prof. Germaine Cornelissen showed the following knowledge, which summarized his important chronobiological studies from cardiovascular physiology and pathophysiology.

In order to gain a better understanding of the blood pressure control and the actual limitations associated with ambulatory blood pressure monitoring (ABPM) limited to 24 the hours, the data from the Brno database were further analyzed by sphygmochron to compare results of analyses of data collected on seven consecutive days with those considering the entire record. The chronobiological studies found different kinds of abnormalities of BP, HR and their variabilities, known as Vascular Variability Anomalies were detected. They include systolic MESOR-hypertension, diastolic MESOR-hypertension, excessive pulse pressure (PP>60 mmHg), systolic circadian hyperamplitude tension (S-CHAT), diastolic circadian hyperamplitude (D-CHAT), deficient heart rate variability (DHRV: HR-SD<7.5 beats/min), systolic ecphasia (S-ecPhi: an odd timing of the circadian variation of BP but not of HR), and diastolic ecphasia (D-ecPhi). These conditions from Brno database were found to occur on at least 1 day in 68 % of subjects, respectively. By comparison, only some subjects were found to have these vascular variability disorders when the whole 7-day record was analyzed. The ability to accurately diagnose these vascular variability disorders is important since they were predictive of overall mortality in this population (28-51).

Figure 2: *Chronobiological study of blood pressure in University of Minnesota, USA, 1995, from the right MUDr. Jiri Dusek, CSc., Professor MUDr. Jarmila Siegelova, DrSc., Professor Dr. Franz Halberg, USA, Professor Dr. Germaine Cornelissen, USA, Dr. Anna Portela, Spain and Professor MUDr. Bohumil Fiser, CSc.*

According to Professor Kenner (2007) in the history of blood pressure measurement, the non-invasive recording of pulsatile arterial blood pressure was developed and described by Richard Wagner in the 1940th. His technique of arterial unloading was a predecessor of the finger-cuff technique by Penaz (1969) (52).
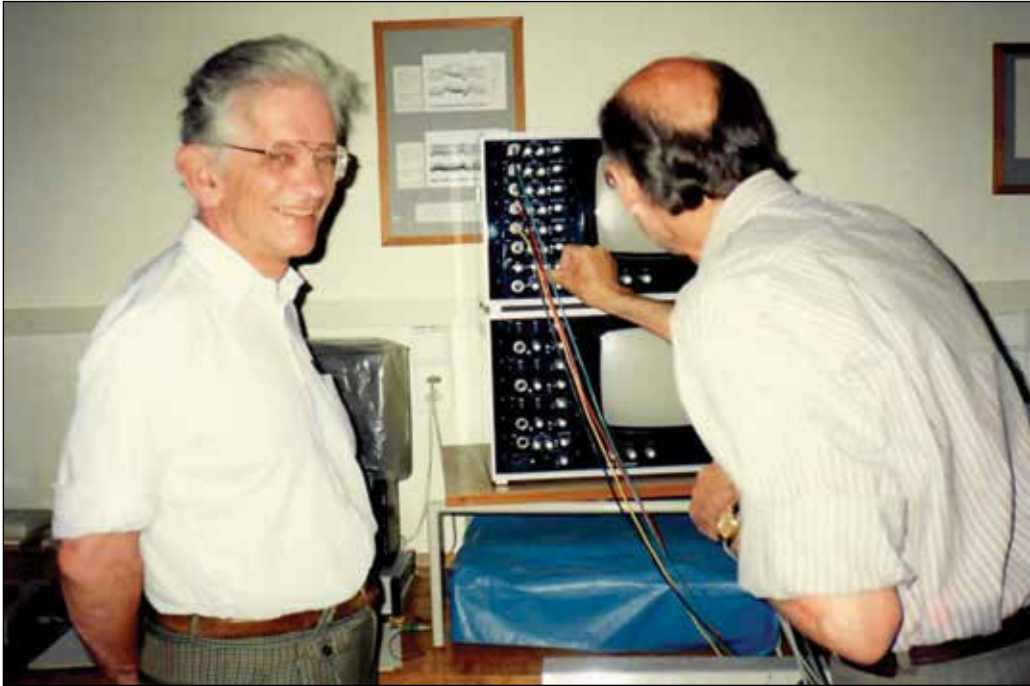
Figure 3: *Prof. Dr. Thomas Kenner, M.D., Dr. h.c. mult. and Prof. MUDr. Jan Penaz, CSc. in Brno Noninvasive Methods in Cardiology*

The arterial unloading technique invented by Penaz was used in a lot of studies of autonomic nervous system by the Brno team Penaz, Fiser, Siegelova, Dusek, Svacinova in healthy subjects and in patients with cardiovascular diseases, in cardiovascular rehabilitation, in patients with hypertension and with therapy of hypertension, in patients with diabetes mellitus (53-60).

The application of the finger cuff as invented by Penaz was technically modified by several authors and companies, and was recently improved by the company CNSystems® for continuous pulsatile pressure recording in a device "Task force monitor"®. The main use of this device is the recording of reactions to orthostatic load by tilt table test.

Syncopal analyses of autonomous nervous system was provided in Graz by Professor Kenner, Professor Moser, Professor Skrabal and Professor Goswami in University of Graz and in Brno. Other devices which are based on the Penaz-technique can be applied in scientific analyses to be applied for short and long term experiments all over the word.

Figure 4: *Prof. MUDr. Jarmila Siegelova, DrSc., Dr. Biaca Brix, Professor Masairo Kohzuki M.D., Prof. PD Dr. med. Nandu Goswami and behind Dr. Jana Svacinova, Masaryk University, Brno 2019*

Nowadays many cuffless blood pressure measuring devices are currently on the market claiming that they provide accurate blood pressure measurements. These technologies have considerable potential to improve the awareness, treatment, and management of hypertension.

"Cuffless blood pressure measuring devices: review and statement by the European Society of Hypertension Working Group on Blood Pressure Monitoring" published in 2022, analyzed very deeply all methods which are described and used (1).

Cuffless blood pressure technologies

In the year 2022 was published "Cuffless blood pressure measuring devices: review and statement by the European Society of Hypertension Working Group on Blood Pressure Monitoring" (1) This publication presents the statement of European hypertension society of the possibilities of noninvasive blood pressure measurement with the cuffless techniques as a new technologies.

The technologies are sumarized on the picture from the publication.
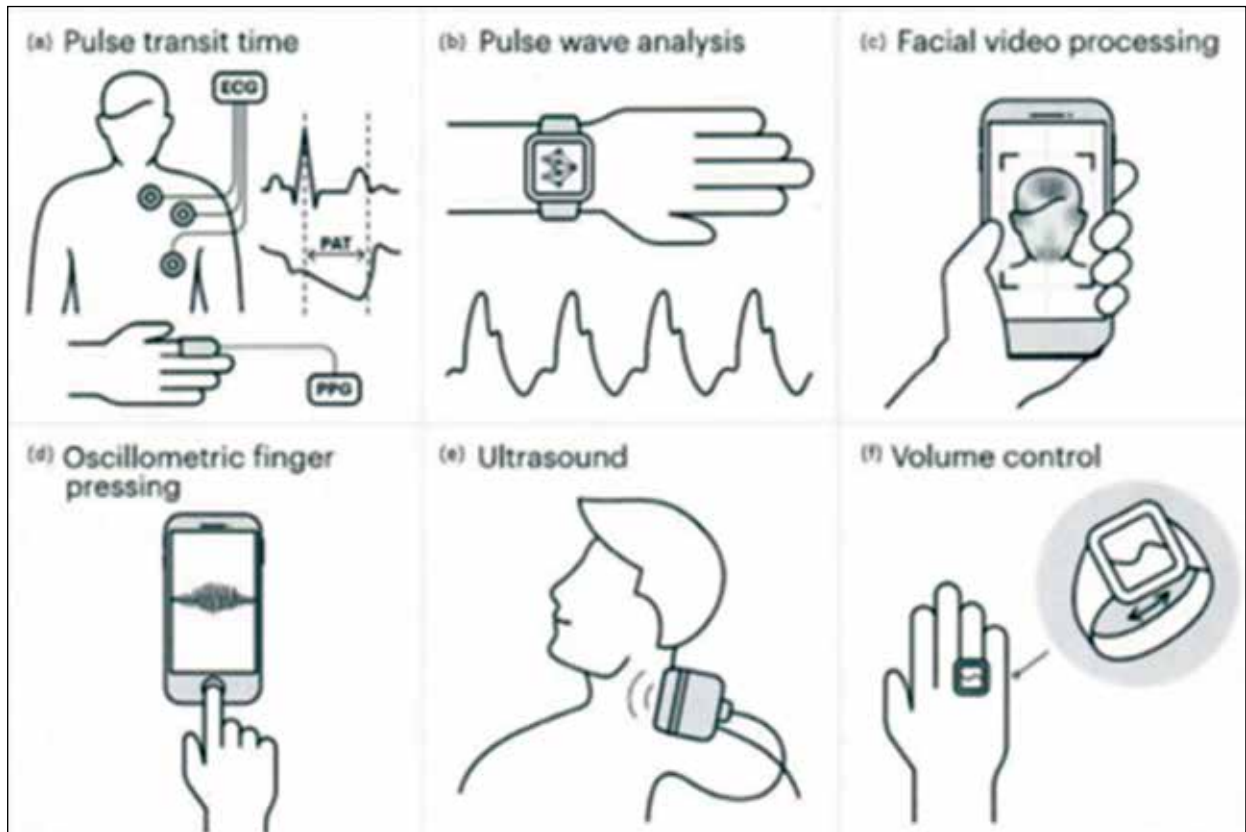
Figure 5: *Example illustrations of cuffless blood pressure technologies on the market (a and b), or in early research stage (c–f). ECG, electrocardiography; PAT, pulse arrival time; PPG, photoplethysmography (1).*

Pulse transit time

Pulse transit time is detected as the time delay between proximal and distal arterial waveforms and may be the only calibrated technology with a generally accepted theory (1).

Pulse wave analysis

Pulse wave analysis extracts blood pressure related features from an arterial waveform. It requires only a single sensor but has little theoretical basis (1).

Facial video processing

Facial video processing extracts pulse waveform features from the facial skin in a video stream and may uniquely allow blood pressure to be measured passively with a common device (e.g. each time someone uses her/his smartphone) (1).

Oscillometric finger pressing

Oscillometric finger pressing extends the automatic cuff principle for blood pressure monitoring via widely available smartphones (1).

Ultrasound

Ultrasound may be the only technology in this group for measuring blood pressure without involving variable pressure application (1).

Volume control

Volume control may currently be the only technology in this group for continuous blood pressure monitoring (1).

These different cuffless method techniques were based on the noninvasive measurements of cardiovascular parameters. Also in our Noninvasive methods of cardiology was included pulse wave analysis was also one study of Prof. Kenner. As described by Wetterer and Kenner (1968) transmission line models can be applied to determine the frequency dependence of the pressure transformation including the so called peripheral amplification which is due to pulse wave reflections (7,8). Part of the results of pulse wave analysis were also presented in cardiovascular medicine in Brno.

In further studies by Prof. K. Shirai was analysis of vascular function using the cardio-ankle vascular index (CAVI). And his co-workers, to whom belong also Prof. Dobsak described the role of monitoring of arterial stiffness with cardio-ankle vascular index. The results on the Czech population were published and presented in Noninvasive Methods in Cardiology (61-64).

Figure 6: *Prof. T. Kenner, Prof. K. Shirai, Prof. J. Siegelova and Prof. P. Dobsak in Brno in 2012.*

In 2022 conclusion of the statement of blood pressure measurement were presented as guidelines by the European Society of Hypertension and they do not recommend cuffless devices for the diagnosis and management of hypertension.

The Original Statement in 2022

This statement by the European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability presents the types of cuffless BP technologies, issues in their validation, and recommendations for clinical practice.

Statements: Cuffless blood pressure monitors constitute a wide and heterogeneous group of novel technologies and devices with different intended uses. Cuffless blood pressure devices have specific accuracy issues, which render the established validation protocols for cuff blood pressure devices inadequate for their validation.

In 2014, the Institute of Electrical and Electronics Engineers published a standard for the validation of cuffless blood pressure devices, and the International Organization for Standardization is currently developing another standard. The validation of cuffless devices should address issues related to the need of individual cuff calibration, the stability of measurements post calibration, the ability to track blood pressure changes, and the

implementation of machine learning technology. Clinical field investigations may also be considered and issues regarding the clinical implementation of cuffless blood pressure readings should be investigated (1).

Cuffless blood pressure devices have considerable potential for changing the diagnosis and management of hypertension. However, fundamental questions regarding their accuracy, performance, and implementation need to be carefully addressed before they can be recommended for clinical use.

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Rhythmometric Analyses in Mathematica

A. Chase Turner, Larry A. Beaty, Germaine Cornelissen

Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA

Correspondence:

Halberg Chronobiology Center
University of Minnesota,
420 Delaware St. S.E.
MMC8609
Minneapolis, MN 55455, USA
turn0412@umn.edu
beaty024@umn.edu
corne001@umn.edu
Website: https://halbergchronobiologycenter.umn.edu/halberg-chronobiology-center

Introduction

Interest in circadian rhythms has grown exponentially since the discovery of clock genes [1] that provided a molecular basis to their ubiquitous presence in most, if not all biological processes. Technological advances have also made it possible to collect large amounts of data at all levels of organization, from investigations of molecular and cellular mechanisms [2] to the monitoring of physiological functions at the organismic level [3]. These advances have necessitated the development of programs for their analysis and characterization.

While methods of time series analysis [4] are widely available, they often assume data to be equidistant. Biological data, however, are notorious for being non-equidistant or having missing values. Reasons are diverse and can stem from equipment error or malfunction, or inability to collect data at certain times, such as during sleep. For research related to circadian rhythms or other biological rhythms of known period, least squares regression can be used to fit models consisting of one or multiple cosine curves. Regression methods do not require

data to be equidistant. They are also well suited for hypothesis testing (rhythm detection) and parameter estimation (rhythm characterization and comparison) [5-7].

Several programs supporting chronobiologic applications have been developed over the years. The performance of several such methods has been compared and published earlier [8]. Other packages providing an integrated suite of functions for rhythm analysis, and new ones were developed or extended since, as reviewed elsewhere [9]. In particular, some extensions of the single and population-mean cosinor techniques are available from CRAN as part of the CATkit library [10].

Despite earlier efforts, there remains a need for rhythmometric techniques to be made widely available for use by chronobiologists and others. The system needs to be flexible and easy to use. To consolidate and extend cosinor-based applications, Mathematica [11] was selected to develop an open-source toolkit for chronobiologists. Mathematica is a computational data science platform available on Microsoft Windows, Apple macOS, Linux operating systems and Web Browsers via Cloud deployment. We here offer a preview of the Mathematica toolkit and illustrate its use based on blood pressure (BP) data collected by ambulatory BP monitoring (ABPM) in a patient examined before and during anti-hypertensive treatment.

Materials and Methods

Analysis and graphics were produced with Mathematica 13.1 running on an Apple macOS 13.0 host with 64 GB of RAM. Other supported Mathematica configurations are documented on the software publisher's website [12].

A 61-year old man diagnosed with high BP provided a 7-day/24-hour ABPM profile before starting treatment with the ACE inhibitor Lisinopril (10 mg/day). After being treated for about 2 weeks, another 7-day/24-hour ABPM profile was obtained during treatment. BP was measured oscillometrically with the TM-2430 monitor from A&D (Tokyo, Japan). Measurements were obtained automatically around the clock at 30-minute intervals.

The following analyses of these data were carried out in Mathematica:

1. Chronograms [13] are plots of the data as a function of time.

2. Plexograms [13] are plots of original data stacked over an assumed period (in this case, 24 hours) to visualize the periodic component's shape (in this case, the circadian pattern of BP).

3. Least Squares Spectral Analysis (LSSA) is a cosinor-based technique approximating results from a Discrete Fourier Transform (DFT) [14]. It consists of separately fitting by least

squares cosine curves with frequencies in the range of 1/T (where T is the record length) to 1/2Dt (where Dt is the average sampling interval), trial frequencies being incremented by 1/T. In the case at hand, T is 7 days and Dt is 30 minutes. The LSSA was computed at trial periods of 7, 7/2=3.5, 7/3, ..., 7/167 days. Spectral lines with large amplitudes indicate the presence of rhythmic components.

4. A single cosinor [13] model consists of fitting a cosine curve with an anticipated period to the data. Results can be used to plot the model together with the data. It is recommended to verify that the anticipated component is a spectral peak in the LSSA.

5. A multiple cosinor [6, 13] model consists of fitting a model consisting of two or more cosine curves with anticipated periods to the data. Results can be used to plot the composite model together with the data. It is recommended to verify that the anticipated components are spectral peaks in the LSSA. For the model to represent a periodicity, components need to be harmonically related. In the example used herein, periods considered are 24 and 12 hours. This model underlies the sphygmochron [15], which is widely used to analyse ABPM data [16].

6. Nonlinear cosinor models consist of fitting a single- or multiple-component model to the data for which the periods are not precisely known. In other words, instead of fixing the period and estimating the MESOR (M, rhythm-adjusted mean), amplitude (A) and acrophase (φ, phase of the maximum in relation to a given reference time), the period (τ) is also estimated. As a parameter in the argument of the cosine function, such models need nonlinear least squares techniques. Guess estimates for the periods are used as initial values. The model is then fitted iteratively to yield estimates for M and (τ, A, φ) of each component in the model [17-19].

Results

Figure 1 illustrates examples of chronograms of systolic (S) and diastolic (D) BP and heart rate (HR) before and during treatment. The decrease in SBP on treatment can readily by seen by the naked eye. The same SBP data are shown in Figure 2 after being stacked over a 24-hour period to visualize its circadian pattern before and during treatment. The lower night-time compared to daytime readings can clearly be observed. Figure 2 is a BarWhiskerChart [20] displaying hourly medians with their inter-quartile range (IQR, colored columns) and upper and lower fences representing Q3+1.5IQR and Q1-1.5IQR, respectively; outliers are pictured as dots.

Results from the plexograms can be displayed in the light of time-specified reference limits (chronodesms, [21]) qualified by gender and age, as done in the sphygmochron [15, 16]. In the

example illustrated in Figure 3, the average circadian profile is displayed together with the original data collected each day (thin gray lines). It can be seen that SBP exceeds the upper 95% prediction limit in clinically healthy peers, suggesting the need for treatment.
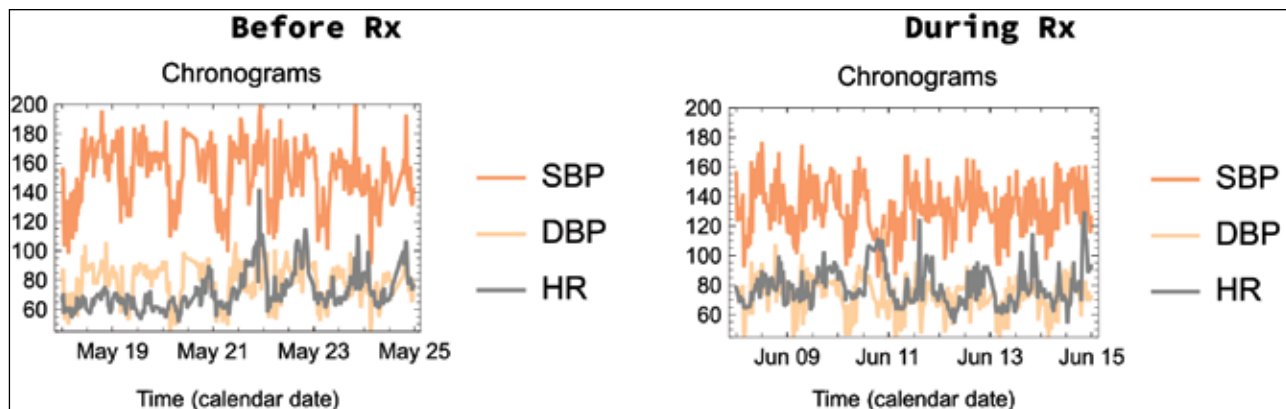

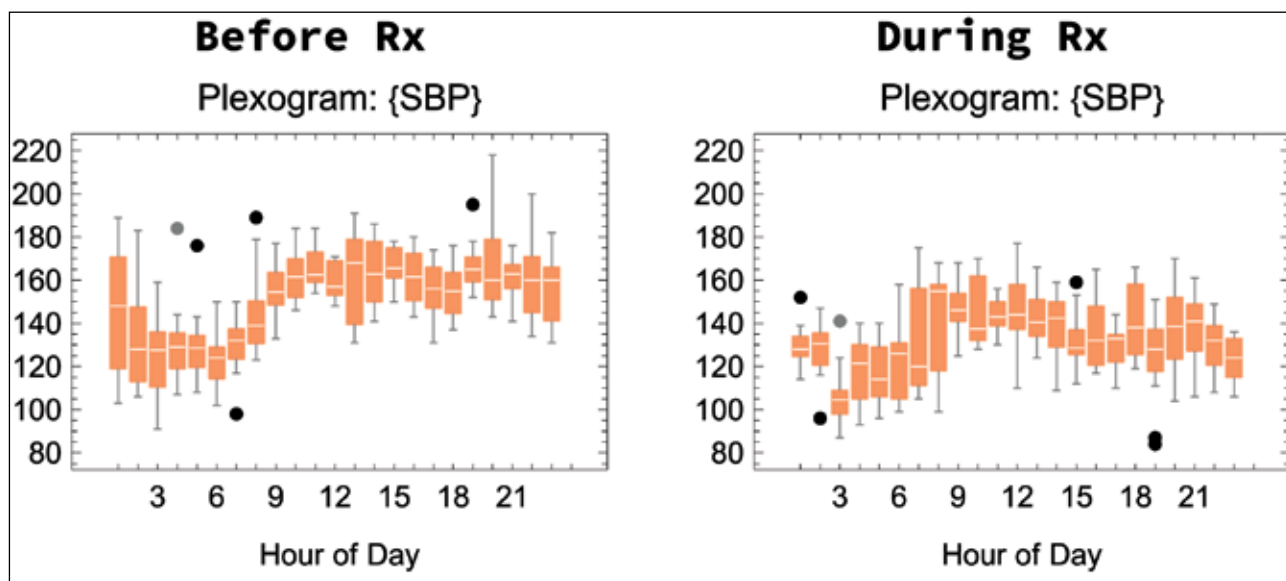**Figure 1:** *Examples of SBP, DBP, and HR chronograms generated in Mathematica*

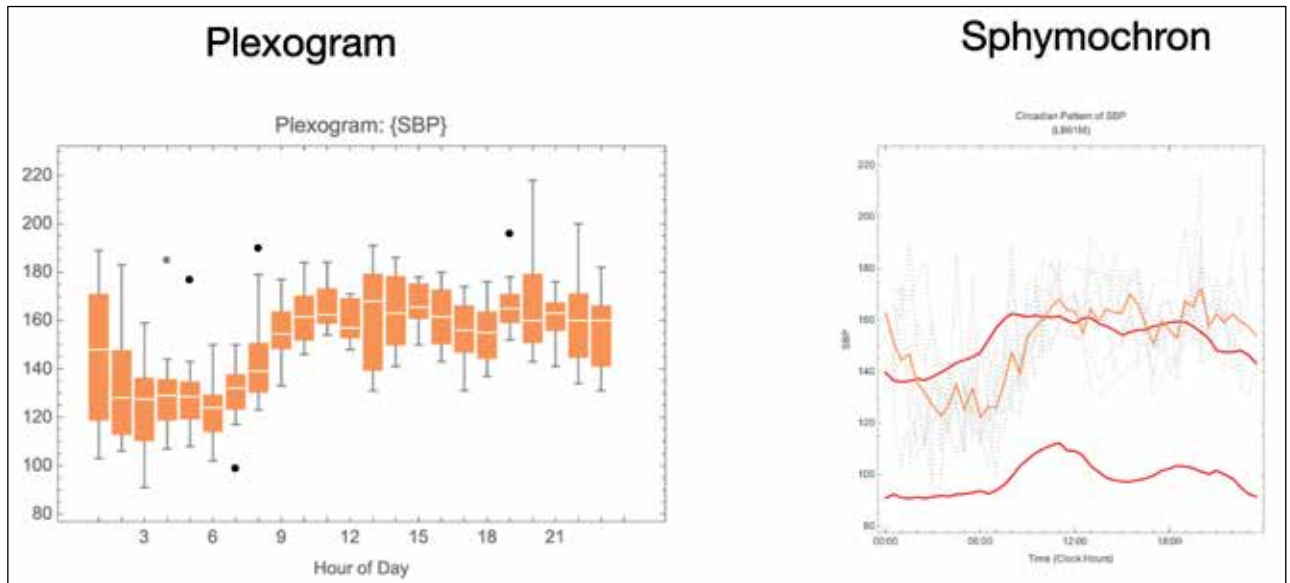**Figure 2:**. *Examples of plexograms of SBP generated in Mathematica*

Figure 3:. *Plexogram results (left) and sphygmochron plot (right) showing average half-hourly data (orange curve) relative to reference values in clinical health (red curves).*
Data collected each day are shown as thin gray lines.

Figure 4 compares the circadian profile of SBP with the chronobiologic reference limits before and during treatment, showing that on treatment, SBP remains within acceptable limits on average, suggesting the efficacy of treatment in this case.

LSSA results confirm the presence of two major spectral peaks corresponding to periods of 24 and 12 hours. These two components constitute the model fitted in the sphygmochron, which also displays acceptable ranges for M, A and ϕ of the 24-hour component of the composite model [15, 16]. Deviations from these ranges have been associated with large increases in cardiovascular disease risk in several outcome studies [22].
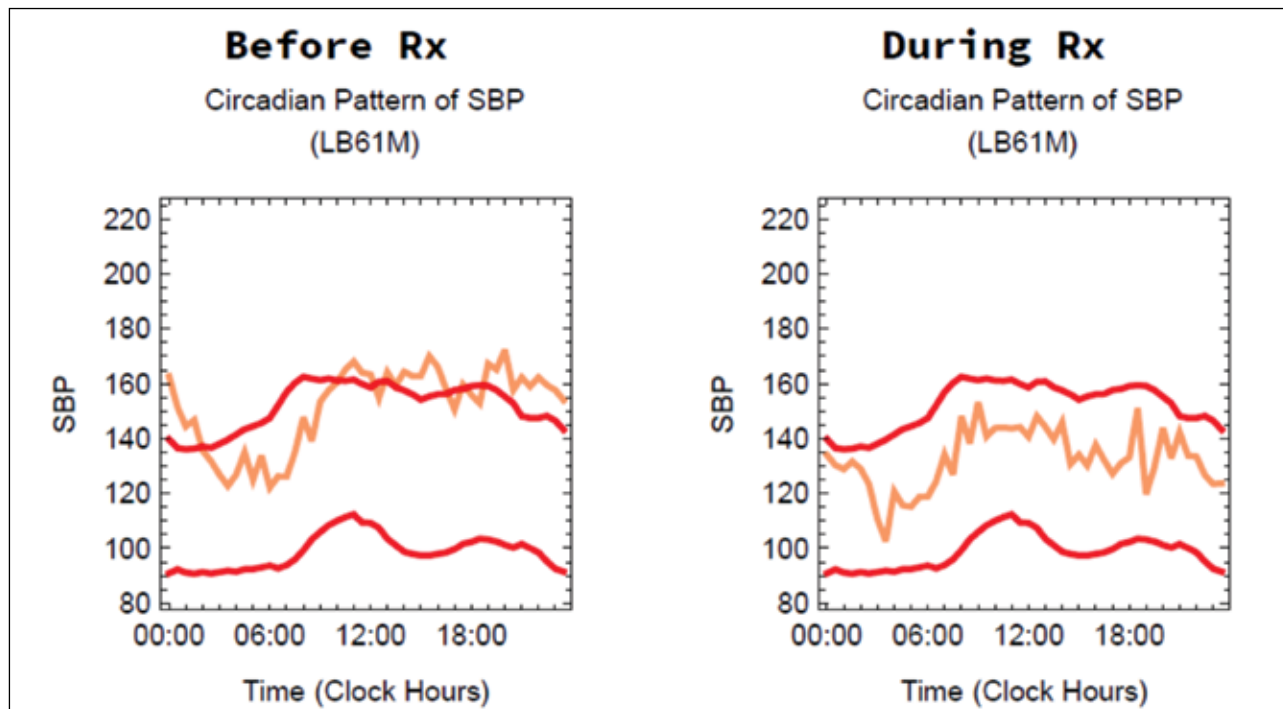
Figure 4: *Comparison of average circadian profile of SBP before and during treatment.*

		SBP: Before Rx				SBP: During Rx		
		Estimate	Standard Error	Confidence Interval		Estimate	Standard Error	Confidence Interval
Cosinor Fixed $M+A[1]\cos\frac{2\pi t}{1\,day}+\phi[1] + A[2]\cos\frac{2\pi t}{0.5\,days}+\phi[2]$	M	151.635	0.916554	{149.832, 153.438}	M	132.447	0.897162	{130.682, 134.213}
	A[1]	16.979	1.30019	{14.4211, 19.5369}	A[1]	9.48483	1.27713	{6.97192, 11.9977}
	φ[1]	1.92716	0.0761127	{1.77742, 2.07689}	φ[1]	2.66545	0.132882	{2.40399, 2.92691}
	A[2]	9.87616	1.29994	{7.31877, 12.4335}	A[2]	8.22722	1.26583	{5.73655, 10.7179}
	φ[2]	0.572081	0.129905	{0.316517, 0.827646}	φ[2]	0.918806	0.154399	{0.615008, 1.2226}
		Estimate	Standard Error	Confidence Interval		Estimate	Standard Error	Confidence Interval
Cosinor Unconstrained $M+A[1]\cos\frac{2\pi t}{\tau[1]}+\phi[1] + A[2]\cos\frac{2\pi t}{\tau[2]}+\phi[2]$	M	151.527	0.916405	{149.724, 153.33}	M	132.337	0.906	{130.554, 134.119}
	A[1]	16.9998	1.30083	{14.4406, 19.559}	A[1]	9.58232	1.281	{7.06174, 12.1029}
	φ[1]	1.95093	0.14473	{1.6662, 2.23567}	φ[1]	2.8374	0.267228	{2.31158, 3.36321}
	A[2]	10.1828	1.30451	{7.61632, 12.7492}	A[2]	8.27415	1.26731	{5.7805, 10.7678}
	φ[2]	0.130735	0.236185	{-0.333886, 0.595356}	φ[2]	0.753333	0.301076	{0.160915, 1.34575}
	τ[1]	0.999616 days	0.00572044 days	{0.988362 days, 1.01087 days}	τ[1]	1.00731 days	0.0104713 days	{0.986711 days, 1.02792 days}
	τ[2]	0.494603 days	0.00234947 days	{0.489981 days, 0.499225 days}	τ[2]	0.497705 days	0.00294069 days	{0.491919 days, 0.503492 days}
		Estimate	Standard Error	Confidence Interval		Estimate	Standard Error	Confidence Interval
Cosinor Harmonic $M+A[1]\cos\frac{2\pi t}{\tau[1]}+\phi[1] + A[2]\cos\frac{2\pi t}{\frac{\tau[1]}{2}}+\phi[2]$	M	151.601	0.915735	{149.799, 153.402}	M	132.451	0.898838	{130.683, 134.219}
	A[1]	16.9791	1.30248	{14.4166, 19.5415}	A[1]	9.4772	1.27907	{6.96044, 11.994}
	φ[1]	1.84413	0.0976495	{1.65202, 2.03624}	φ[1]	2.63808	0.170292	{2.30301, 2.97316}
	A[2]	10.0907	1.30161	{7.52998, 12.6514}	A[2]	8.25321	1.26751	{5.7592, 10.7472}
	φ[2]	0.386575	0.177503	{0.0373882, 0.735783}	φ[2]	0.876246	0.266105	{0.352645, 1.39985}
	τ[1]	0.994913 days	0.00336232 days	{0.988298 days, 1.00153 days}	τ[1]	0.998697 days	0.00511618 days	{0.98863 days, 1.00876 days}

Table 1: *Assessment of 2-component model (24- and 12-hour periods) of SBP before and during treatment.*

Table 1 illustrates the characterization in Mathematica of the 2-component model used in the sphygmochron. This model considers cosine curves with periods of about 24 and 12 hours. In the currently available version of the sphygmochron, the 24- and 12-hour periods are fixed. Deviation from 24 hours of the circadian period, however, is recognized as a potential additional cardiovascular risk factor [23]. It is hence desirable to determine whether the circadian period is synchronized to 24 hours by estimating the period as well as the MESOR, amplitude(s) and acrophase(s), using nonlinear least squares.

Mathematica's NonlinearModelFit (nlm) function [24] can be used in several ways, as shown in Table 1. The periods can be fixed, as currently done in the shygmochron (Table 1, top part). The periods of the two components can be estimated separately (Table 1, middle part). In the example considered herein, the circadian period does not deviate from 24 hours (1 day), but the circasemidian component is synchronized to 12 hours only during treatment. Before treatment, it is slightly shorter than 12 hours (0.5 day), as evidenced from the 95% confidence interval of the period not covering 12 hours. A third option consists of estimating the period of both components, but adding the constraint that the second component is the second harmonic of the circadian period (Table 1, bottom part). In this case, only the circadian period is estimated and the circasemidian period is constrained to be half the circadian period. In the example considered, the circadian period is 24-hour synchronized as evidenced by the 95% confidence interval of the period covering 1 day. These different options of fitting the same kind of model can also be coded mathematically, as shown by the formulae listed in Table 1 (left). Figure 5 visualizes how the models differ using these three options in the case of SBP.

Discussion and Conclusion

Once this Mathematica toolkit is complete, researchers will be able to upload Excel data for analysis via their web browsers, and to download reports in Excel, PDF and various graphic standards. Such open-source computational documents are known to foster collaboration. They also promote reproducible research on a multi-platform technology. Additionally, Mathematica's report generator [25] is available to researchers to develop reports that help medical practitioners to translate dense biometric datasets into actionable results.
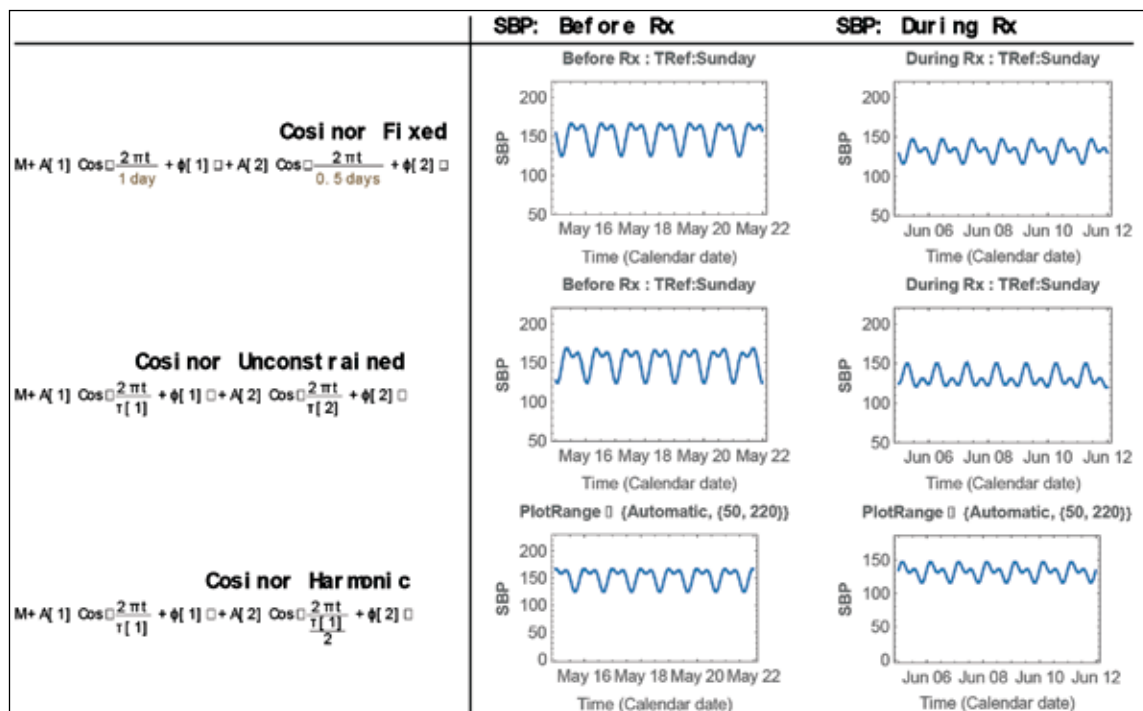
Figure 5: *Visualization of models, using three different options to fit the same 2-component model to SBP data before and after treatment.*

In this preview of the HCC Mathematica toolkit for chronobiologists, the side-by-side plots of the patient's "Before Rx" and "During Rx" sphygmochron plots is a powerful visualization for the clinician to share with the patient to show promising results of continuing to take anti-hypertensive medication. However, development of the sphygmochron plot required considerable analysis of underlying biometric data and on that score, Mathematica's superior analytics and data processing are a promising addition to this toolkit.

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The Current Place of Tranexamic Acid in the Elective Hip Arthroplasty

Luboš Nachtnebl, Vasileios Apostolopoulos

1st Department of Orthopaedic Surgery, St. Anne's University Hospital, Faculty of Medicine, Masaryk University Brno, Czech Republic

Introduction

The total hip arthroplasties (THA) are associated with perioperative blood losses exceeding 500 mL, some studies claim average blood losses of up to 1500Ml [1]. Blood loss volumes are certainly strongly dependent on the chosen surgical approach and technique. Approximately 30% of patients that undergo elective hip replacement receive at least one blood unit in postoperative care [2]. Reducing blood losses has a beneficial impact on recovery time, length of stay, and health economics. In the last decade, there has been an increasing interest in the efficacy of tranexamic acid (TXA) in orthopaedics and especially in elective major joint replacements. Highlighted advantages of the TXA in hip replacement surgery are the systemic or local prevention of hemorrhage and the prevention of heterotopic ossification formation. This paper presents an overview of our current understanding of the use of TXA in elective hip replacement.

Pharmacology of tranexamic acid

Tranexamic acid (trans-4-aminomethyl cyclohexane carboxylic acid) is an anti-fibrinolytic substance that chemically belongs to the group of e-carboxylic acids. TXA is a synthetic amino acid derivative of lysine that competitively inhibits the activation of plasminogen to the serine protease, plasmin. Furthermore, tranexamic acid is a competitive inhibitor of tissue plasminogen activator, blocking the lysine-binding sites of plasminogen, resulting in inhibition of plasminogen activation and fibrin binding to plasminogen and therefore impairment of fibrinolysis [3]. Tranexamic acid can also directly inhibit plasmin activity, but higher doses are required to reduce plasmin formation. Tranexamic acid is distributed throughout all body tissues and the plasma half-life is 120 min [4].

Posology of tranexamic acid

Over the last decade, numerous clinical trials have investigated the use of TXA to minimize operative bleeding. There was no official consensus on the timing, route, and dose of TXA until the American Association of Hip and Knee Surgeons (AAHKS) published a clinical practice guideline recommending routine use of TXA; low dose intravenously (< 20 mg·kg^{-1} or ≤ 1 g), high dose intravenously (≥ 20 mg·kg^{-1} or > 1 g), high dose intra-articular (> 1.5 g), or combined intravenous/intra-articular TXA for hip arthroplasty [5]. Although there is a wide variation in dosing regimens in clinical practice with intra-articular doses of TXA ranging from 15 mg·kg^{-1} to 3 g and intravenous TXA doses ranging from 500 mg to 3 g [1].

Current standing

The original application of TXA was to prevent bleeding in dental procedures, nowadays TXA has become a widely adopted drug in several medical specialties including orthopaedic surgery [6]. The Revolution in the use of TXA in trauma and orthopaedic surgery was the publishing of the CRASH-2 trial in 2013, a randomized placebo-controlled trial of two hundred and seventy-four hospitals in 40 countries. The main outcome was the significantly reduced risk of death in bleeding compared to a placebo and the safe administration of TXA in patients undergoing trauma [7]. A network meta-analysis compared intravenous, oral, topical, and combined methods of TXA application concluding that all significantly reduce the amount of blood loss and the risk of transfusion compared to placebo. Also, all of the compared application methods were recorded to have similar outcomes in patients undergoing primary THA. The use of TXA provided reductions of 180 ml blood loss compared to placebo with mean differences of 295 ml to 432 ml for the various TXA treatments. The only exception was the low-dose topical TXA which was not statistically superior to the placebo as well as oral TXA [8]. With the increasing use of tranexamic acid in THA, safety concerns remain. An up-to-date literature review suggests that the thromboembolic risk does not increase with low-dose, short-term administration for these indications within normal clinical use of thromboembolic prophylaxis [9]. Using national claims data, one study examined tranexamic acid use in 765.011 total hip/knee arthroplasties from 2013 to 2016 in patients with preexisting comorbidities. Results of this study showed that despite being effective in reducing blood transfusions, TXA was not associated with increased complications, irrespective of the patient's high-risk status at baseline [10]. Intra-articular application of TXA brings about lower plasma concentration as a result is expected to have a reduced risk of thromboembolic events. This consideration was tested by a meta-analysis of randomized controlled trials which compared the effect of topical and intravenous administration of TXA on blood loss and rates of transfusion. The topical route was found to be superior in terms of thromboembolic event risk than the intravenous

route [9]. Although authors claim that research is required to find the optimum dose for topical use [11]. Intra-articular hematoma following THA is a risk factor of an early periprosthetic infectious event. Another theoretical consideration is that the use of TXA could have a beneficial effect on the periprosthetic infection incidence as reduces the postoperative intra-articular hematoma. There is currently no evidence evaluating this point and probably could be the subject of further investigation in future works. There is strong evidence that TXA reduces postoperative blood losses and consequently leads to less frequent blood transfusions. This fact has an impact on the economic burden for hospital management and the health system. The cost of a unit of blood component transfusion cost in the Czech Republic is 110 - 135 USD erythrocyte suspension. On the contrary, a package of TXA containing 5 ampoules costs only 10 USD. Also, increased blood loss could lead to longer lengths of stay at the hospital and the connected economic consequences. Heterotopic ossification is a frequent complication after THA, presented as bone in soft tissue where bone normally does not appear. The diagnosis is based on x-ray postoperative scans (Figure 1). A recent retrospective study analyzed the effect of intravenous TXA on the incidence of heterotopic ossifications in elective hip arthroplasty. In their cohort, the TXA protocol significantly reduced the incidence of heterotopic ossification regardless of the fixation of endoprosthesis [12].

Our experience

In the 1st Department of Orthopaedic Surgery, St. Anne's University Hospital in Brno, we routinely use TXA in elective hip arthroplasty to reduce blood losses. Since using TXA, we recorded a post-operative reduction of blood loss in the surgical drain. Currently, with the use of TXA (mostly intravenously) the average post-operative losses in the drain are approximately 300ml. In most cases, we use a preoperative dose of 1g TXA intravenously (2 amp. 0.5g/5ml per amp.). In high-risk patients, we prefer using intra-articular administration of TXA. At the end of the procedure, after the fascia suture and insertion of a surgical drain, we apply an intra-articular dose of 1g TXA (2 amp. 0.5g/5ml per amp.). In rare cases of diffuse bleeding, we combine topical and intravenous administration. We prefer the intravenous TXA route immediately before the procedure and intra-articular application at the end of the procedure.
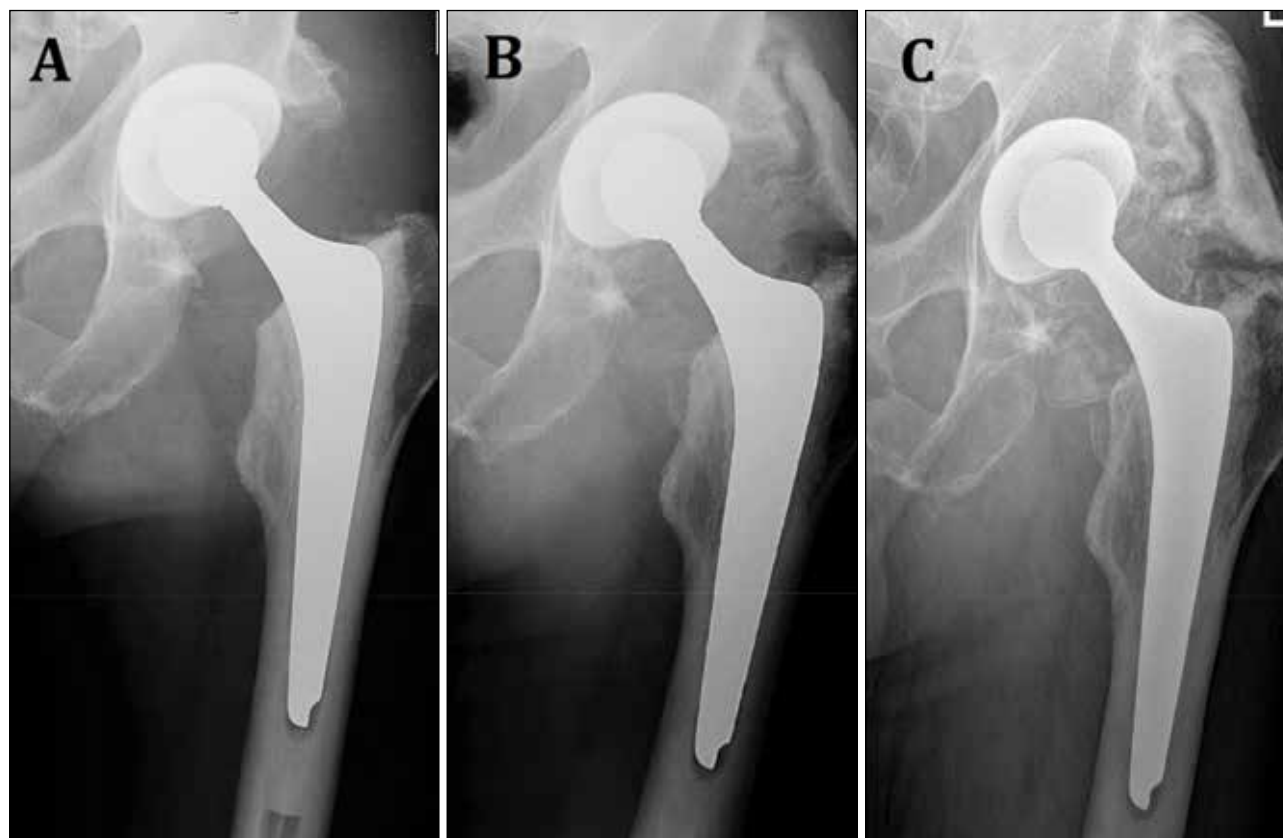
Figure 1: *Heterotopic ossification formation after elective primary hip arthroplasty A. Post-operative x-ray scan B. Radiographic imaging at the 1-year follow-up C. Heterotopic ossification presence 2 years after the procedure* (Source: own matrial).

Summary

The prophylactic use of TXA limits the amount of bleeding in elective primary hip replacement, reduces the likelihood of blood transfusion, and lowers length and cost of hospitalization. Intravenous and topical intra-articular administrations of TXA were proved to be equal in terms of effectiveness. Topical administration of TXA achieves a higher therapeutic concentration at the site of bleeding, effectively reducing blood loss with limited systemic absorption and subsequent systemic side effects. According to the current evidence TXA is not associated with an increased rate of complication, even when applied to a high-risk patient.

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Effect of Physiotherapy on Subjective Evaluation Post-COVID Symptoms

[1,2]Adam Vajčner, [2]Klára Černá, [2]Jitka Jaroňová, [2]Zuzana Koyšová, [1,2]Libor Dobšák, [3]Luboš Nachtnebl, [1,2]Michaela Sosíková

[1]*Department of Sport Medicine and Rehabilitation, Faculty of Medicine, Masaryk University and St. Anne's University Hospital, Brno, Czech Republic*
[2]*Department of Physiotherapy and Rehabilitation, Faculty of Medicine, Masaryk University, Brno*
[3]*First Department of Orthopedic Surgery, Faculty of Medicine, Masaryk University and St. Anne's University Hospital, Brno, Czech Republic*

Abstract

Present paper introduces results of effect evaluated by the self-reported subjective questionnaires of persistent symptoms for outpatients after COVID-19.

The most frequent symptoms typical for post-COVID syndrome are dyspnea, fatigue, myalgia, sleep disorders, arthralgia, caught, headaches etc. We called these symptoms persistent post-COVID-19 symptoms and these maintain 12 and more weeks after infection of COVID-19. Because rehabilitation must also be effective for a larger number of patients, we assume that presented novel group physiotherapy protocol, will be very effective in subjective evaluation of patients´ well-being. The program is based on recommended rehabilitations´ standards for pulmonary rehabilitation in COVID-19 based on recent literature. We evaluated effectivity of specific rehabilitation program of group physiotherapy for outpatients after COVID-19 disease at the rehabilitation clinic of the Department of Sports Medicine and Rehabilitation of St. Anne's University Hospital in Brno.

We currently included 36 patients to participate in this program and statistically analyzed 28 of them by COPD Assessment Test for subjective assessment of pulmonary functions and Multidimensional Assessment of Fatigue Scale for evaluation of fatigue. Both are standardized self-reported questionnaires.

We found strong statistically significant improvement after group physiotherapy in subjective evaluation of dyspnea and fatigue. Based on presented data we assumed that also group physiotherapy program can be well effective tool for patients with persistent post-COVID symptoms.

Introduction

Present paper introduces results of physiotherapy effect evaluated by a self-reported subjective questionnaire of persistent symptoms for outpatients after COVID-19. Physiotherapy is based on novel protocol of group outpatient physiotherapy after COVID-19 with post-acute COVID syndrome or post-COVID syndrome. Persistent symptoms are occurred after COVID-19 very frequently [5, 19, 20, 23, 25]. Recent literature highlights early pulmonary rehabilitation as necessary part of interdisciplinary cooperation [2, 3, 6].

The most frequent symptoms typical for post-COVID syndrome are dyspnea (40 %), fatigue (44 %), myalgia (34 %), sleep disorders (33 %), arthralgia (13 %) [25]. 5–10 % of patients over 12 weeks after COVID-19 reported residual symptoms after COVID-19. Symptoms are developed over 5 – 10 % of patients after COVID-19 even more than 12 weeks after disease [19, 20]. 4 152 997 infected patients were infected in Czech Republic to October 2022 and 4 101 132 of them were recovered [1]. Theoretically it means approximately 205 000 - 410 000 post-COVID patients in Czech Republic with persistent symptoms who's potentially can be indicated to pulmonary rehabilitation. Therefore, rehabilitation must also be effective for a larger number of these patients, and we assume that presented group physiotherapy protocol, will be very effective in subjective evaluation of patients´ well-being.

Aim of this study is to evaluate effect of group physiotherapy on the most frequent persistent symptomatology after COVID-19 – dyspnea and fatigue. We hypothesize that, 4-weeks group physiotherapy with complex therapeutically approaches significantly improve condition of patients after COVID-19 with persistent symptoms evaluated by self-reported assessment of dyspnea and other respiration complications and fatigue. We used well published assessment questionnaires for dyspnea called **COPD Assessment Test** and for fatigue **Multidimensional Assessment of Fatigue Scale**.

## Materials and methods

Group of patients

Study group of patients consists from 36 subjects, with average age of 57,3 years (SD 12.5; median 58, min 29, max 82). 17 men and 19 women are involved. Average BMI is 29.7, between pre-obesity grade and first obesity grade (non-statistically significant difference between hospitalized and non-hospitalized patients during ongoing COVID-19 infection - 0.9597, Mann-Whitney U test). 52.7 % of patients had 3 or more dominant symptoms (dyspnea, fatigue, joint pain). Only 4 % were smokers. 58.3 % of patients during active infection had to be on oxygen therapy and hospitalized. 5 patients (13.9 %) were on mechanical ventilation (during

active infection). 32 patients finished more than 50 % of sessions during this program. Data were collected since April 2021 to December 2021. All patients signed inform consent. For descriptive statistic see Table 1.

Table 1: Study group of patients

	Total number of patients (N=36)	At least 50 % of therapies (N=32)
	average (SD); median (min;max)	
Age	57,3 (12.5); 58 (min 29; max 82)	58.2 (12.1); 58.5 (min 29; max 82)
Gender	17 M/19 F	14 M/18 F
Weight	87.9 (SD 21.8); 87 (min 54; max 140)	87.0 (SD 21.6); 85.0 (min 54; max 140)
Height	1.7 (SD 0.1); 1.7 (min 1.6; max 1.9)	1.7 (SD 0.1); 1.7 (min 1.6; max 1.9)
BMI	29.7 (SD 5.8); 29.3 (min 19.1; max 43.2)	29.9 (SD 5.9); 29.3 (min 19.1; max 43.2)
	n; %	
1-2 symptoms	17; 47.3 %	14; 43.8 %
3 < symptoms	19; 52.7 %	18; 56.3 %
Smoking	4; 11 % (+ 1, el.cig)	3; 9.4 %
Hospitalization	21; 58.3 %	19; 59.4 %
Hospitalization – mechanical ventilation	5; 13.9 %	4; 12.5 %
Non-hospitalization	15; 41.7 %	13; 40.6 %
Comorbids +	26; 72.2 %	24; 76.0 %
Comorbids -	10; 27.8 %	8; 25.0 %

The Graph 1 shows most frequent symptoms in presented study group. The frequencies of dyspnea 77.8 % and fatigue 55.6 % correspond with recent published reviews [5, 25].
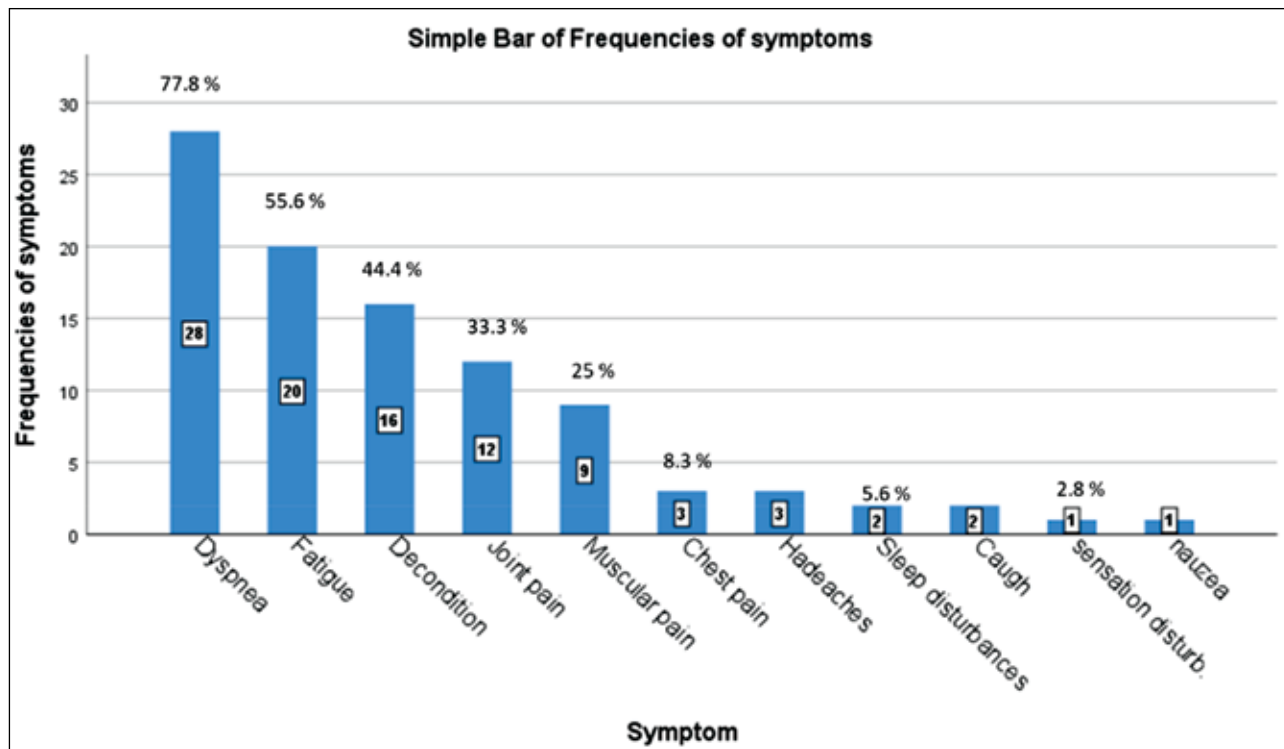
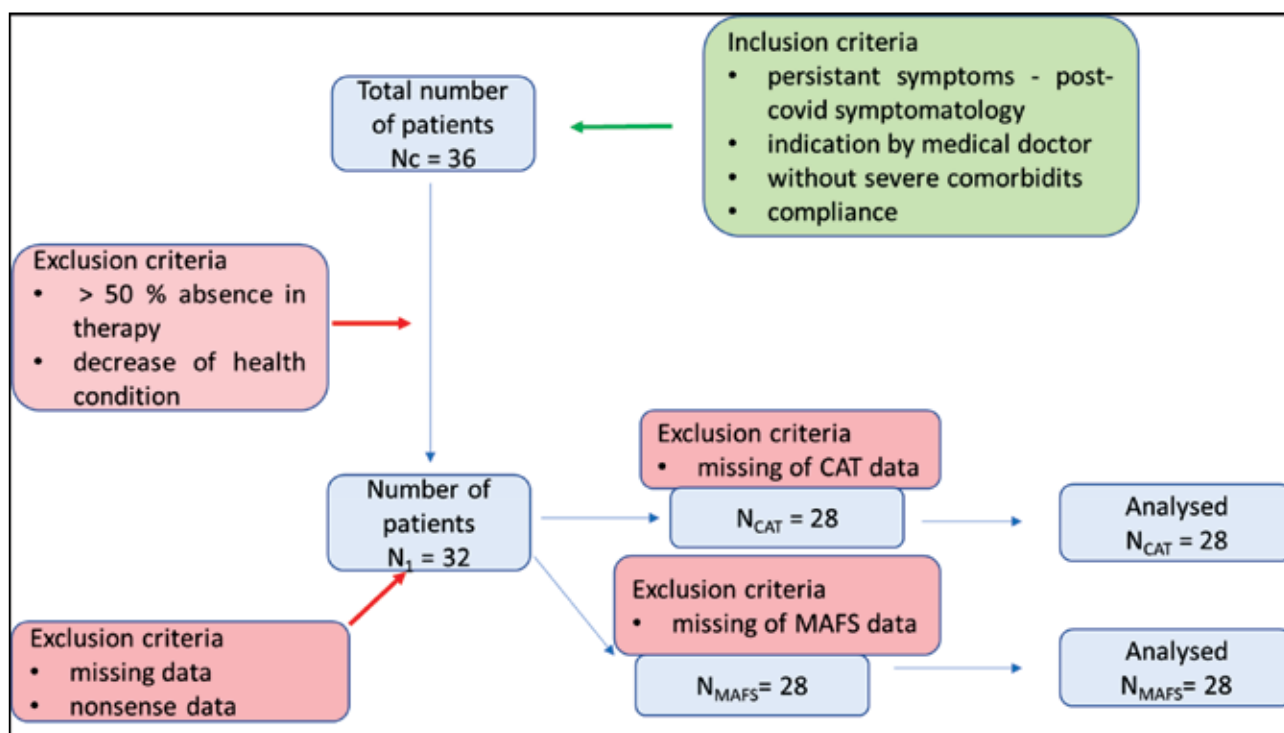**Graph 1:** *Frequency of persistent symptoms in study groups*

Figure 1: *Diagram of study group criteria*

All patients were evaluated through inclusion and exclusion criteria. All patients were indicated for group physiotherapy by well experienced medical doctor specialized in rehabilitation. All patients had to have some of persistent symptomatology which was the reason why they looked out our physiotherapy program. All patients had to be without severe comorbid and be capable cooperate during group physiotherapy. Exclusion criteria were more than 50 % of absence on therapy sessions and decompensation of their health condition (exclusion 4 patients). For statistical analysis we excluded 4 patients because of missing questionnaires data. For the diagram see Figure 1.

Methods and Protocol of Physiotherapy program

The program is based on standardized recommendations for pulmonary rehabilitation in COVID-19 [2, 3, 4, 12, 13]. It is conducted as outpatient group physiotherapy for 2 to 4 patients. Physiotherapy took place at Outpatient ward in Department of Sport Medicine and Rehabilitation, St. Anne's University Hospital, Brno, Czech Republic. Each session of physiotherapy took 60 minutes. The program last for 4 weeks, total 8 sessions, twice a week. 2 well-experienced physiotherapists were presented in each group. The therapy group was heterogeneous, including patients who were symptomatic, hospitalized and non-hospitalized during ongoing COVID-19 positivity. Each patient was monitored continuously during each therapy by blood oxygenation, heart rate and subjective perceiving of dyspnea and perceiving of exertion using standardized Borg scales.

Program was based on specific therapeutic schedule of increasing intensity and consists of 5 therapeutic parts. These specific parts of program were: Warm-up phase, Stretching and Mobilization phase, Postural-Breathing phase, Endurance phase – Nordic Walking, Relaxation and Stretching phase. Methodology was set up according recent guidelines and recommendations for pulmonary rehabilitation and post-COVID treatment [2, 4, 8, 9, 10, 16, 11, 21, 22]. The Figure 3 illustrates the course of therapy program.
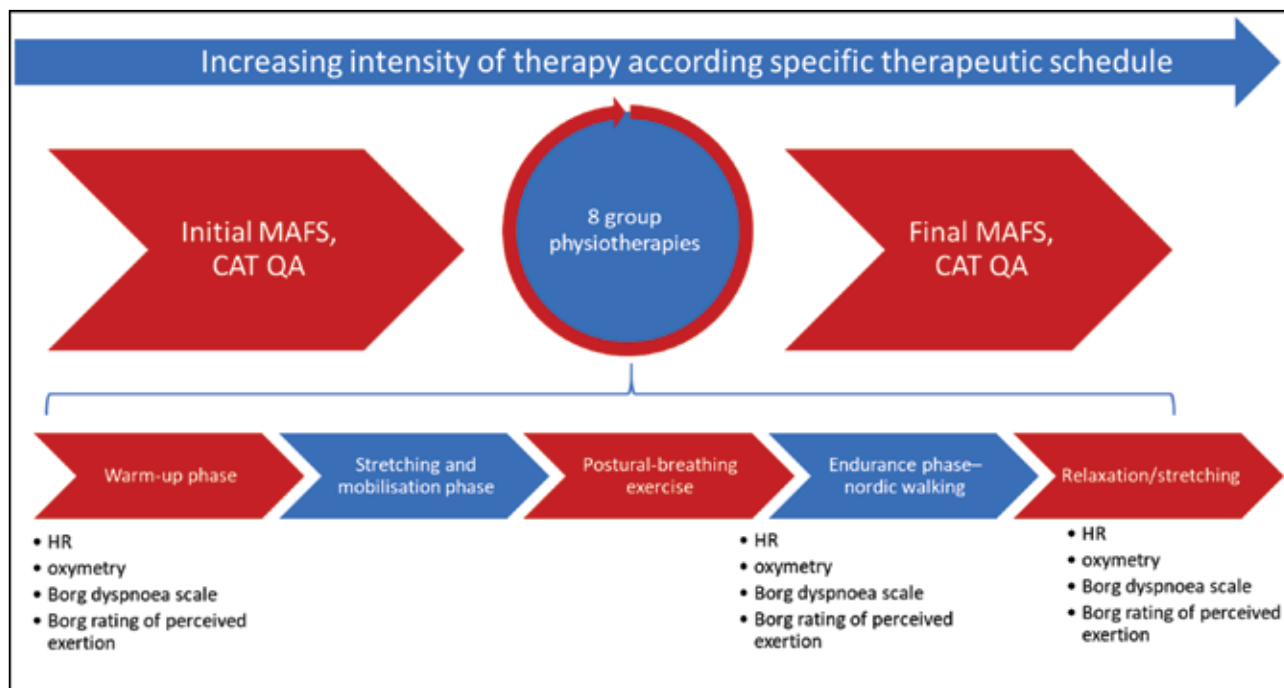
Figure 2: *Protocol of group physiotherapy program for post-COVID outpatients*

All patients were examined by self-reported questionnaires for evaluation dyspnea and pulmonary functions **COPD Assessment Test** (CAT) and fatigue **Multidimensional Assessment of Fatigue Scale** (MAFS). Evaluation took place at the beginning before first session and at the end of the physiotherapy program after the last session. MAFS and CAT questionnaires are standardized for Czech language and both are established to evaluate symptoms during or after COVID-19. Both questionnaires are evaluated by 2 points change as the significant improvement of health [3, 7, 14, 15].

CAT evaluates mainly rate of dyspnea and other 7 categories of pulmonary functions: Caught, Mucus in Chest, Chest Tightness, ADL (activities at home), Activities outside (socializing), Sleep, Fatigue and energy. Scoring is from 0 to 5 were 5 is the most severe condition [7, 15].

MAFS assesses different aspects of fatigue and its influence to the Activity to Daily Life. QScoring is from 0 (no fatigue) to 10 (most severe condition). Assessment is divided to 4 Dimensions of fatigue consist from 16 Questions: *I-dimension* - Severity of Fatigue (1. Question); *II-dimension* - Discomfort during Fatigue (2.–3. Questions); *III-dimension* - Limitations in ADL (4.–14. Questions); *IV-dimension* - Length of Fatigue, frequency and Change during last week (15. and 16. Question). Total score is called *Global Fatigue Index (GFI)* and scoring is computation = 1. Question + 2. Question + 3. Question + Average of 3. dimension (ADL Activities) + 2,5 x 15. Question [2, 14].

Non-parametrical Wilcoxon Signed-Rank Test was used for statistical analysis after evaluation of data´s normality. Computation was conduct in program Statistics 12.
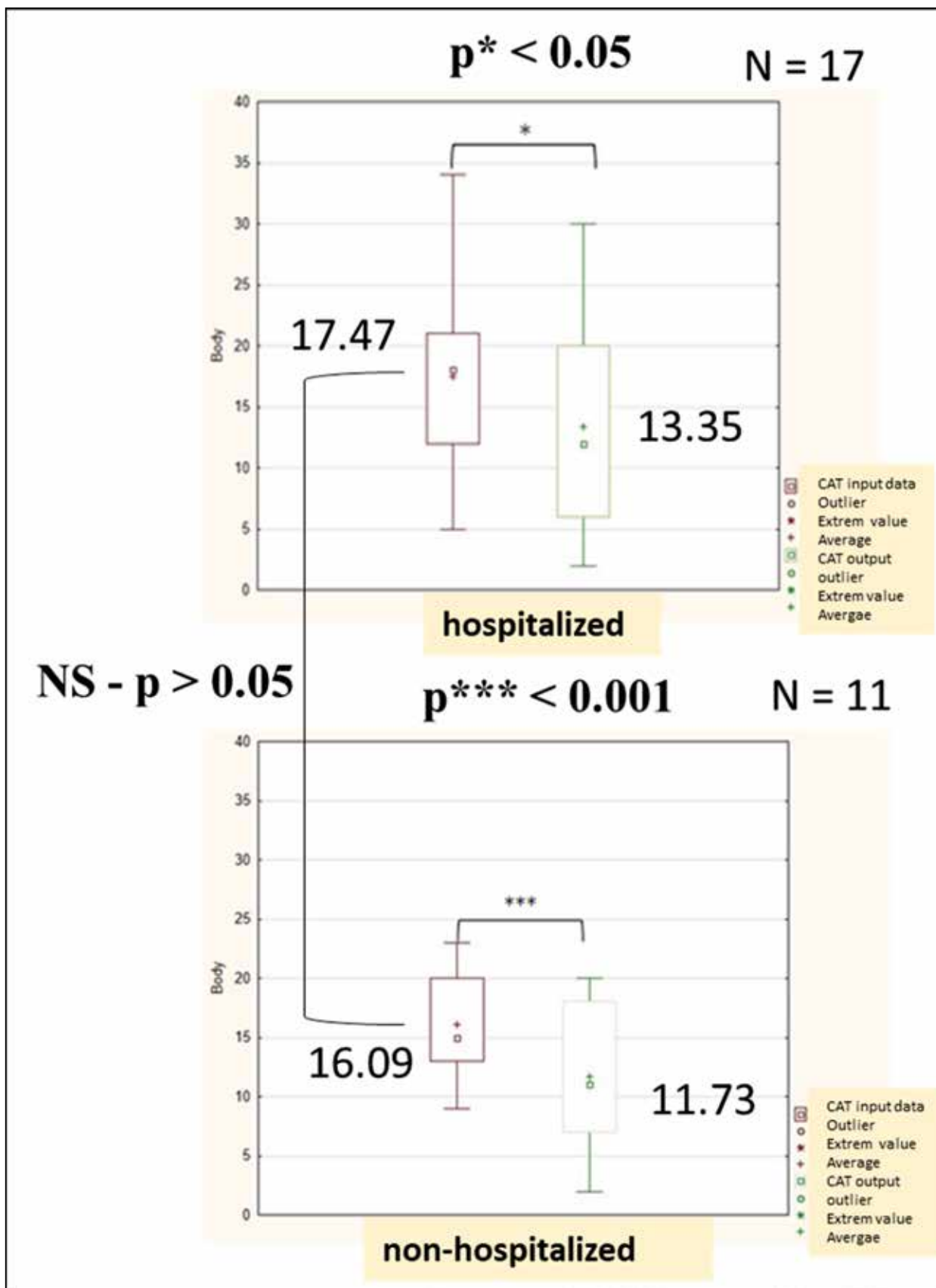
Results

We found statistically significant improvement in total CAT score by an average of 4.12 points on significance $p < 0.001$ (significance level α 0.05, N_{CAT} = 28 patients). Average Total score before therapy was 16.93 ± 6.67 (Median 17 (min 5; max 34)) and after therapy 12.71 $\pm$ 7.66 (Median 12 (min 2; max 30). Statistically more significant improvement was in the subgroup with mild ongoing COVID-19 (non-hospitalized) $p = 0.001$.

Statistic results for individual items of CAT assessment were for dyspnea $p < 0.001$; performing ADL $p = 0.001$; activity outside the home - socialization $p < 0.05$; fatigue and energy $p < 0.001$.

The rehabilitation program has positive effect on self-assessment of respiration complications and its influence of ADL.
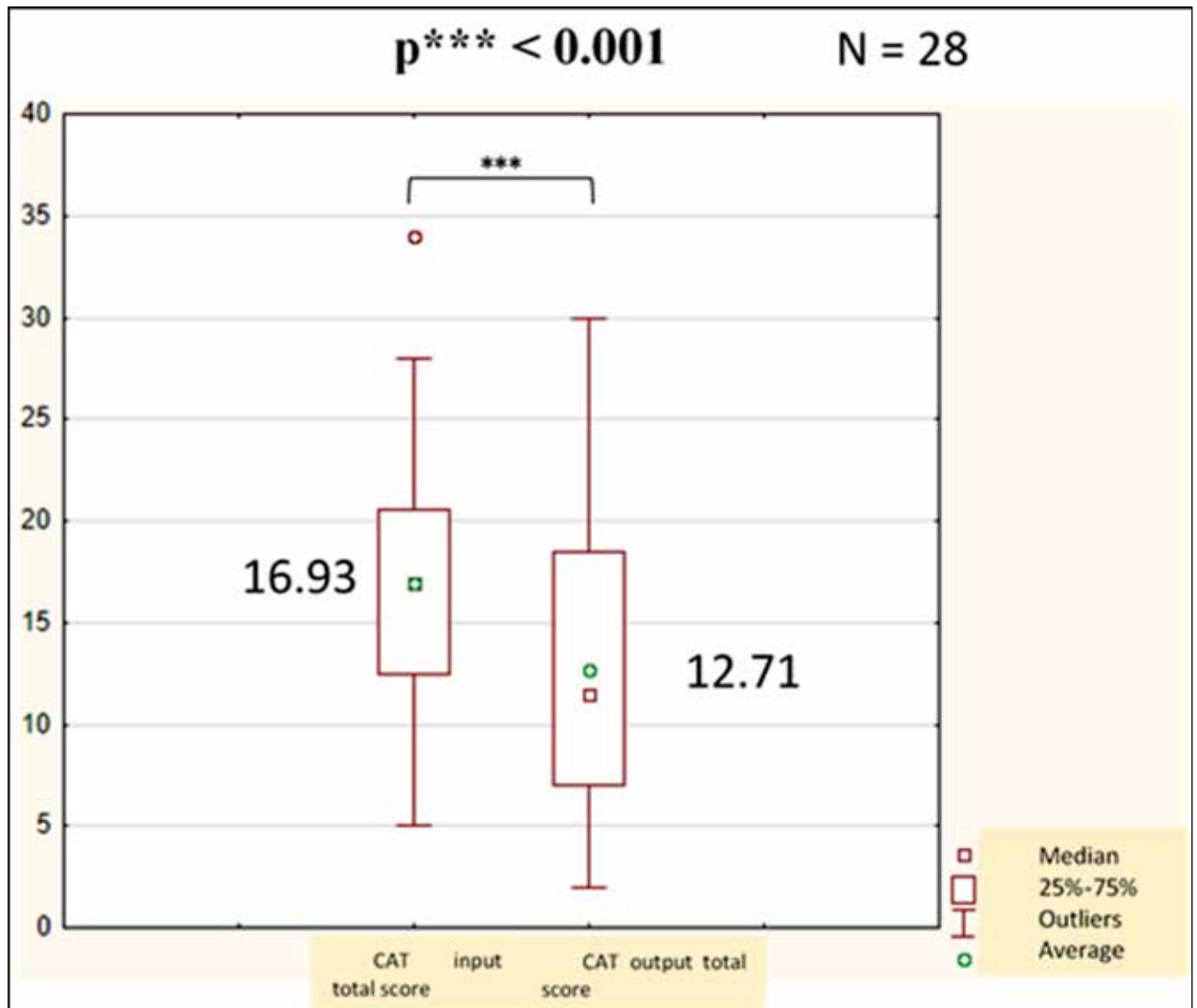
Graph 2: *Statistically significant improvement of dyspnea after physiotherapy evaluated by subjective COPD of Assessment Test*

Graph 3: *Statistically significant improvement of dyspnea after physiotherapy in hospitalized (N=17) and non-hospitalized (N=11) patients during ongoing COVID-19; NS – non-significant result between input data of both groups*

Table 2: Statistics of single pulmonary functions in COPD of Assessment Test

| n = 28 CAT Assessment Questions	Input Average; median	Output Average; median	P-value	Significance
CAT$_1$ – Caugh	1.82 ± 1.56; 1 (min 0; max 5)	1.43 ± 1.23; 1 (min 0; max 5)	0.064	p > 0.05
CAT$_2$ – Mucus in Chest	1.43 ± 1.2; 1 (min 0; max 4)	1.36 ± 1.31; 1 (min 0; max 4)	0.675	p > 0.05
CAT$_3$ – Chest Tightness	1.82 ± 1.33; 1.5 (min 0; max 5)	1.57 ± 1.32; 2 (min 0; max 4)	0.191	p > 0.05
CAT$_4$ – Dyspnea	3.39 ± 0.96; 3 (min 1; max 5)	2.39 ± 1.23; 2.5 (min 0; max 5)	0.000	p*** ≤ 0.001
CAT$_5$ – ADL (activities at home)	2.43 ± 1.32; 3 (min 0; max 4)	1.57 ± 1.26; 1.5 (min 0; max 4)	0.001	p*** ≤ 0.001
CAT$_6$ – Activities outside (socializing)	0.86 ± 1.15; 0.5 (min 0; max 5)	0.43 ± 0.63; 0.0 (min 0; max 2)	0.028	p* ≤ 0.05
CAT$_7$ – Sleep	2.11 ± 1.45; 2 (min 0; max 5)	1.79 ± 1.57; 1 (min 0; max 4)	0.171	p > 0.05
CAT$_8$ – Fatigue	3.07 ± 1.09; 3 (min 0; max 5)	2.18 ± 1.6; 2 (min 0; max 5)	0.000	p*** ≤ 0.001
Total score CAT	16.93 ± 6.67; 17 (min 5; max 34)	12.71 ± 7.66; 12 (min 2; max 30)	p = 0.0003	p*** ≤ 0.001

We found statistically significant improvement in Global Fatigue Index of MAFS by an average of 8.8 points on significance < 0.001 (significance level α 0.05, N_{MAFS} = 28 patients). Average GFI before therapy was 31.8 ± 8.48 (Median 33.8 (min 14.4; max 42.2)) and after therapy 23.0 ± 9.98 (Median 20.6 (min 7.8; max 42.9). Statistically significant improvement was in both subgroup (non-hospitalized and hospitalized patients) p = 0.01.

Statistic results for individual items MAFS assessment: level of fatigue p < 0.001; magnitude of fatigue p < 0.001; distress caused by fatigue p < 0.01; fatigue during individual ADL items (walking and exercise, leisure and recreational activities, housework, sexual activity, shopping, errands, exercise) p < 0.05 to p < 0.001; frequency of occurrence of fatigue p < 0.05.

The rehabilitation program has positive effect on self-assessment of fatigue and its influence of ADL.
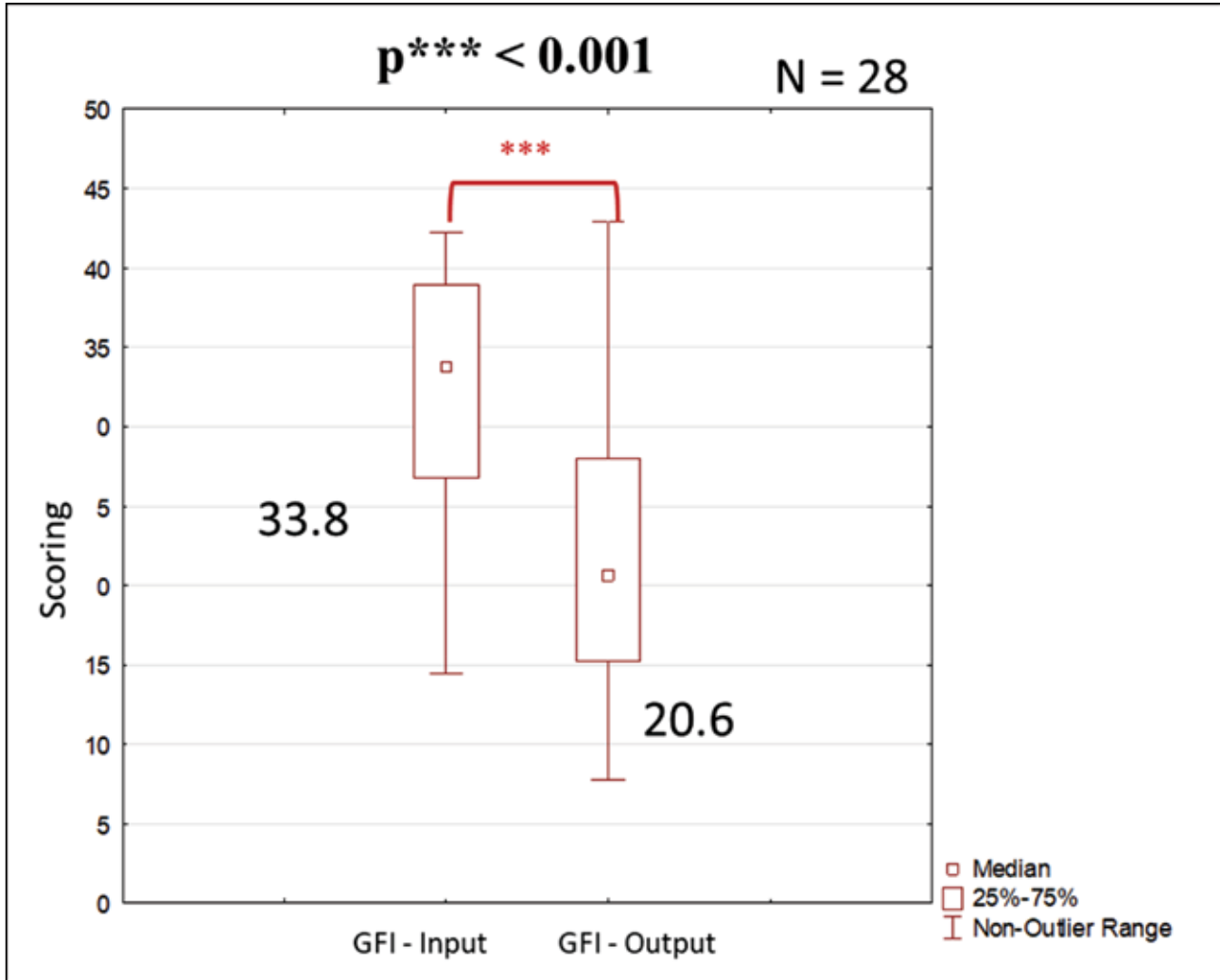
Graph 4: *Statistically significant improvement of fatigue after physiotherapy evaluated by subjective Global Fatigue Index (GFI) in Multidimensional Assessment of Fatigue Scale*
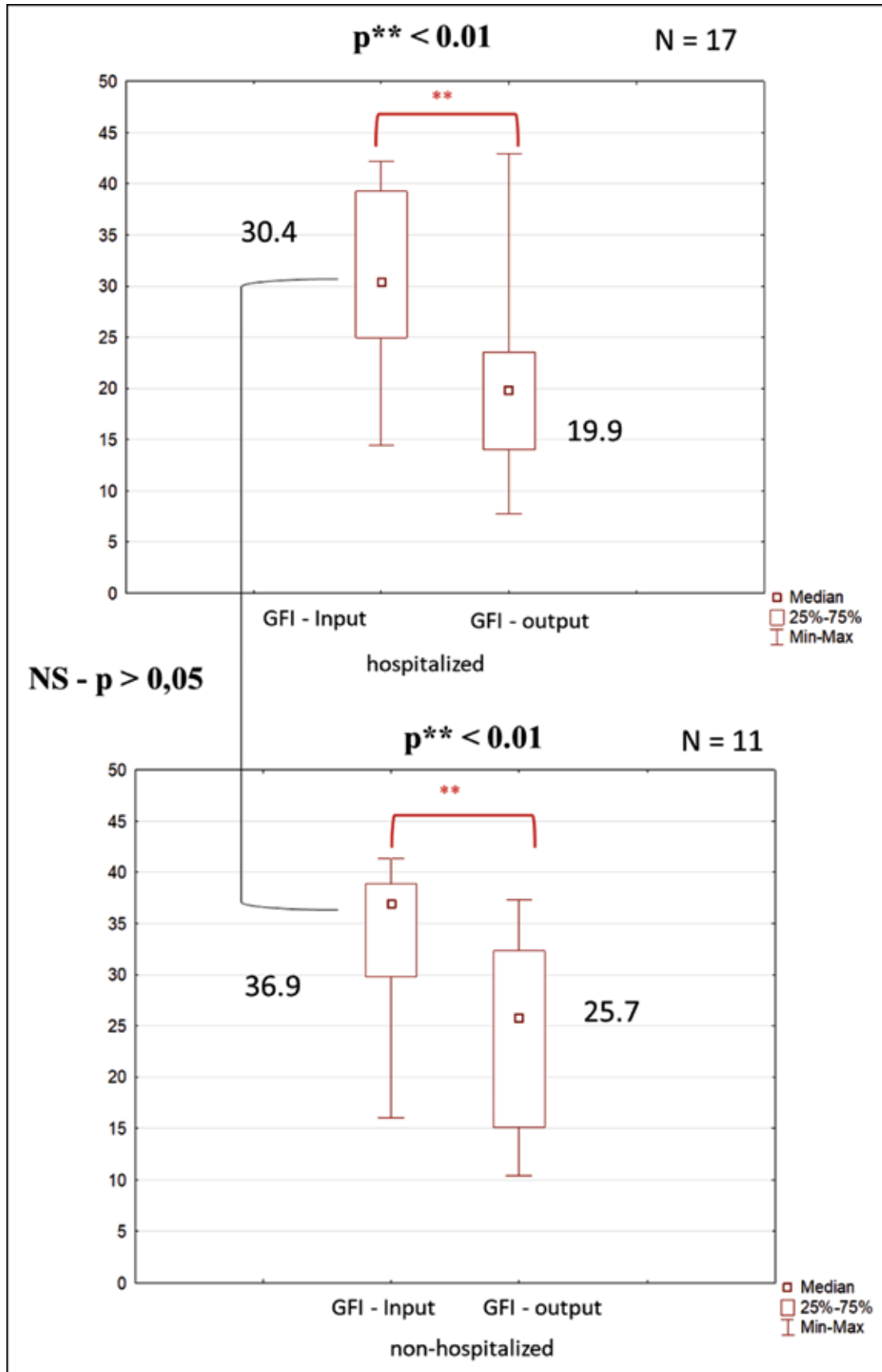
Graph 5: *Statistically significant improvement of fatigue after physiotherapy in hospitalized (N=17) and non-hospitalized (N=11) patients during ongoing COVID-19 evaluated by Global Fatigue Index (GFI); NS – non-significant result between input data of both groups*

Table 3: Statistics of dimensions of fatigue in Multidimensional Assessment of Fatigue Scale

n = 28		Input Average; median	Output Average; median	Difference Average; median	P-value	Sign.
1. d.	Degree of fatigue	6.9 ± 2.1; 7 (min 2; max 10)	4.7 ± 2.3; 4 (min 2; max 9)	2.2; 3.0	p = 0.0001	p*** ≤ 0.001
2. d	Severity of fatigue	6.5 ± 2.1; 7 (min 2; max 9)	4.6 ± 2.5; 4 (min 1; max 9)	1.9; 3.0	p = 0.0001	p*** ≤ 0.001
	Distress caused by fatigue	5.9 ± 2.2; 7 (min 1; max 10)	4.3 ± 2.6; 3 (min 1; max 10)	1.6; 4.0	p = 0.0025	p** ≤ 0.01
3. dimension — degree of fatigue	Household activity (washing dishes etc.)	4.9 ± 2.5; 5 (min 1; max 9)	3.5 ± 2.1; 3 (min 1; max 9)	1.4; 2.0	p = 0.0208	p* ≤ 0.05
	Cooking	4.3 ± 2.2; 4 (min 1; max 8)	2.7 ± 1.6; 2 (min 1; max 7)	1.6; 2.0	p = 0.0020	p** ≤ 0.01
	Bathing or washing	3.1 ± 2.4; 2 (min 1; max 10)	2.7 ± 2.0; 2 (min 1; max 9)	0.4; 0.0	p = 0.1706	p > 0.05
	Dressing	2.9 ± 2.5; 2 (min 1; max 10)	2.4 ± 1.9; 1 (min 1; max 8)	0.5; 1.0	p = 0.1208	p > 0.05
	Working	5.5 ± 2.9; 6 (min 1; max 9)	4.5 ± 2.9; 3.5 (min 1; max 10)	1; 2.5	p = 0.1034	p > 0.05
	Visit or socialize with friends or family	4.3 ± 2.6; 4 (min 1; max 9)	3.5 ± 2.3; 2 (min 1; max 9)	0.8; 2.0	p = 0.1262	p > 0.05
	Sexual activity	4.5 ± 2.9; 4 (min 1; max 10)	3.1 ± 2.1; 3 (min 1; max 9)	1.4; 1.0	p = 0.0113	p* ≤ 0.05
	Leisure and recreational activities	6.1 ± 2.3; 6.5 (min 2; max 9)	3.8 ± 1.8; 3 (min 1; max 7)	2.3; 3.5	p = 0.0005	p*** < 0.001
	Shoping	5.4 ± 2.6; 6 (min 1; max 9)	3.8 ± 2.2; 3 (min 1; max 8)	1.6; 3.0	p = 0.0080	p** ≤ 0.01
	Walk	5.4 ± 2.3; 6 (min 1; max 9)	3.5 ± 1.9; 3 (min 1; max 7)	1.9; 3.0	p = 0.0010	p*** ≤ 0.001
	Exercise, other than walking	5.7 ± 2.5; 6 (min 1; max 10)	4.2 ± 2.2; 3.5 (min 1; max 9)	1.5; 2.5	p = 0.0420	p* ≤ 0.05
4. dimension	Frequency of fatigue over the past week	8.1 ± 2.0; 7.5 (min 5; max 10)	6.9 ± 2.1; 7.5 (min 5; max 10)	1.2; 0.0	p = 0.0052	p* ≤ 0.05
	Fatigue changed during the past week	2.4 ± 0.7; 2 (min 1; max 3)	2.3 ± 0.9; 2 (min 1; max 3)	0.1; 0.0	p = 0.6661	p > 0.05
GFI ± SD		31.8 ± 8.48; 33.8 (min 14.4; max 42.2)	23.0 ± 9.98; 20.6 (min 7.8; max 42.9)	8.7; 13.2	p = 0.000038	p*** ≤ 0.001

Discussion

Our methodology is well set up for post-COVID patients which has been statistically proven. Significant effect form of therapy is group physiotherapy for outpatients, 4-week therapy lasting 60 minutes per session, overall 8 therapies, complex physiotherapy - dynamic warm-up, stretching, postural and respiratory exercises in ontogenetic positions, endurance training

– Nordic Walking, relaxation – mindfulness. The group can be heterogeneous, thus consists of patients with severe and mild symptoms. COPD Assessment Test (CAT) and Multidimensional Assessment of Fatigue Scale (MAFS) are suitable tools for subjective assessment in post-COVID symptomatology in Physiotherapy. For more complex analysis we need collected more subjects to find correlation between characteristics of study group and results in evaluated parameter.

Conclusion

Presented physiotherapy group program is effective on the most frequent persistent post-COVID symptoms – dyspnea and fatigue. Conclusion is based on the significant improvement after physiotherapy on subjective evaluation of well-being influenced by persistent symptoms after COVID-19 infection.

The presented group physiotherapy program significantly improves the quality of life after COVID-19 and can be used for large number of post-COVID patients, based on our data.

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A Brief Physiology of Ion Balance in Mammal Cardiomyocytes

Luboš Nachtnebl, [1] **Petr Filipenský,** [1, 2] **Magda Krechlerová,** [3] **Helena Bedáňová,** [4,5] **Alena Sedláková,** [4,5] **Adam Vajčner,** [5] **Michal Pohanka,** [4,5] **Petr Dobšák**

[1st] Department of Orthopedic Surgery, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno
[1] Department of Urology, [2nd] Department of Internal Medicine, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno
[2] Department of Public Health, Faculty of Medicine, Masaryk University Brno
[3] Center of Cardiovascular and Transplant Surgery, Faculty of Medicine, Masaryk University Brno
[4] Department of Sports Medicine and Rehabilitation, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno
[5]Department of Physiotherapy and Rehabilitation, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno

Introduction

The muscle cells (cardiomyocytes) that make up all of the heart muscle contract in a repetitive, organized and adapted way in order to ensure the final function of circulatory support. The coordination of the contractile function is ensured thanks to the syncitium structure of the cardiac tissue which allows the propagation of the electrical activity from one cardiac cell to another. This electrical activity translates into an action potential (AP) which represents the result of a cascade of ion transfers (entry of Na^+ and Ca^{++} ions, exit of K^+ ions), largely depending on the variations in permeability of the sarcolemma and succeeding from the diastolic potential. The latter, located between -80 and -90 mV, depends on the characteristics of the sarcolemma which, at rest, is almost exclusively permeable to K^+, and on the variations in ionic concentrations (Na^+ and K^+) on either side of this membrane. However, in diastole, the sarcolemma is slightly permeable to Na^+ and the concentration gradients are maintained thanks to active transport ensured by an electrogenic ATP-dependent Na^+/K^+ membrane pump.

The cytoplasmic membrane is equipped with a number of channels of different types, some of which allow the entry of calcium ions from the extracellular space. Two of them also serve as systems for Ca^{++} expulsion from the cell: one of them is the Na^+/Ca^{++} exchanger (NCX) and the other is the ATP-dependent cytoplasmic membrane Ca^{2+} pump (PMCA). The amount and proportion of individual NCX and PMCA channels varies by cell type.

For example, cardiomyocytes contain the NCX exchanger in high concentration. The main role of PMCA is to stabilize the Ca^{++} content in the cytosol, while NCX channels are an important rapid regulatory mechanism preventing extreme fluctuations in intracellular calcium content (especially in cardiomyocytes). Currently, however, this traditional view of the role of both channels is changing, primarily in connection with the discovery of specific functional properties of different PMCA isoforms. However, a detailed view is beyond the scope of this text.

During the action potential, membrane depolarization triggers the opening of specific Ca^{++} channels in the sarcolemma (sarco/endoplasmic reticulum Ca^{++} ATP-ase II or SERCA II). This mechanism, although necessary, is however not sufficient to cause muscle contraction in the mammalian heart. Indeed, this calcium influx induces the release of Ca^{++} stored in the sarcoplasmatic reticulum (SR) by a channel sensitive to ryanodine (ryanodine receptor or RyR) and activated by Ca^{++}. The mechanism of this autocatalytic release of Ca^{++} is called "Ca^{++}-induced calcium release". The rise in the intracellular calcium concentration (Ca^{++}_{INT}) of about 10^{-7} to 10^{-6} M (calcium transient), triggers the contraction. The different steps between the genesis of the membrane action potential and the initiation of the contractile phenomenon characterize the mechanism of "excitation-contraction coupling".

The blood inflow into the ventricular cavities takes place during the myocardial relaxation phase. This occurs during the reduction of cytosolic Ca^{++} in the cardiomyocytes. Three mechanisms contribute to this. On the one hand, the Ca^{++} ions are actively recaptured into the inner structures of the SR thanks to SERCA II. On the other hand, Ca^{++} is expelled into the extracellular space by the Na^+/Ca^{++} pump (NCX) of the cytoplasmatic membrane, which is energetically supplied by the transmembrane sodium gradient. Finally, the third mechanism comprises the ATP-dependent calcium pump (PMCA) of the cytoplasmatic membrane which, however, plays only minimal role in the expulsion of Ca^{++} from the cell.

All of these processes require an energetic supply. H^+ protons are thus continuously generated in the cytosol of cardiac cells by their intense metabolic activity, contributing to lowering their intracellular pH (pH_{INT}). In particular, glycolysis, synthesis and degradation of glycogen and triglycerides, lipolysis and hydrolysis of ATP are some examples of biochemical reactions associated with H^+ production. Although the precise mechanisms at the origin of the effects of pH_{INT} on contraction are not completely elucidated, it nevertheless appears that all the stages of the coupling between the genesis of the membrane action potential and the production of power by the myofilaments are affected by the changes in pH_{INT}. The control of H^+ homeostasis is therefore crucial to ensure normal conductivity of ion channels and the efficiency of the contractile machinery. In a normal myocardium the pH_{INT} is maintained around 7.0 - 7.2 by mechanisms that buffer or expel excess H^+ following their passive entry into the cell and the continuous production of metabolic acids (exchanges Na^+/H^+ and $Cl^-/$

HCO_3^-). The main source of energy, on which cardiac contractile function depends, is the cytosolic (intracellular) ATP which is complexed with Mg^{++}. In the cells, the concentration of ATP is physiologically about 7 - 10 mM and the total Mg^{++} represents about 17 mM, of which 40 - 45% is bound to ATP. Therefore, under conditions where ATP is decreased, the loss of this important Mg^{++} binding site can lead to an elevation of Mg^{++}_{INT}. However, Mg^{++} plays also an important role in the regulation of ion channels, the activation of enzymes, and the movements of calcium through the SR. Therefore, several mechanisms for regulating Mg++ are present, which involve intracellular buffers, transport systems through intracellular organelles and some membrane transporters that are still not fully understood (Na+/Mg++ exchanger ?). Thus, from a functional point of view, the contractile activity of the cardiac muscle depends first of all on the contractile machinery, but also on the regulation of ionic movements.

2. Regulation of intracellular ionic activities in human cardiomyocytes

In the cardiac cells, the intracellular ionic activities are maintained at different values from those expected, if the ions would be distributed passively on both sides of the cell membrane. This implies the existence of mechanisms that buffer, compartmentalize, capture or expel ions following their passive entry/exit into/from the cell. Fig. 1 illustrates the complexity of these mechanisms involved in the regulation of ionic species in the myocardium. Their interaction is so intense that any disturbance of just one mechanism affects directly or indirectly the others.

A. ATP-dependent transporters or pumps (ATP-ases)

These mechanisms use the energy released by the hydrolysis of ATP into ADP to transport the ions on either side of the sarcolemma or to store them in the SR.
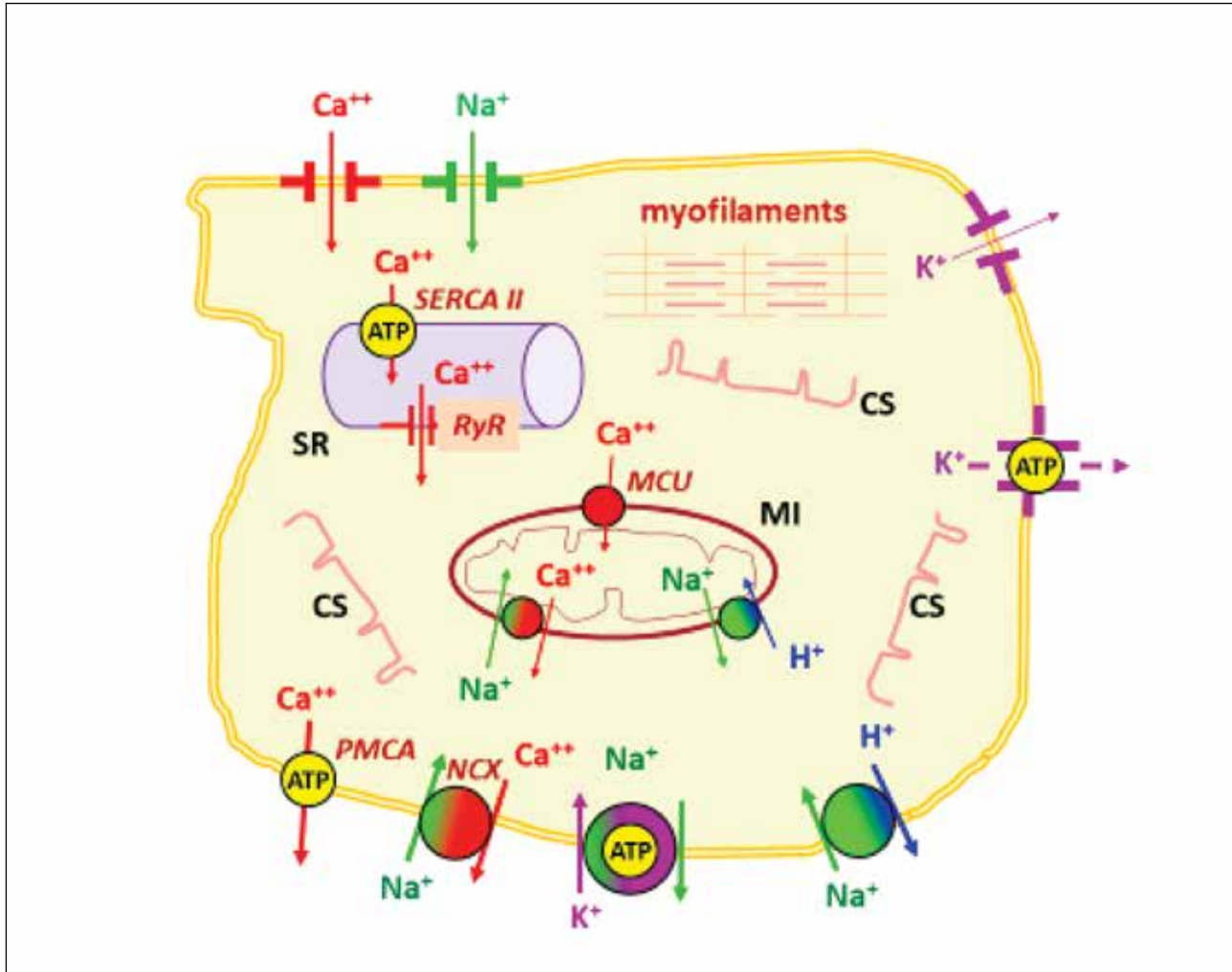
Figure 1: *Schematic view of the ion transfers in the cardiomyocyte in physiological conditions. The entry of Ca^{++} into the SR is secured by the ATP-dependent calcium channel (SERCA II) and its exit by Na$^+$/ Ca^{++} exchanger (NCX) and sarcolemmal RyR. The entry of Ca^{++} into the mitochondria (MI) takes place via the calcium uniport (MCU) and its exit via Na$^+$/Ca^{++} exchange. The mitochondrial sodium gradient is maintained by Na$^+$/H$^+$ exchanger (CS = cytoskeleton; MI = mitochondria; SR = sarcoplasmic reticulum) (Source: own material).*

1. The ATP-ase or Na$^+$/K$^+$ pump of the sarcolemma is responsible for the simultaneous transport of 3 Na$^+$ ions from the inside to the outside of the cell and of 2 K$^+$ ions in the opposite direction. Due to the unequal number of charges transported (an overall positive charge), this exchange is capable of generating a current called "pump current" and, conversely, can be modulated by the membrane potential. Among the most important physiological activators of this ATP-ase belong intracellular ions of Na$^+$ and catecholamines. The major physiological role of the Na$^+$/K$^+$/ATP-ase consists in regulating the efflux of intracellular Na$^+$ against the passive entry of Na$^+$ in diastole or during depolarization, via the sodium current. Thus, this ionic pump allows the maintenance of the electrochemical gradient of Na$^+$ which is essential for electrical activity and the operation of other mechanisms such as

Na$^+$/Ca^{++} or Na$^+$/H$^+$ exchanges. Finally, the pump current contributes to the establishment of the resting membrane potential.

2. Calcium ATP-ase of the sarcolemma regulates Ca$^{++}_{INT}$ in diastole or in conditions of prolonged inactivity. The lower rate of Ca^{++} transport does not allow expulsion of this ion in large quantities into the extracellular space based on kinetics compatible with those of contraction. The rhythmic entry of calcium ions into the SR occurs through the ATP-dependent calcium system (SERCA II, controlled by phospholamban) and their release through the SR ryanodine-sensitive channels (RyR) in high concentration to the contractile apparatus. Potassium channels are activated by calcium and lead to slow repolarization. Their early activation shortens the plateau phase.

3. Calcium ATP-ase of the SR transports Ca^{++} from the cytosol into the lumen of SR.

B. Electrochemical ion gradient-dependent transporters

These mechanisms use transmembrane ion gradients as an energy source for ion transport. The Na$^+$ gradient maintained by the Na$^+$/K$^+$ pump is fundamental because many mechanisms use the potential energy contained in this gradient to ensure transport, either in the same direction as the sodium gradient (case of the Na$^+$/glucose co-transporter), or in the opposite direction (antiports or counter-transports Na$^+$/H$^+$ and Na$^+$/Ca^{++}).

1. The Na$^+$/Ca^{++} exchange of the sarcolemma is electrogenic (3 Na$^+$ ions are simultaneously exchanged for one Ca^{++} ion) and transports Ca^{++} (calcium efflux coupled with the development of a transient inward current). This exchange also allows the entry of Ca^{++} essentially at the beginning of the action potential, and thus participates in the net influx of Ca^{++}. In diastole, the Na$^+$/Ca^{++} exchange works in the direction of calcium efflux and allows the Ca^{++} gradient to be maintained.

2. The transporters of the mitochondrial inner membrane: calcium mitochondrial uniporter (MCU), Na$^+$/H$^+$ and Na$^+$/Ca^{++} exchangers participate in the regulation of intra-mitochondrial ionic species, particularly Ca^{++}, which presence stimulates the oxidative activity of mitochondria. Under physiological conditions, the mitochondrial Ca^{++} cycle is dependent on extra-mitochondrial concentration of Na$^+$. Mitochondrial transporters are mediated diastolic calcium signal relays for intra-mitochondrial Ca^{++}. These mechanisms ensure the setting of the energy production in the mitochondria according to the contractile activity of the muscle.

3. Na^+/H^+ exchange of the sarcolemma. It is an electroneutral and reversible antiporter, whose primary function - under physiological conditions - is to exchange an internal H^+ for an external Na^+. The 1:1 stoichiometry results in the independence of this exchanger with respect to the membrane potential. Exchange activity is weak or non-existent at physiological pH. The system is activated by a drop in pH_{INT} and its essential function is to prevent excessive acidification. The Na^+/H^+ antiporter is activated by intracellular H^+, and on the contrary, inactivated by extracellular concentration of H^+. The inhibition of activity by external H^+ is close to 50% for an extracellular pH (pH_{EXT}) between 7.0 and 7.5, which suggests that changes in pH_{EXT} may play an important modulating role (under physiological conditions). Besides its role in the regulation of pH_{INT}, Na^+/H^+ exchange can intervene in the control of Na^+_{INT} and Ca^{++}_{INT}. It represents also an important entry way for Na^+ and is capable of modifying the activity of the Na^+/Ca^{++} exchange. Therefore, it is also indirectly involved in the control of contractile activity.

4. Sarcolemma-independent Cl^-/HCO_3^- exchange. The content of Cl^-_{INT} in the cardiomyocytes is 20 to 30 mM, i.e. 5 times greater than that expected from the electrochemical gradient. Under physiological conditions, this anionic, electroneutral and reversible antiporter allows the efflux of HCO_3^- and the influx of Cl^-. Besides its role in the control of Cl^-_{INT}, it intervenes in the regulation of pH_{INT} following an intracellular alkalinization by transporting acidic equivalents. Its activity is inhibited when pH_{INT} becomes acidic.

5. The $Na^+/HCO3^-$ symport of the sarcolemma. This mechanism at the origin of the removal of acids and depending on HCO^- and extracellular Na^+, has been identified in mammalian cardiac tissue samples. The recovery of pH_{INT}, following an acid load, depends on external Na^+, but is independent of internal content of Cl^-. Sarcolemmal Na^+/HCO_3^- symport is electroneutral, responsible for about 20-30% of the total efflux of acidic equivalents (at a pH_{INT} of 6.6) and can participate in the control of Na^+_{INT} and Ca^{++}_{INT}.

6. The H^+-lactate symporter of the sarcolemma. The transport of lactate is carried out for the most part by this co-transport, which occurs in particular during the removal of lactic acid from the cells in a situation of hypoxia.

7. Na^+/Mg^{++} exchange of the sarcolemma. The existence of such an exchange in cardiac cells remains unclear. The plasma membrane is poorly permeable to Mg^{++} ions. The regulatory mechanisms of Mg^{++} very likely involve membrane transporters, intracellular buffers and transport systems through intracellular organelles. Some studies suggest that the binding of Mg^{++} to intracellular sites and/or the transport of Mg^{++} through organelles are modulated by Ca^{++} and H^+ ions. Changes in pH_{INT} or Ca^{++}_{INT} could then induce variations in $Mg^{++}_{INT,}$ and thus influence Mg^{++} homeostasis and consequently the cellular processes that depend on it.

C. Buffer systems

1. Adsorption of ions at the sarcolemma. Beside different sarcolemmal mechanisms involved particularly in the movements of Ca^{++}, there is a microenvironment of anionic phospholipids which adsorb this ion, and thus are able to modulate the activity of Ca^{++} transporters.

2. Soluble cytoplasmic proteins - calmodulin (CM) and troponin C (TN-C). CM is a Ca^{++} and Mg^{++} binding peptide. Once these ions are fixed, the CM can regulate the functioning of several enzymes, known as CM-dependent, such as some protein kinases, or even the calcium ATP-ase of the sarcolemma. TN-C is important for cardiac contractile activity. Indeed, in the absence of Ca^{++}, TN-C interacts with other troponins such as troponin T (TN-I) and troponin T (TN-T); the formation of this macromolecular complex prevents the interaction of actin with myosin and therefore contraction. In the presence of Ca^{++} ions, TN-C undergoes a conformational change that is transmitted to TN-T and TN-I, ends actin inhibition and allows the formation of actin-myosin bridges, leading to the development of contractile activity. If it is correct to attribute to these proteins a role of intracellular calcium "buffer", the binding of Ca^{++} on CM and TN-C is accompanied by above mentioned conformational changes leading to the activation of other target proteins. These molecules can therefore be considered as transduction elements of the "calcium messenger", a chemo-chemical signal for CM and a chemo-mechanical signal for TN-C.

3. Intracellular buffering capacity (β) in response to acid overload. The cell can involve several processes which absorb protons in a rapid and reversible way. These are transient and saturable mechanisms (because they have a limited capacity), allowing the cell to react almost instantaneously to minimize a variation in pH_{INT}, and include:

a) The physico-chemical buffering power of the pH. It is probably the most important component of β by its ability to absorb protons. This is a unique property that allows weak acids and bases to moderate pHINT changes by combining with protons according to the reaction: B + H+ → BH (B = base). These ionic reactions occur in a fraction of a second. An example of weak intracellular base is the HCO_3^- ion. In situations of increased acidification, its combination with H^+ leads to formation of H_2O and CO_2. The cell can also behave as an open system for other weak acids or bases, but only provided that the plasma membrane is sufficiently permeable to their uncharged forms. In their presence, the intracellular concentration of the buffers will increase.

b) Biochemical buffering mechanisms. Certain metabolic reactions participate in pH_{INT} homeostasis. A good example can be the conversion of weak non-volatile acid, such as lactic acid, into a diffusible product in the form of CO_2. Under normal (physiological) conditions, inorganic phosphate (P_i) exists in two forms near neutral pH: HPO_4^- and $H_2PO_4^-$ (acid dissociation constant pKa = 6.8) with a total concentration of approximately 1 to 2 mM. The

contribution of phosphate compounds to the buffering capacity remains low, but can increase in situations such as ischemia.

Also the neutralization by H^+ ions of ionizable groups of many intracellular molecules, in particular proteins, participates in the adsorption of protons. Although the chemical nature of these buffers is not completely identified, the greatest contribution could come from the ionizable groups of intracellular proteins, more particularly the imidazole groups which have a pKa in the range of physiological pH. The increase in β with intracellular acidification suggests that the average pKa of the intrinsic cellular buffers is lower than the physiological pH_{INT} and could represent a protective mechanism enabling the myocardium to fight against acid overload, in particular under hypoxic/ischemic conditions.

c) Organelle buffering mechanisms. These mechanisms concern the transfer of acids or bases in intracellular organelles.

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Destabilization of Ionic Transport Systems in Cardiomyocytes During Hypoxia and Ischemia

Luboš Nachtnebl, [1] Petr Filipenský, [1, 2] Magda Krechlerová, [3] Helena Bedáňová, [4,5] Alena Sedláková, [4,5] Adam Vajčner, [5] Michal Pohanka, [4,5] Petr Dobšák

1st Department of Orthopedic Surgery, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno
[1] Department of Urology, 2nd Department of Internal Medicine, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno
[2] Department of Public Health, Faculty of Medicine, Masaryk University Brno
[3] Center of Cardiovascular and Transplant Surgery, Faculty of Medicine, Masaryk University Brno
[4] Department of Sports Medicine and Rehabilitation, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno
[5] Department of Physiotherapy and Rehabilitation, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University Brno

A. Introduction

In 1994, one hundred and thirty-six years after the introduction of the term "ischemia" by Virchow, the journal Cardiovascular Research conducted a survey among eminent cardiologists to find out "their" definition of myocardial ischemia (Hearse, 1994). The results showed the responses ranged from 3 to 404 words (!). This clearly shows that a generally accepted definition does not currently exist. However, it is undisputed that ischemia is necessarily associated with changes in the energy metabolism and ionic disturbances of the myocardium.

It is difficult to study ischemia experimentally and in particular in isolated muscle preparations. For this reason, many studies concerning the effects of ischemia on contractile function have used experimental protocols that reproduce only some of the metabolic, ionic and mechanical aspects of ischemia. The most used models are hypoxia or anoxia, based on partial or total suppression of oxygen (O_2) in the perfusion medium (replacement of O_2 by nitrogen or application of a reducing agent, such as sodium dithionate), or even on the use of blockers of oxidative phosphorylation (OP) such as cyanide (CN).

However, ischemia is associated not only with a reduction in oxygen supply but also in energetic substrates, as well as an accumulation of metabolic products such as lactate, protons and potassium. To more accurately reproduce the situations of ischemia, hypoxia or anoxia, it

seems to be advantageous to associate them with an inhibition of anaerobic glycolysis. Under these conditions, although the mechanisms involved are probably less complex, the mechanical responses are similar to those observed in real pathologic situations. Therefore, with the aim of providing an overview of the ionic alterations occurring during ischemia and their consequences on cardiac contractility, the following text is based on studies carried out both in hypoxic conditions in presence or absence of functional glycolysis, anoxia and ischemia, and using multicellular preparations or isolated myocytes (Fig. 1).
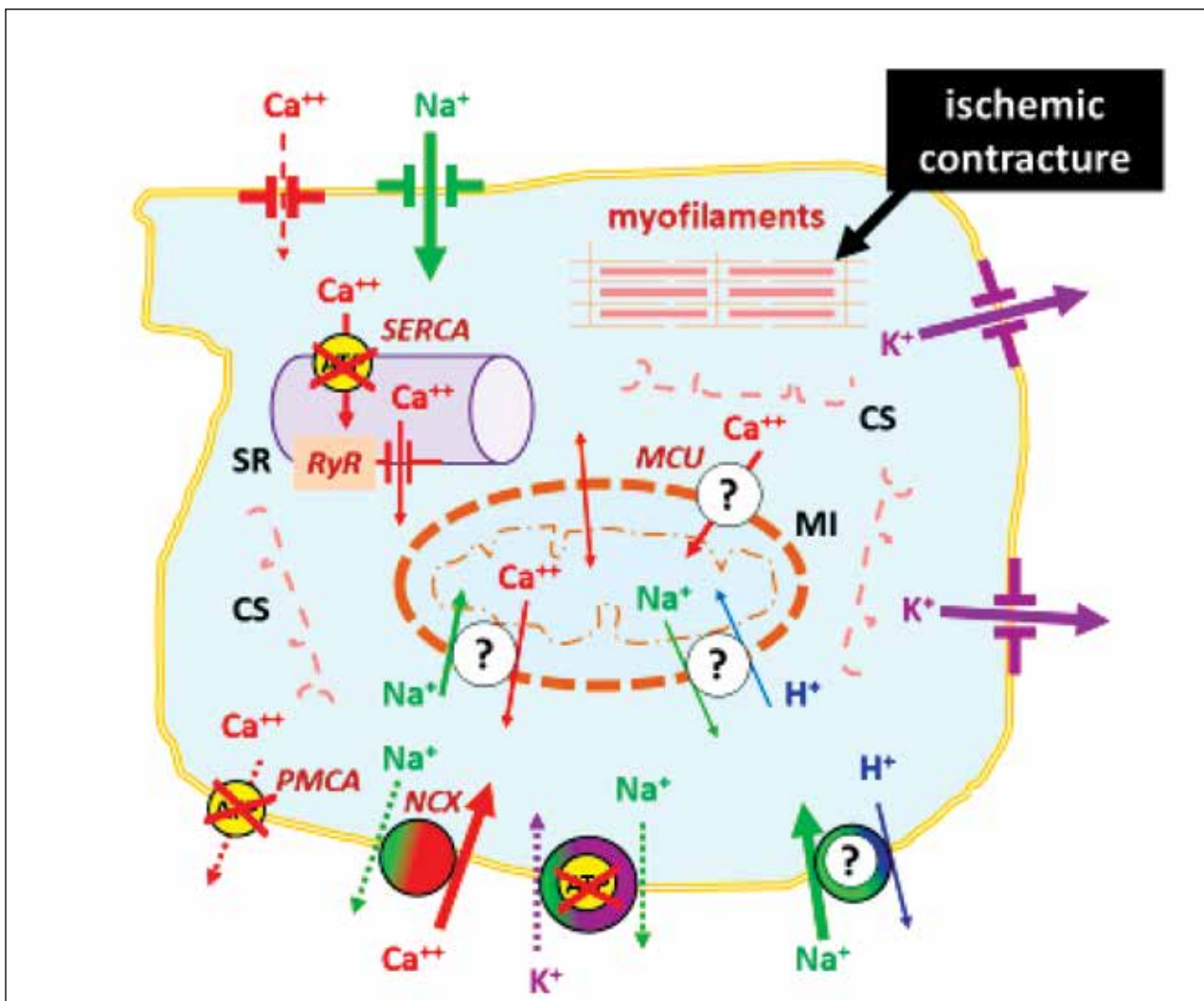

Figure 1: *Schematic overview of ionic imbalance during cardiomyocyte ischemia. Following ATP depletion, $Na^+/K^+/ATP$-ase is inhibited and sodium efflux reduced. The entry of Na^+ is enhanced by Na^+/H^+ exchange (activated by the acidification of pH_{INT}). Intracellular Ca^{++} rises following the reduction (and/or reversal) of Na^+/Ca^{++} exchanger (NCX). Mitochondrial Ca^{++} increase is realized by a still poorly defined mechanism. Mitochondria begin to show signs of edema. K^+ efflux is massive, via ATP-sensitive potassium channels. The actin-myosin bridges are no longer detached (state of rigor or "stunning") and the cytoskeleton begins to break down (CS = cytoskeleton; MI = mitochondria; MCU = calcium mitochondrial uniporter; PMCA = ATP-dependent cytoplasmic membrane Ca^{++} pump; SR = sarcoplasmic reticulum) (Source: own material).*

B. Changes in intracellular pH (pH_{INT})

Changes during hypoxia-ischemia of pH_{INT} and its short-term (intracellular buffering capacity) and long-term (membrane transporters) regulatory mechanisms will be discussed.

1. Changes in pH_{INT}

a) In the presence of functional glycolysis, pH_{INT} measurements carried out in experiments with perfused hearts or multicellular heart preparations have shown the existence of a transient alkalinization which is associated with the rapid hydrolysis of phosphocreatine (PCr) to creatine (Cr) and Pi, a reaction consuming protons. This brief alkalosis is followed by an intracellular acidification which stabilizes after approximately 5-10 minutes. This acidosis is the consequence of the stimulation of anaerobic glycolysis and the production of lactic acid when the oxidative phosphorylations (OP) are blocked.

b) Experiments in isolated rat ventricular cells, using fluorescent indicators, did not show any acidification following the addition of cyanide (CN). On the other hand, if glycolysis is completely inhibited (f.e. by the iodo-acetic acid), a very rapid and strong acidosis (about 0.5 to 1 upH) occurs after the application of cyanide. In the presence of CN, the magnitude of acidosis may depend on the rate at which lactic acid leaves the tissue, which is likely to be greater in isolated cells. In the latter situation, where no lactic acid production is detectable, a rapid hydrolysis of ATP is entirely responsible for the acidosis.

c) During ischemia, more intense intracellular acidification than that encountered during hypoxia or anoxia develops. Its amplitude depends on the duration and severity of the ischemia. Intra- and extracellular acidification is determined by the buffering capacity of intra- and extracellular systems, as well as by the activity of membrane ion transporters. The results published until now differ according to the methods and animal species used. However, there is a general agreement that the acidification of pH_{INT} depends on the buffer present in the perfusion medium. It seems also, the physiological buffer system CO_2/HCO_3^- provides better protection against intracellular acidosis compared to organic buffers.

2. Intracellular buffering capacity and membrane transporters

a) In a situation of hypoxia, there is no change in the value of the intracellular buffering power. In ischemia, intra- and extracellular lactate accumulation, PCr hydrolysis, and Pi rise contribute significantly to buffering capacity. In addition, in perfused isolated hearts subjected to ischemia, several studies demonstrated an increase in the intracellular buffering capacity for a pH_{INT}, probably linked to an increase of the buffering capacity of the intracellular proteins. By limiting the extent of cytosolic acidification, this mechanism could be a form of protection of cardiac cells against the risk of functional damage associated with ischemia.

b) In hypoxia, intracellular acidification induces an increase in Na^+_{INT}, following the activation of the Na^+/H^+ antiporter and of Ca^{++}_{INT} via Na^+/Ca^{++} exchanger (NCX). This mechanism, therefore, makes it possible to minimize and compensate for the negative inotropic effect of acidosis. It is likely that the increase in Na^+_{INT} during hypoxia is mainly related to the activation of the Na^+/H^+ exchange following the cytosolic acidification. During ischemia, the intracellular pH drops significantly more and under these conditions, the activity of the Na^+/H^+ exchange is slowed down and the effect of acidosis on the contraction is not sufficiently compensated by an entry of Ca^{++} via the Na^+/Ca^{++} antiport (NCX).

c) Regarding other membrane transporters involved in the regulation of pH_{INT}, it has been suggested that the increased release of nucleotides (such as Mg^{++}-ATP complex) into the coronary circulation during hypoxia and cardiac ischemia would participate in alteration of pH_{INT} by stimulation of the acidifying Cl^-/HCO_3^- exchange mechanism.

C. Intracellular calcium

Although the available results are not always homogeneous, complex modifications of the intracellular distribution of Ca^{++}_{INT}, as well as of the calcium transient, have been observed during ischemic/anoxic periods or metabolic inhibitions. Changes in diastolic Ca^{++} and regulatory mechanisms during hypoxia/ischemia will now be discussed.

1. During experimental hypoxia, in papillary muscles and isolated perfused hearts, an increase in diastolic Ca^{++} was observed parallel to the drop in developed pressure. Modifications of the membrane potential, an inhibition of the reuptake of Ca^{++} by the SR and of its redistribution in the internal compartments could be at the origin of the alterations of Ca^{++} described.

2. The inhibition of glycolysis and OP is accompanied by a marked reduction in the tension developed, as well as a strong reduction in the amplitude of the calcium transient and an increase in diastolic Ca^{++}.

3. In anoxia, the intracellular acidification following the increased production of lactic acid is at the origin of the increase in the calcium transient. The competition of H^+ and Ca^{++} ions for common intracellular binding sites would cause an elevation of Ca^{++}_{INT}, and consequently of the Ca^{++} load of the SR and the amplitude of the transient. Following several anoxic episodes, glycogen stores are depleted and acidification due to lactic acid is reduced. In addition, under these conditions, anoxia causes a strong shortening of the duration of the action potential. These modifications then contribute to reducing the calcium transient.

4. Several experimental measurements of Ca^{++} using fluorescent techniques in isolated rat heart ventricular cells showed, the calcium transient in the presence of cyanide is either

unchanged or increased. However, when glycolysis is inhibited, the addition of cyanide causes a decrease in systolic calcium transient and an increase in diastolic Ca^{++}. A shortening of the AP is probably at the origin of the decrease in the calcium transient. The increase in $Ca^{++}_{INT,}$ which precedes the development of a contracture, could be explained by a decrease in the expulsion of Ca^{++} by Na^+/Ca^{++} exchanger (NCX) following the elevation of Na^+_{INT}.

5. Much greater consistency exists in the results examining changes in intracellular Ca^{++} during ischemia. In general, Ca^{++} measurements taken during ischemia revealed an increase in the amplitude of the calcium transient, as well as an increase in diastolic Ca^{++}. After 20 to 30 minutes of ischemia, the calcium transient decreases to eventually disappear and spontaneous oscillations of Ca^{++} develop. Several factors having opposite effects are probably at the origin of these modifications: a reduction in the pool of ATP, an alteration of the action potential and the development of acidification following the production of lactic acid. The latter presumably increases (moderately) diastolic Ca^{++} by displacing Ca^{++} from intracellular binding sites. By promoting the release of Ca^{++} from the SR and the entry of Ca^{++} into the sarcolemma by Na^+/Ca^{++} exchanger, acidosis could also contribute in this way to an increase in Ca^{++}_{INT}.

Insufficient Ca^{++} sequestration and expulsion when ATP stores are very low, would be responsible for the later increase in Ca^{++}_{INT}. After complete disappearance of calcium transients due to stimulation, reflecting an inability of the SR to recapture Ca^{++} and a considerable depletion of ATP, a gradual increase in Ca^{++} intracellular content appears. All the underlying mechanisms are not fully understood, but although intracellular acidosis and decreased ATP exert an inhibitory effect on Na^+/Ca^{++} exchanger. It has been suggested that increased Na^+_{INT} would lead to an elevation of Ca^{++}_{INT} via Na^+/Ca^{++} exchanger (NCX).

D. Intracellular sodium

1. In the ventricular muscles, when the oxidative phosphorylations are blocked by cyanide or during the replacement of oxygen by nitrogen, an increase in Na^+ is observed accompanied by a slight depolarization. The reversibility of these modifications is obtained with reoxygenation. The sodium overload is to be related to the activation of the Na^+/H^+ exchange by the acidosis which develops under these conditions.

2. If glycolysis is inhibited by deoxyglucose, cyanide or nitrogen, a more rapid increase in Na^+ follows. In this case, the slowing of the activity of the $Na^+/K^+/ATP$-ase due to the decrease in intracellular ATP, would also participate in the elevation of Na^+.

3. In some experiments with heart hypoxia, a reversible increase in Na^+_{INT} was observed, which is responsible for a Ca^{++} via Na^+/Ca^{++} exchanger.

4. During global ischemia in animal experiments with perfused isolated heart, intracellular Na^+ increases to about 5 times the control value after approximately 20 minutes.

There seems to be a temporal dissociation between the increase in Na^+_{INT} and Ca^{++}_{INT} during ischemia, since Na^+ is already elevated after few minutes of ischemia, while Ca^{++} only begins to increase after 15 to 20 minutes. According to previously published experiences, this uncoupling could come from an inhibition of the activity of Na^+/Ca^{++} exchange by intracellular acidification.

E. Intra- and extracellular potassium

Hypoxia, metabolic inhibition, or ischemia are accompanied rapidly (within about 30 seconds) by an increase in cardiac cell K^+ efflux, resting potential depolarization, and progressive shortening of the duration of the action potential (AP). This results in a loss of cellular K^+ and an accumulation of this ion in the extracellular spaces during ischemia, which can reach up to 18 mM. Several mechanisms can be at the origin of these alterations. Elevated extracellular K^+ suggests increased efflux and/or decreased influx of K^+. However, a slowing down of $Na^+/K^+/ATP$-ase activity (which would lead to a reduction in active K^+ transport into the cell) is unlikely, at least in the early stages of ischemia since the levels of ATP are still high enough. The efflux of K^+ could be associated with the outflow of lactate via a co-transport, but the most studied hypothesis at present is the opening of a potassium channels sensitive to intracellular ATP content (K^+-ATP or ATP-sensitive K^+ channels). These channels are normally inhibited by physiological concentrations of ATP; a reduction in ATP leads to their activation. Some specific blockers of these channels prevent the shortening of the action potential during hypoxia and partially or totally reduce the accumulation of extracellular K^+ during ischemia. Moreover, the ATP concentrations measured during an inhibition of OP and glycolysis remain higher than those allowing the activation of the K^+-ATP channels when the action potential is already shortened. The density of K^+-ATP channels in the membrane of cardiac cells is high (between 0.5 and $10/\mu m^2$). Some authors suggest that the activation of a small percentage of K^+-ATP channels produced by a slight decrease in cytosolic ATP, is able to cause a shortening of AP duration and increase the efflux and extracellular accumulation of K^+ during hypoxia or ischemia. In addition, other factors are likely to modulate the activity of K^+-ATP channels. The increase in intracellular ADP during hypoxia or ischemia decreases the sensitivity of these channels to the inhibitory effect of ATP. A similar effect is also achieved by a decrease in pH_{INT} which could therefore contribute to the shortening of the action potential in conjunction with the decrease in intracellular ATP during ischemia. Finally, it should be noted that a reduction in incoming calcium currents during hypoxia or ischemia can also decrease the duration of AP.

F. Intracellular magnesium

Several NMR studies have shown, in perfused rat or rabbit hearts, an increase in intracellular Mg^{++} (Mg^{++}_{INT}) during hypoxia and ischemia, in parallel with the decrease in intracellular ATP content.

1. An elevation of Mg^{++}_{INT} of approximately 40% was observed in experiments in isolated heart myocytes following metabolic inhibition.

2. The increase in Mg^{++} after longer heart ischemia remains lower than those expected following the hydrolysis of ATP. Since total Mg^{++} is unchanged, this suggests that some of the Mg^{++} released from ATP is buffered by other cellular components. Given the possible roles of Mg^{++} in the regulation of ion channels (potassium and calcium), enzymes and other cellular functions, changes in its concentration during ischemia and reperfusion could have important consequences that remain to establish.

G. Conclusion

Hypoxia and ischemia are accompanied respectively by partial and total depression of contractile function. It is now well established that contractile failure during hypoxia and ischemia can be partly explained by P_i accumulation and intracellular acidosis. In fact, P_i and the acidification decrease the calcium sensitivity of contractile proteins and the production of maximum force. The effects of P_i are predominant at the beginning of the period of ischemia or hypoxia, while the effects of pH are accentuated when the ischemia progresses. A shortening of AP duration is observed during hypoxia, metabolic inhibition or ischemia. Depending on the glycogen reserves and/or the intensity of glycolysis, the shortening of the AP could occur more or less quickly and participate in the decline of contractile activity by a reduction in the supply of calcium to the myofilaments.

Hypoxic or ischemic contracture is an elevation of the resting tension that occurs during prolonged ischemia or metabolic inhibition. Two main factors can participate in its establishment: a) an increase in the concentration of intracellular Ca^{++} which activates the contractile proteins, and b) a reduction in ATP_{INT} which, when it is sufficient (at <1 mM), prevents the actin-myosin bridges from to detach (state of rigor). Arrhythmias that occur during ischemia are the well known cause of sudden death. Electrophysiological changes (membrane depolarization and slowing of conduction) represent some factors for the appearance of arrhythmias. In addition, the elevation of Ca^{++}_{INT} which can also lead to depolarization, as well as Ca^{++} oscillations and intracellular acidification, favor the genesis of arrhythmias.
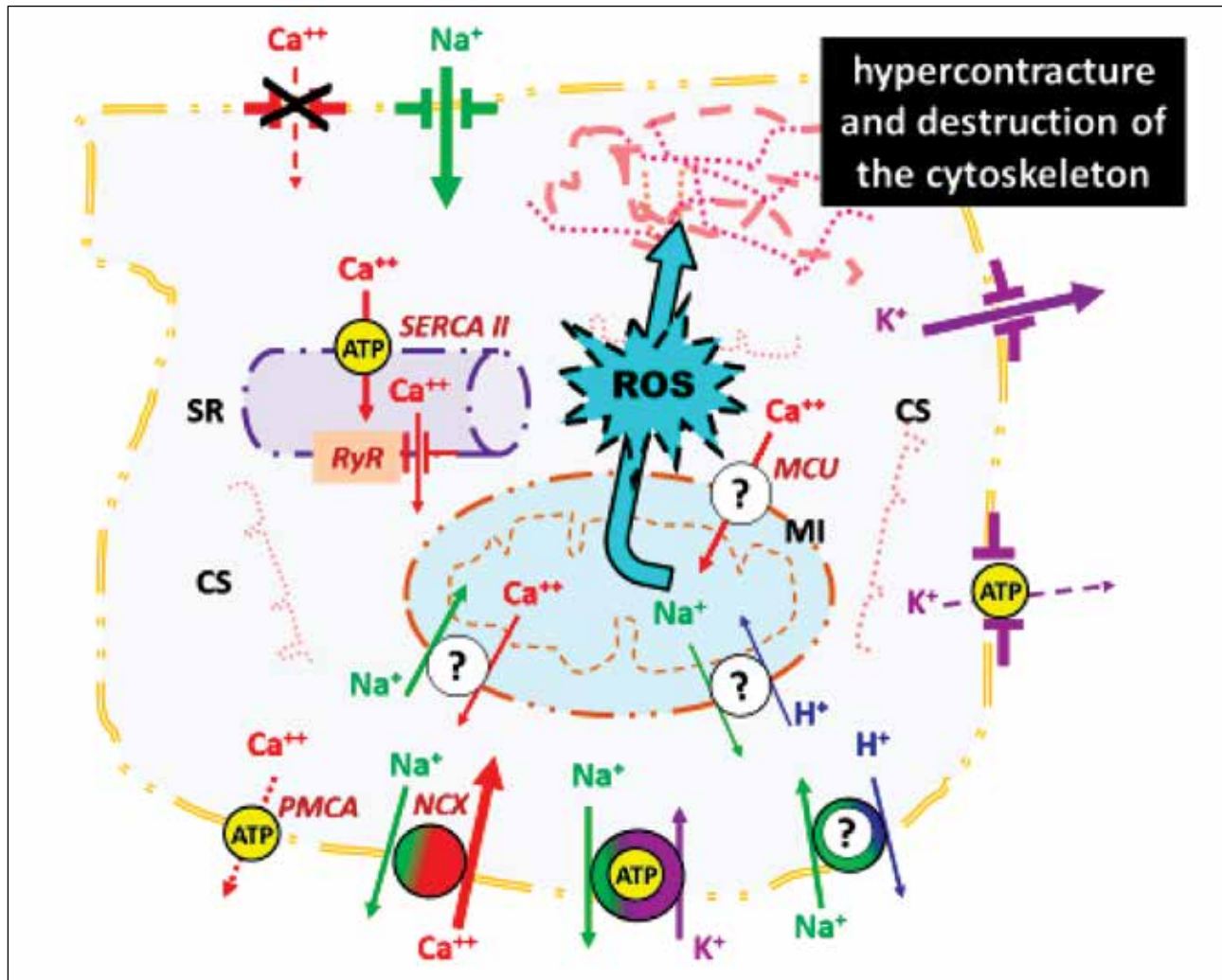
Figure 2: *Schematic overview of ionic imbalance during reperfusion/reoxygenation. During reoxygenation, ATP is partially resynthesized, inducing the closure of K^+-ATP channels. Activation of myofilaments can lead to the development of hypercontracture. Despite Ca^{++} uptake by SR and mitochondria, Na^+/Ca^{++} exchange continues to further contribute to Ca^{++} accumulation (CS = cytoskeleton; MI = mitochondria; ROS = reactive oxygen species; SR = sarcoplasmic reticulum) (Source: own material).*

Reoxygenation-reperfusion is accompanied by a significant elevation of intracellular Ca^{++} causing cell damage and arrhythmias (Fig. 2). The most likely mechanism is Ca^{++} entry via Na^+/Ca^{++} exchange. Indeed, the accumulation of Na^+ during ischemia and reperfusion following the reactivation of the Na^+/H^+ pump stimulates the Na^+/Ca^{++} exchange and the accelerated entry of Ca^{++}. This hypothesis is supported by the demonstration of a protective effect exerted by inhibitors of Na^+/H^+ exchange against damage to reoxygenation and reperfusion (overproduction of reactive oxygen species or ROS). In long-lasting ischemia, reperfusion causes a massive intracellular increase in Ca^{++} accompanied by the development of an irreversible contracture and destruction of cytoskeleton.

Note. This chapter is mainly focused on the description of ionic pathological changes during hypoxia and ischemia. The above text, mentioning the processes taking place in cardiomyocytes during reperfusion, should be understood as only a very brief supplement to the discussed topic. A detailed description of all the effects of oxidative stress during ischemia-reperfusion syndrome is beyond the scope of this chapter.

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Blood Pressure Control during Exercise Training: 24-h / 7-day Ambulatory Blood Pressure Monitoring

Lecture in Word Congress on Chronomedicine
7th Annual Konference on Indian Society of Chronomedicine

Jarmila Siegelova, [1]Germaine Cornelissen, Jiri Dusek, Michal Pohanka, Leona Dunklerova, Dita Rakova, Alena Havelkova, Petr Dobsak

Department of Physiotherapy, Department of Sports Medicine and Rehabilitation, Faculty of Medicine, Masaryk University, St. Anna Teaching Hospital, Brno, CZ, [1]University of Minnesota, USA

World Congress on Chronomedicine (WCC 2021)
7th Annual Conference on
Indian Society of Chronomedicine
Organized by
Association of Physicians of India Noida Chapter
Theme: Chronobiology of Non Communicable Disease

API Registration No.: 60586

Day-1, Friday, 17th December 2021			
TIMING	**TOPIC**	**SPEAKER**	**CHAIRPERSON**
1:00pm-1:30pm	Registration		
1:30pm-1:45pm	Welcome Address By Dr. S Chakravorty, Dr. A K Shukla, Dr. Meenakshi jain		
1:45pm-2:00pm	Opening ceremony- Guest of honour DR. PURSHOTAM LAL		
2:00pm-2:15pm	Early Morning exacerbation of severe asthma- Changing Paradigm in Treatment	Dr. Rahul Sharma	Dr. K C Sood Dr Kuldeep Dhar
	Chronobiology In Diabetes		
	Moderator Dr. Vandana Garg		
2:15pm-2:35pm	Nutraceutical as an Emerging Natural Chronomedicine in the Management of Type 2 Diabetes caused by Circadian Dysrhythmia"	Dr Pradeep Visen	Dr. Partha Biswas Dr. Amitesh Agarwal
2:35pm-2:55pm	Heart Failure Challenges in Type 2 DM	Dr.Osama Elmaraghy	Dr. Rakesh Kumar Dr. Sanjay Tandon
2:55pm-3:15pm	Circadian clocks in Endocrinology: Clinical Implications	Dr. Saurav Shishir	Dr. B D Sharma Dr. V B Dhaka
3:15pm-3:35pm	Shift work associated metabolic and genetic dysregulation among Indians - Novel Insights	Dr. S. V. Madhu	Dr. P K Dhawan Dr. R K Prasad
3:35pm-3:55pm	Meal timing in Health and Disease	Dr. Saurabh Srivastava	Dr. Neelu Gupta Dr. Ajay Agarwal
3:55pm-4:15pm	Continuous Glucose Monitoring System (CGMs)-Work shop	Dr. A K Shukla	
4:15pm-4:35pm	TIR- How to interpret CGM Record and Its implication	Dr. Keshore R	Dr. Veerendra Singh Dr. Meenakshi Jain
4:35pm-4:55pm	TEA		
	Chronobiology of Inflammatory Disease		
	Moderator- Dr. Meenakshi Jain		
4:55pm-5:15pm	Circadian changes in oxidative stress in relation to cardiac dysfunction	Dr. M A Manal	Dr. Abla Smail Dr. A K Shukla
5:15pm-5:35pm	Therapeutics on the clock: Chronomedicine in treatment of Chronoinflammatory disease	Dr. Anubha Verma	Dr. Ajay Gupta Dr. B S Pandey
5:35pm-5:55pm	Pitfalls of treating arterial hypertension in patients with metabolic syndrome	Prof. Ludovit Gaspar,	Dr. Tamnosh Bhattacharya Dr. Anant Panday
5:55pm-6:15pm	Chronobiology of Fertility Potential	Dr. Amit K. Singh	Dr. Anupam Biswas Dr. Bhawan Banga
6:15pm-6:35pm	Blood Pressure Variability: How does it make a difference ?	Dr. Anuj Maheswari	Dr. Rajeev Garg Dr. N K Soni
6:35pm-6:55pm	Chronomedicine: A Cardiac surgeon perspective	Dr Vaibhav Mishra	Dr. Parneesh Arora Dr. Amitabh Yaduvanshi
	Chronobiology of Insulin Therapy		
	Moderator- Dr. A K Shukla		
6:55pm-7:15pm	Individualization of insulin therapy: Choosing the right solution for your varying patients needs in relation to time	Dr. S. Chakravorty	Dr. G C Vaishnava Dr Ravi Kant
7:15pm-7:35pm	Gen 2.0 basal – Circuit Breaker for the Vicious Cycle of Hyperglycemia & Hypoglycemia"	Dr. R. K, Prasad	Dr. Manju Tyagi Dr. Meenakshi Jain
7:35pm-7:55pm	Retarding the progression of diabetic kidney disease: Newer insight into SGLT2 inhibitor	Dr. Bijay Patni	Dr. Nishesh Jain Dr. S.C. Chabra
8:00pm	Dinner		

TIMING	TOPIC	SPEAKER	CHAIRPERSON
	Day-2, Saturday, 18th December 2021		
	Chronobiology In Cardiology		
	Moderator: Dr. Kuldeep Dhar		
9:00am-9:30am	Diabetes mellitus as risk factor of Heart Failure	Prof. Ghazi M. Halabi	Dr. A K Shukla Dr. Payal Jain
9:30am-10:00am	Emergence of Cardiometabolic risk factors in shift workers	Dr. Ravi Kant	Dr. B C Bansal Dr. K D Kotlia
10:00am-10:20am	Stiffness of endothelium and risk of CVDs	Dr. Kohji Shirai	Dr. K K Tripathi Dr. Amitabh Yaduvanshi
10:20am-10:40am	Chronobiology and sleep pathophysiology and treatment of circadian rhythm sleep disorder	Dr. Ajitesh Rai	Dr. Vinay Labroo Dr. S. Chakravorty
10:40am-11:00am	Assessment of Myocardial-related Chronobiology Changes Using Echocardiography	Dr. Brian Mendel	Dr. R B Singh Dr. Amitabh Yaduvanshi
11:00am-11:20am	Blood Pressure Control during Exercise Training: 24-h / 7-day Ambulatory Blood Pressure Monitoring	Prof. Jarmila Siegelova	Dr. MH Sanwarwalla Dr. OM Kumari Gupta
11:20pm-11:40pm	Recombinant Drug adversity On Circadian Blood Pressure And Multiple Organ Damages As Compared With Pure Human Gene Products	Dr. Jong Lee	Dr. Ghizal Fatima Dr. Saurabh Srivastav
11:40am-12:00pm	Natural Interventions to Treat Sleep Deprivation as a Risk Factor for Coronary Artery Diseases"	Dr. Adrian Isaza	Dr. Ravi Kant Dr. Amit Gupta
12:00pm-12:20pm	Chronomedicine in acute coronary syndrom	Dr. Sameer Gupta	Dr. R K Gattani Dr. Sanjay Mahajan
12:20pm-12:40pm	History of Nutrition and Cardiovascular Disease	Dr. Galal Elkilany,	Dr. Neelu Jain Gupta Dr. Sunil Chauhan
12:40pm-1:00pm	Circadian changes in the ventricular function	Dr. Krassimira Hristova	Dr. Smith Nath Dr. Deeraj Gandotra
1:00pm-1:50pm	**Lunch**		
1:50pm-2:30pm	Shyama Krishna Memorial Oration award of the Indian society of Chronomedicine	Dr. (Prof). Till Roenneberg	Prof. Meenakshi Sinha Dr. Om Kumari Gupta
2:30pm-2:50pm	**International Fellowship of the Indian society of Chronomedicine Oration Award**	Dr Robert Dallmann	Dr. Mary D'Cruz
2:50pm-3:10pm	**Prof Franz Halberg Medal Oration award of the Indian society of Chronomedicne**	Dr. Aarti Jagannath	Dr. Shipra Bharadwaj
3:10pm-3:30pm	**Honorary National Fellowship of the Indian Society of Chronomedicine Oration Award**	Dr. Neelu Jain Gupta	
3:30pm-3:50pm	**Presidential Oration**	Dr. OM Kumari Gupta	Prof Meenakshi Sinha
	Circadian Rhythm in Kidney Disease		
	Moderator: Dr. Manisha Dassi		
3:50pm-4:10pm	Preventing progression of chronic kidney disease	Dr. Manik Chhabra	Dr. L. K Jha Dr. Naresh Dang
4:10pm-4:30pm	Clock Genes and carbon	Dr. Suresh Tyagi	Dr. N K Sharma Dr. S K Gupta
4:30pm-4:50pm	Diabetic Kidney Disease -Time to Bell the Cat	Dr. Manoj Singhal	Dr. Kuldeep Dhar Dr. Dilip Bhalla
4:50pm-5:10pm	Nocturnal Hyperglycemia as Manifestation of Glucose Variability Disorder and Risk Factor of Cardiovascular Diseases.	Dr. R B. Singh	Dr. Om Kumari Gupta Dr. S. Chakravorty
5:10pm-5:30pm	SHF HI: HFrEF in India-Can ARNI impact outcome in the real world scenario in India	Dr. S K Aggarwal	Dr. Anand Pandey Dr. Pawan Gupta
5:30pm-5:50pm	Clinical chronobiology: A Timely consideration in critical care medicine	Dr. Meenakshi Jain	Dr. Gulab Gupta Dr. N P Singh
	Chronobiology in GI		
	Moderator: Dr. Gunjan Mittal		
5:50pm-6:00pm	Chronic Irritable Bowel Syndrome	Dr. K K Tripathi	Dr. Vinod Vashisht Dr. Pankaj Tyagi
6:00pm-6:20pm	Fasting therapy and Intermittent fasting-bursting the myths	Dr. Arvind Gupta	Dr. Kiran Seth Dr. Mayank Anand
6:20pm-7:00pm	Inaguration (Chief Guest- Dr. Mahesh Sharma, Guest of Honour- Shree Suhas LY)		
	Hypertension & Chronobiology		
	Moderator: Dr. A K Shukla		
7:00pm-7:20pm	Role of rhythm in pain perception	Dr. Meenakshi Sinha	Dr. Sameer Balia Dr. Sanjay Mahajan
7:20pm-7:40pm	Sleep wake cycle and cancer- Role of immuno therapy	Dr. Meenu Walia	Dr. Gopal Sharma Dr. Meenakshi Jain
7:40pm-8:00pm	Ambulatory BP Monitoring and Circadian rhythm	Dr. Amitabh Yaduvanshi	Dr. Yogesh Valecha Dr. S K Agarwal
	Gala Dinner		

TIMING	TOPIC	SPEAKER	CHAIRPERSON
**Day-3, Sunday, 19ᵗʰ December 2021**		
	Chronobiology in Neurology		
	Moderator: Dr. Kiran Seth		
10:00am-10:30am	Deciphering the role of Circadian Rhythm Disruption on Mental Health and Physiological responses among shift workers and non shift worker	Dr. Ghizal Fatima	Dr. Amit Batra Dr. Ashish Jain
10:30am-10:50am	Infant colic diagnosis and therapy	Dr Douglas Wilson	Dr. N K Safaya Dr. Prabhjot Kaur
10:50am-11:10am	Chronotherapy in epilepsy	Dr. Jasmine Parihar	Dr. Vandana Garg Dr. Kapil Singhal
11:10am-11:30am	Degenerative spinal disorders : Chronobiology and clinical relevance	Dr. Prankul Singhal	Dr. S.k Plaha Dr. Akash Garg
11:30am-11:50am	TEA		
	Other Non Communicable Disease & Chronobiology		
	Moderator: Dr. Amitesh Aggarwal		
11:50am-12:10pm	Precision medicine in Hematology: The time for next generation gene sequencing	Dr. Pravas Mishra	Dr. Esha Kaul Dr. Mukesh Mehra
12:10pm-12:30pm	Timing for the admistration of and antihypertensive drug in the treatment of essential hypertension	Dr Varun Verma	Dr. Pankaj Gaur Dr. Amrit Singh
12:30pm-12:50pm	Application of chronomedicine in day to day practice	Dr. Narsingh Verma	Dr. R B Singh Dr. Anuj Maheswari
12:50pm-1:10pm	Circadian dysfunction in obesity	Dr. Eszter Halmy	Dr. S K Sahoo Dr. Neeru Gera
1:10pm-1:30pm	Chronobiology and ageing	Dr. Ashish Goel	Dr. Nishant Raizada Dr. Madhur Rastogi
1:30pm-1:50pm	Circadian rhythm & cardiorespiratory parameters'	Dr. Arun Goel	Dr. Alpana Raizada Dr. Kapil Mahendru
1:50pm-2:10pm	Chronopharmacology of hypolipidemic drugs- new insight and therapeutic implication	Dr. Mohit Bhagwati	Dr. Viresh P Mehta Dr. Vidhi Sharma
2:10pm-2:30pm	Effect of time on Genitacy encoded important biological rythme	Dr Fabien De Meester	Dr. R B Singh Dr. Narsingh Verma
2:30pm	Lunch		

Blood Pressure Control during Exercise Training: 24-h / 7-day Ambulatory Blood Pressure Monitoring

Introduction

Franz Halberg and Germaine Cornelissen using ambulatory blood pressure monitoring showed the need to account day-to-day changes of blood pressure and heart rate and the necessity to circadian assessment of the hour-to- hour variability in cardiovascular parameters. Together with the Chronobiology center of Minnesota we participate on the international project BIOCOS. The presentation in May 2017 adds new results to this project BIOCOS (1). In the guidelines for diagnoses of hypertension, fixed limits of 140/90 mmHg (systolic/ diastolic BP) were used to diagnose hypertension in all adults 18 years and older. The circadian rhythm in BP was thought to primarily reflect the rest-activity schedule rather than being in part endogenous (2). While this is no longer the case, ambulatory BP monitoring is still restricted to "special cases", often limited to 24 hours. Evidence is presented herein for the need to routinely screen for BP and heart rate (HR) variability, and for continued monitoring in patients in need of treatment. According to a consensus meeting held at St. Anna Hospital, Masaryk University, Brno, Czech Republic in 2008 Franz Halberg with Germaine Cornelissen, Thomas Kenner, Bohumil Fiser, Jarmila Siegelova and others proclaimed Vascular Variability Disorders. Determination of Vascular Variability Disorders – MESOR hypertension, circadian hyper-amplitude-tension excessive pulse pressure deficient heart variability and deviation of circadian rhythm is best to diagnose from seven day/24-h ambulatory blood pressure monitoring (3, 4, 5, 6).

Our previous studies analyzed from seven day/24-h ambulatory blood pressure monitoring immediate effect of exercise on circadian blood pressure profile. The question of long exercise during 3-4 months will show the effect on seven day/24-h ambulatory blood pressure monitoring.

The purpose of the study

The aim of the study was to compare the 7-day/24-h blood pressure monitoring before and after exercise training lasting 4 months in healthy subjects.

Methods

We examined 20 healthy subjects, mean age 23.1±2.2 years (from 19 to 29 years, 7 men and 13 women), mean body weight was 72.8±13.4 kg, mean height 174±8 cm.

For exercise training we used walking activity at the level of 70 % maximum heart rate. The exercise training lasted 4 months. The subjects were recruited for seven-day ambulatory blood pressure monitoring before and after 4 months. Medical Instruments TM2431 (A&D, Japan) were used for ambulatory blood pressure monitoring (oscillation method). One-hour means of systolic and diastolic blood pressure were evaluated. We calculated mean systolic and diastolic blood pressure for seven days and every 24-hour profile.

The regime of measurement of blood pressure was done for 7 days repeatedly every 30 minutes from 5 to 22 h during the day time and once in an hour from 22 to 5 h at night.

The study was approved by local ethical committee.

One-hour means of systolic and diastolic blood pressure were evaluated for every hour from seven-day/24-h ambulatory blood pressure monitoring.

We evaluated every day mean of 24-hour profile and 7-day mean systolic and diastolic pressure.

We analyzed day-to-day variability before and after 4 months with exercise training.

Results

In healthy subjects, the seven day blood pressure profile (mean ± SD) in systolic blood pressure was 122±9 mmHg before the exercise training and after 4 months of exercise training was 123±9 mmHg. The seven-day blood pressure profile (mean ± SD) in diastolic blood pressure was 70±3 mmHg before the exercise training and after exercise was 71±3 mmHg in the whole group.

In healthy subjects, the seven-day heart rate profile (mean ± SD) was 72.3±4.9 cycle per minute before the exercise and after exercise was 72.9±4.8 cycle per minute in the whole group.

Circadian variability in the following results is presented as everyday 24-h value in the black points and seven-day/24-h mean as a red line in blood pressure and heart rate.

Seven-day systolic blood pressure variability in 20 healthy subjects before exercise training is shown in Fig. 1a. The lowest systolic blood pressure was 113 ± 2 mmHg, the highest systolic blood pressure 143 ± 2 mmHg.

Seven-day systolic blood pressure variability in 20 healthy subjects after exercise is shown in Fig. 1b. The lowest systolic blood pressure was 107 ± 2 mmHg, the highest systolic blood pressure 144 ± 4 mmHg.

Seven-day diastolic blood pressure variability in 20 healthy subjects before exercise is shown in Fig. 2a. The lowest diastolic blood pressure was 64 ± 1 mmHg, the highest diastolic blood pressure 76 ± 4 mmHg.

Seven-day diastolic blood pressure variability in 20 healthy subjects after 4 months exercise training is shown in Fig. 2b. The lowest diastolic blood pressure was 64 ± 2 mmHg, the highest diastolic blood pressure 77 ± 3 mmHg.

Seven-day heart rate variability in 20 healthy subjects before 4 months exercise training. The lowest heart rate was 58 ± 4 mmHg, the highest heart rate 80 ± 6 mmHg. Seven-day heart rate variability in 20 healthy subjects after 4 months exercise training. The lowest heart rate was 54 ± 4 mmHg, the highest heart rate 87 ± 5 mmHg. 24-h profile variability before and after 4 months of exercise training was present in our healthy subjects in cardiovascular parameters, measured from seven day/24-h ambulatory blood pressure monitoring. The variability of 24-h systolic blood pressure profile was large and was present also after 4 months of exercise training. In 24-h diastolic blood pressure profile we have seen also large blood pressure variability before and after 4 months of exercise training. 24-h of heart rate also showed variability before and after exercise training. The highest and lowest values in the seven day means are also in all subjects similar before and after training.

Discusion

Systolic and diastolic 24-h blood pressure profile varied largely and the variability of 24-h profile was not changed after 4 months of exercise training in our healthy subjects. Our previous results we have shown that in patients with increased sympathetic activity with chronic coronary artery diseases. The eight weeks exercise training in patients decreased sympathetic activity and increased parasympathetic activity. Our results on healthy subjects in this study do not showed changes in mean values of heart rate during seven day/24-h ambulatory blood pressure monitoring. There are also differences in the age of both groups and perhaps also different living style of young students of physiotherapy and patients with chronic heart failure could play role in our results.

Conclusion

The study showed the seven day/24-h blood pressure monitoring before and after exercise training lasting 4 months in healthy subjects was not different. 4 months lasting exercise training, based on walking at the 70 % maximum heart rate, does not affected mean 7-day/24-h of heart rate before and after training.

Day-to-day changes of 24-h blood pressure profile systolic and diastolic blood pressure analyzed from 7-day ambulatory blood pressure monitoring, were not different before and after 4 months lasting exercise training.

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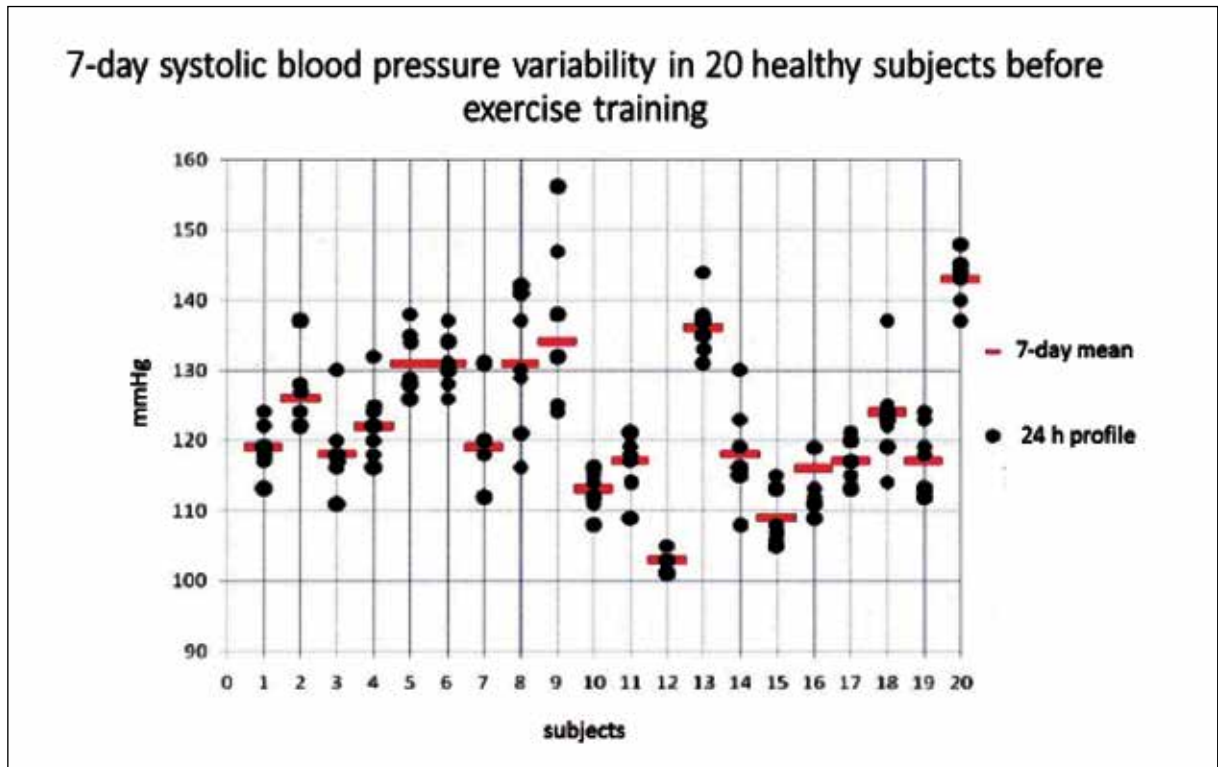
Figure 1a: *Seven-day systolic blood pressure variability in 20 healthy subjects before exercise training*
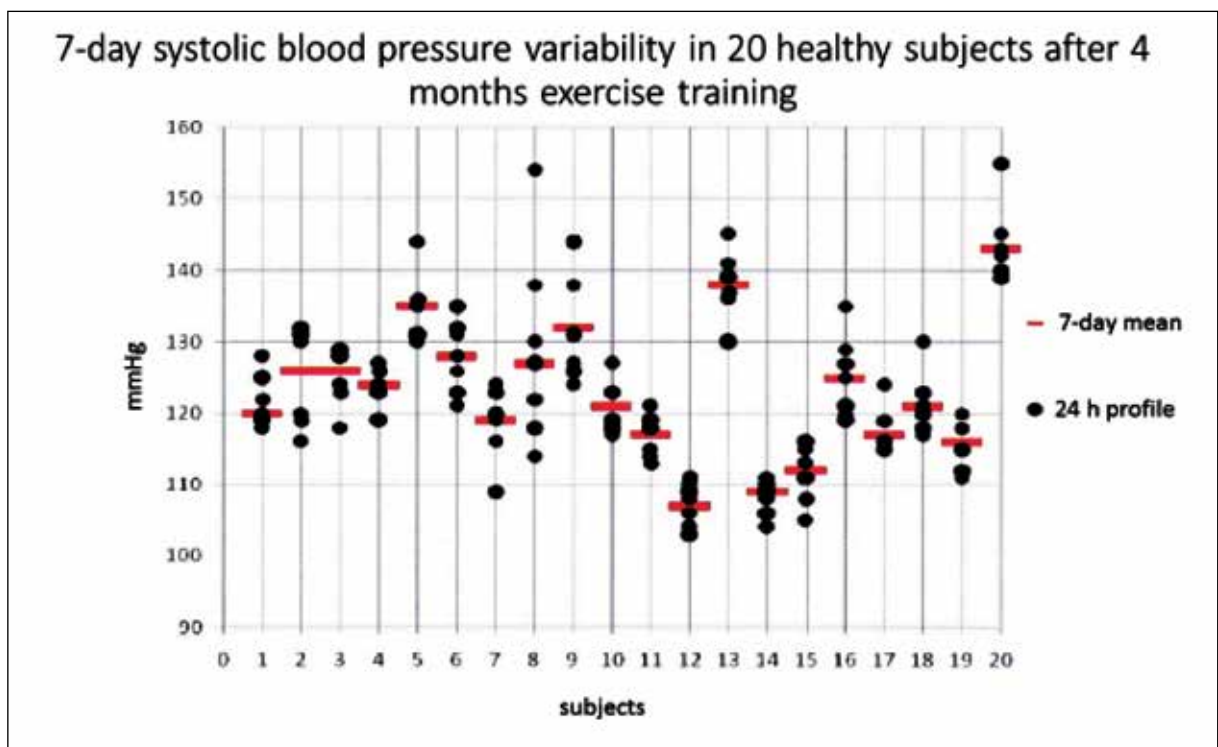

Figure 1b: *Seven-day systolic blood pressure variability in 20 healthy subjects after 4 months exercise training*
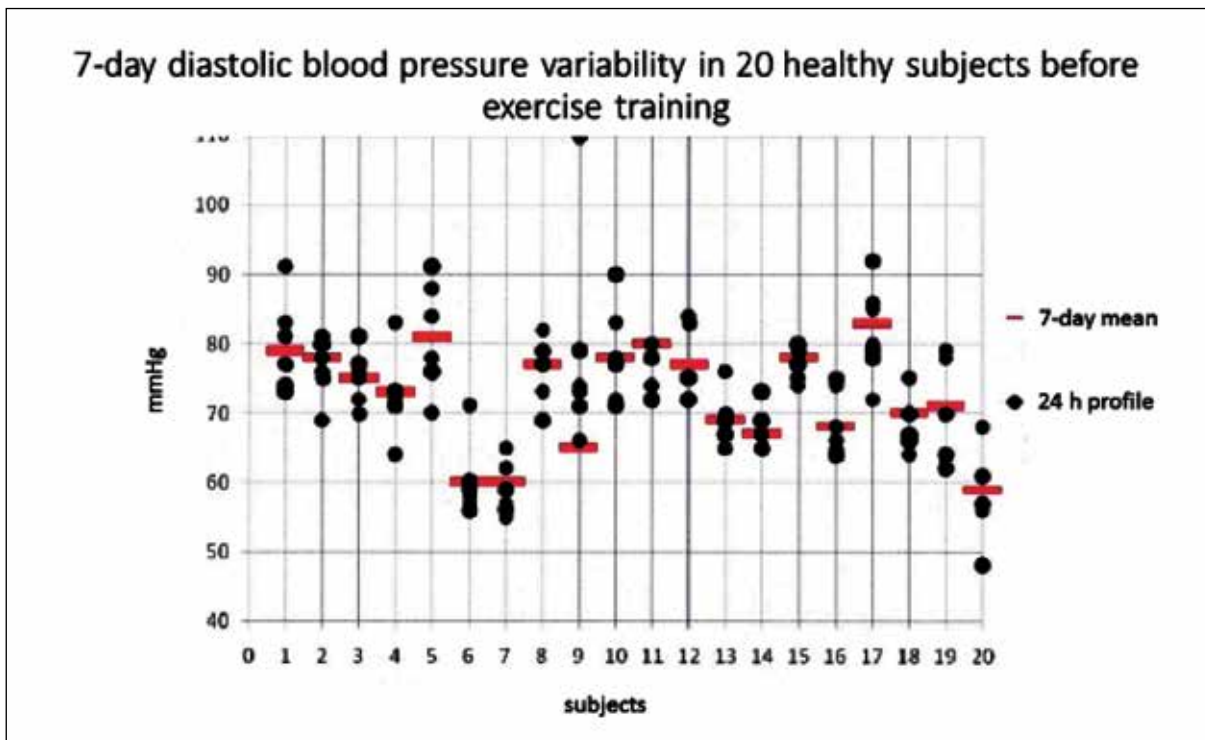
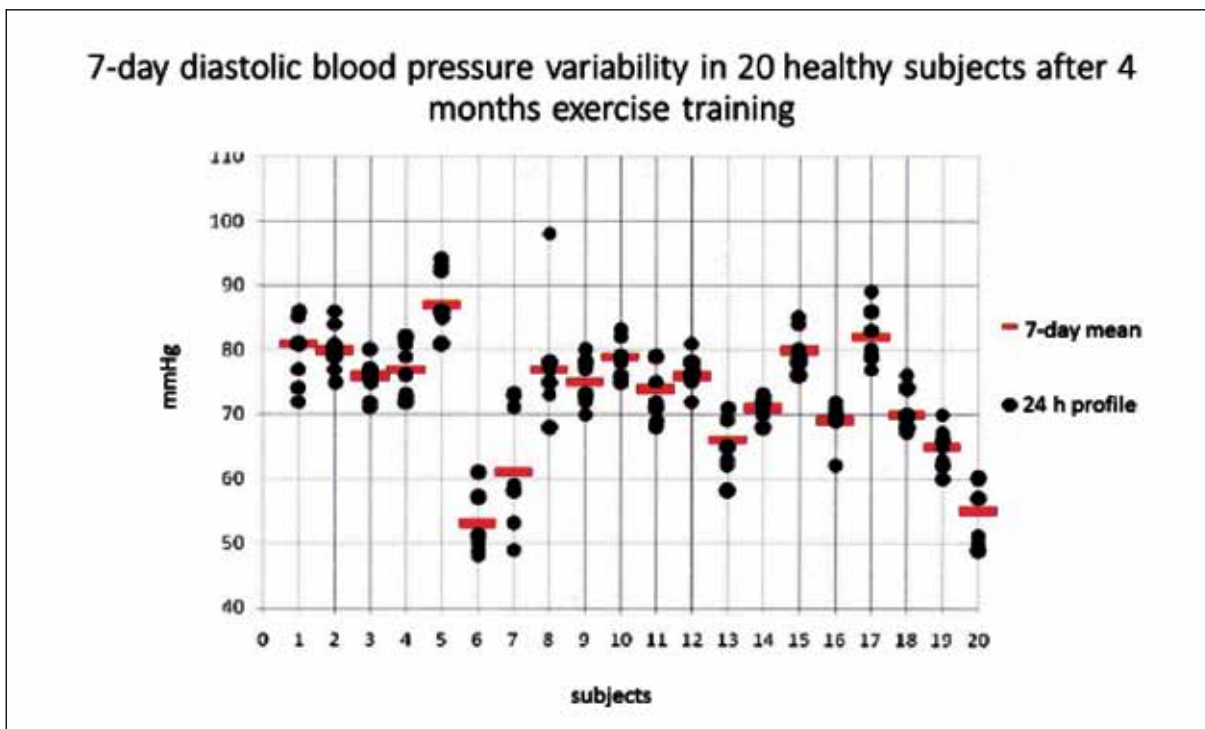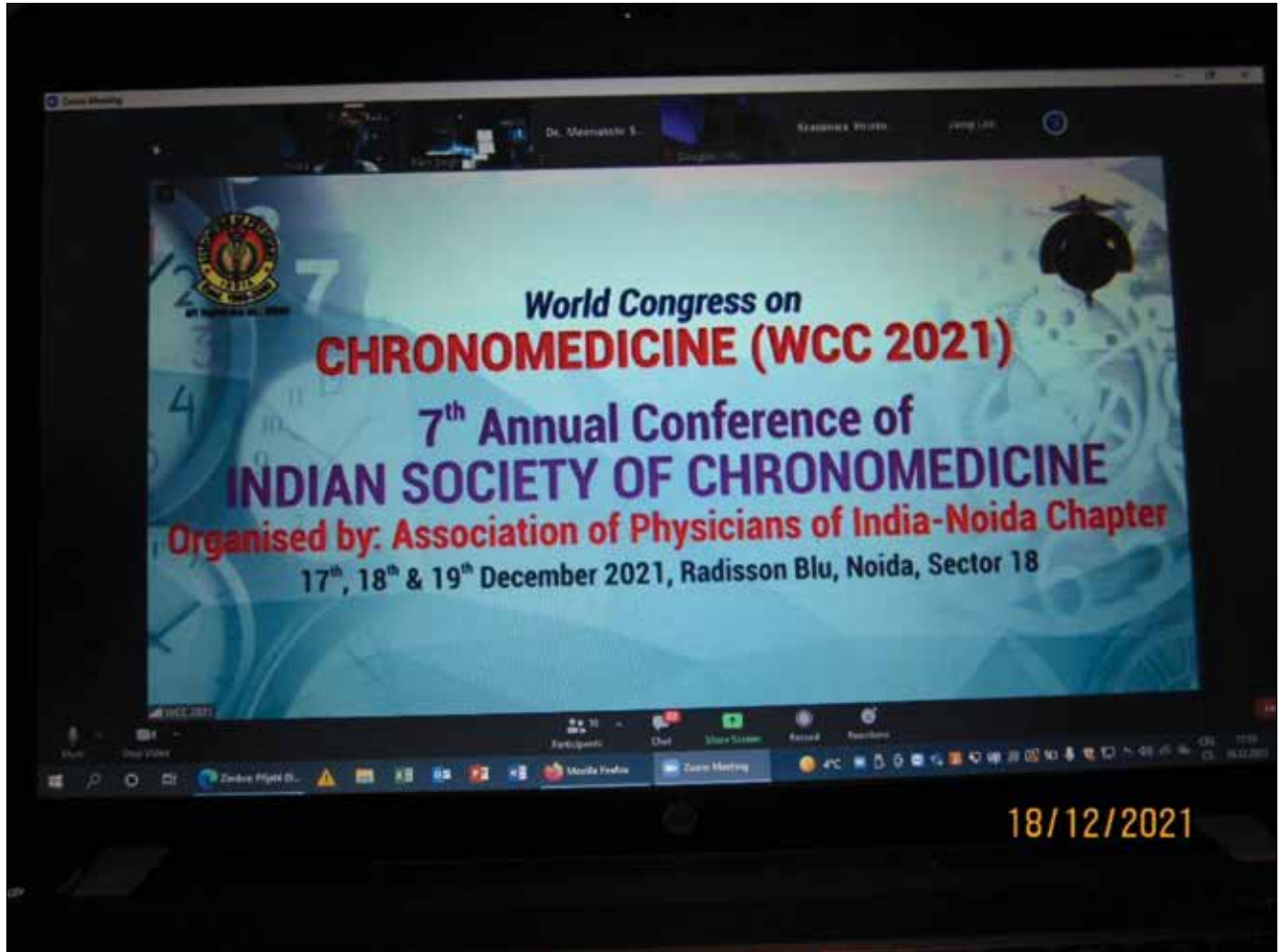**Figure 2a:** *Seven-day diastolic blood pressure variability in 20 healthy subjects before exercise training*

Figure 2b: *Seven-day diastolic blood pressure variability in 20 healthy subjects after 4 months exercise training*

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Edited by: **Cornélissen G., Siegelová J., Dobšák P.**