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MUSCLE ERGOREFLEX ACTIVITY AND AUTONOMIC BALANCE ASSESSED IN THE VERTICAL AND HORIZONTAL BODY POSITIONS IN YOUNG HEALTHY MEN

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We investigated whether indices of the reflex regulation of the cardiovascular system, i.e., muscle ergoreflex activity (ERGO) and characteristics of the autonomic balance, differ from each other while measured in the vertical vs. horizontal positions in healthy humans. Ten young healthy men (mean age 28 ± 1 years; BMI 23.6 ± 0.8 kg/m^2) were examined. In each subject, the ERGO (ventilatory response to a control handgrip exercise, with repetitive isometric contractions at 50% of the maximal strength), baroreflex sensitivity (BRS, the sequence method), heart rate variability (HRV), and a few hemodynamic parameters were measured in two positions, horizontal and vertical. In the latter position, the heart rate, and diastolic and mean blood pressures (BP) were higher, while the stroke volume was smaller (all $P < 0.05$) with no differences in the systolic BP, cardiac output, and systemic vascular resistance (all $P > 0.2$). The HRV time domains, as well as BRS, decreased, whereas HRV-LF increased (all $P < 0.05$) in the vertical position as compared to the horizontal one. There were no differences between other HRV parameters (all $P > 0.2$) in the two examined positions. Minute ventilation during the exercise increased, while ERGO was reduced in the vertical position (all $P < 0.05$). Thus, the body position noticeably affects both reflex regulation of the cardiovascular system and autonomic balance. This observation has to be taken into account during physiological testing.

Keywords: ergoreflex, vertical and horizontal body positions, minute ventilation, heart rate variability, baroreflex sensitivity, hemodynamic parameters.

INTRODUCTION

Obtaining an upright body position has been a significant breakthrough in the evolution of the *Homo* species, which brought a huge advantage over other mammals and enabled further exceptional evolutionary progress [1]. It is not surprising that the ability to change positions from horizontal to upright stigmatized physiological reactions, in particular the reflex control of the cardiovascular system. Moreover, this ability has a set of structural and physiological consequences, which also distinguishes humans among other creatures [1].

Surprisingly, most physiological experiments and measurements in humans are performed while the subject is lying down [2–4]. It can be hypothesized that the respective measurements performed in an upright position would provide additional information regarding physiological regulations within the human body, and valuable information about the fitness of the examined subjects in different physiological contexts should be obtained considering that an upright position accompanies nearly all human active actions [1].

It is logical to believe that there may be differences between the physiological parameters, including those reflecting the cardiovascular reflex control, when

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measured in the horizontal vs. vertical positions in humans. We compared muscle ergoreflex activity (ERGO), arterial baroreflex sensitivity, and indices of the autonomic balance measured in vertical and horizontal positions in young healthy men.

METHODS

Examined Subjects. We examined 10 young and healthy men with the following inclusion criteria: (i) age 18 to 30 years; (ii) no history of acute/chronic illnesses, and (iii) no smoking within 12 hours preceding the study.

General Conditions and the Study Protocol. The tests were performed between 8 and 12 AM, in a quiet air-conditioned room. All examined subjects were advised to eat a light breakfast, avoid coffee or tea at least 6 hours before the test, and do not be after a sleepless night.

During the whole examination, the subjects breathed a room air through a mouthpiece with a nose clip. The mouthpiece was connected to a respiratory gas analyzer (Ultima, USA), and the continuous minute ventilation (VE, l/min) was measured.

Simultaneously, a non-invasive finger recording of the arterial pressure synchronized with ECG recording (R–R intervals, msec) was performed (Nexfin HD device, BmEye, the Netherlands). Systolic, diastolic, and mean arterial pressures (SBP, DBP, and MAP, respectively, mm Hg), as well as other hemodynamic parameters, were derived from the Nexfin measurements using the arterial pressure waveform analysis [5–7].

The Concept of Ergoreflex Assessment. This concept is based on the assumption that metabolites generated in exercising muscles, when trapped immediately in the tissue after the exercise cessation, stimulate the ergoreceptors [3, 8, 9]. To generate the respective metabolites and activate muscle ergoreceptors, controlled muscle effort (e.g., repetitive strong isometric exercise) needs to be performed. In order to extend the influence of metabolites on ergoreceptors in the skeletal muscles beyond the exercise stage, local circulatory occlusion is commonly used, which does not allow the metabolites to be washed out by circulating blood immediately after the exercise cessation [3, 9, 10]. Therefore, ERGO activity assessment in physiological tests is based on calculation on difference between the measured parameters (most commonly, minute ventilation values) within the time period directly after cessation of a standardized exercise with circulatory occlusion (the so-called post-handgrip local circulatory occlusion, PCO) and the period immediately after cessation of such standardised exercise without circulatory occlusion [3, 8–10]. In our study, we used the typical protocol of ERGO activity assessment, which included eight stages accompanied by eight recordings described below (Table 1).

Study Protocol. We performed two recordings with identical protocols in each examined subject. The first recording was performed in a horizontal position (after an aprox. 20-min-long resting), while the second recording was performed in a vertical position. The two recordings were separated by an approx. 20-min-long break. In order to help the subject to maintain the vertical position during the whole recording we used the tilt table. The protocol is presented in the Table 1.

Noninvasive Assessment of the Hemodynamic Parameters. Based on 5-min-long resting synchronised recordings of the finger arterial pressure waveform and R–R intervals performed during stage 1 in both horizontal and vertical positions, the following hemodynamic parameters were estimated: heart rate corresponding to the mean duration of R–R intervals, msec; systolic and diastolic blood pressures (SPB and DBP, mm Hg), and mean arterial pressure (MAP, mm Hg).

The remaining hemodynamic parameters were estimated automatically by the Nexfin HD device, which has been previously validated with an invasive assessment using a Swan–Ganz catheter in our physiological laboratory [7]. These are the stroke volume (SV, ml), stroke volume index (SVI, ml/m^2), cardiac output (CO, l/min), cardiac index (CI, l/min·m^2), systemic vascular resistance (SVR, dyn·sec/cm^5), and systemic vascular resistance index (SVRI, (dyn·sec·m^2/cm^5).

Non-Invasive Assessment of the Heart Rate Variability and Baroreflex Sensitivity. The heart rate variability (HRV) indices and baroreflex sensitivity (BRS) were calculated using noninvasive 5-min-long continuous resting recording of the finger arterial pressure waveform and R–R intervals performed during stage 1 in both horizontal and vertical positions.

The HRV characteristics included time domain measures: standard deviation of the R–R intervals (SDNN, msec), square root of the mean of the sum of the squares of differences between the adjacent R-R intervals (RMSSD, msec), number of the differences between successive R–R intervals greater than 50 msec (NN50), and proportion derived by dividing NN50 by the total number of R–R intervals (pNN50) [4, 11].

The frequency domain analysis allowed us to estimate spectral components distinguished in the HRV spectrum, low (LF, 0.04 to 0.15 Hz) and high (HF, 0.15 to 0.4 Hz) frequencies expressed in absolute values, $msec^2$ [4, 11].

The BRS (msec/mm Hg) was calculated using a sequence method. Sequences of changes in the SBP (with a difference of ≥1.0 mm Hg) accompanied by changes in the R–R intervals (with a difference of ≥5.0 msec) were selected and plotted. The BRS was calculated as the averaged regression slope relating the BP to R–R intervals [2, 12, 13].

Ergoreflex Activity. The ERGO activity, interpreted the activation of the muscle metaboreceptors on VE, was computed as the difference between the averaged VE values recorded during the 2-min-long post-exercise recovery with PCO and during the analogous 2-min-long post-exercise recovery without occlusion [3, 10].

Statistical Analysis. Continuous variables are presented as means ± s.e.m. The statistical significance of differences between the analyzed parameters measured in the horizontal vs. vertical position was assessed using a paired-sample sign test. Differences with $P < 0.05$ were considered statistically significant.

Table 1. The Study Protocol

Таблиця 1. Протокол дослідження

№	Stage	Technical description	Aim in the protocol of ERGO assessment	Duration, min
1	resting	Resting in a constant position.	Recording performed at this stage is used for calculation of the resting/baseline values (i.e., minute ventilation and hemodynamic parameters)	5
2	a control occlusion	A forearm tourniquet inflation of ≥30 mm Hg above the systolic BP	Recording performed at this stage is compared with the baseline values recorded during resting in order to fins a possible effect of the occlusion itself on physiological reactions observed during the proper test	3
3	resting	Resting in a constant position	Recording performed at this stage is for separation of the subsequent stages, and not for any analyses	3
4	exercise	A handgrip exercise based on a repetitive palm contraction of a dynamometer at 50% of the predetermined maximal contraction in a pre-established pace (30 min^{-1})	Recording performed at this stage is usually analysed in three separate sub-stages (the first two minutes, the second two minutes, and the last minute interpreted as that with maximal exercise ventilation)	5
5	recovery with the circulatory occlusion	A forearm tourniquet inflation of ≥30 mm Hg above the systolic BP (the so-called post-exercise recovery with post-handgrip circulatory occlusion)	Recording performed at this stage reflects ventilatory and hemodynamic responses of the muscles to trapped metabolites after the exercise along with the persistent effect of the recent exercise stage	3
6	recovery	Resting in a constant position	Recording performed at this stage is also for separation of the subsequent stages, and not for any analyses	4–7
7	exercise	A handgrip exercise based on a repetitive palm contraction of a dynamometer at 50% of the predetermined maximal contraction in a pre-established pace (30 min^{-1})	Recording performed at this stage is usually also analysed in three separate sub-stages (see point 4)	5
8	recovery without PCO	Resting in a constant position (the so-called post-exercise recovery without post-handgrip circulatory occlusion)	Recording performed at this stage reflects ventilatory and hemodynamic response to the recent exercise stage	2-3
	total, min			**28–33**

RESULTS

Differences in Hemodynamic Parameters, HRV, and BRS. The averaged hemodynamic parameters, HRV characteristics, and BRS values measured in the two body positions are presented in Table 2.

The heart rate, diastolic BP, and mean BP were significantly higher in the vertical position as compared to those in the horizontal position (all $P < 0.05$). The SV (SVI) was reduced in the vertical position as compared to the horizontal one ($P < 0.05$). Neither the systolic BP and CO (CI) nor SVR (SVRI) differed significantly between both positions (all $P > 0.2$).

All time domains of the HRV, and also BRS were decreased, whereas HRV-LF was increased in the vertical position in comparison with those values measured in the horizontal one (all $P < 0.05$).

Differences in VE and ERGO Activity. There were no differences between the averaged VE values in the two examined positions at rest and during recovery without and with PCO (all $P > 0.1$) (Table 3).

Values of VE during the exercise were greater in the vertical position as compared to those in the horizontal position ($P < 0.05$) (Table 3).

As was expected, isolated occlusion did not affect VE significantly in both horizontal and vertical positions (in both cases $P > 0.2$).

The ERGO activity was significantly lower in the vertical position as compared to that in the horizontal one ($P < 0.05$).

T a b l e 2. Averaged Hemodynamic Parameters, HRV, and BRS in the Vertical vs. Horizontal Positions in the Examined Group

Т а б л и ц я 2. Усереднені гемодинамічні параметри, показники варіабельності серцевого ритму та барорефлекторна чутливість, виміряні у вертикальному та горизонтальному положеннях тіла у 10 молодих здорових чоловіків

Variables	Horizontal position	Vertical position	Difference (vertical – supine)	P value
Hemodynamic parameters				
mRR, msec	969 ± 26	749 ± 40	–220 ± 22	**0.04**
SBP, mm Hg	116 ± 5	120 ± 6	5 ± 3	0.68
DBP, mm Hg	68 ± 3	76 ± 3	8 ± 2	**0.04**
MAP, mm Hg	84 ± 3	91 ± 4	7 ± 2	**0.04**
SV, ml	110 ± 4	84 ± 3	–27 ± 2	**0.04**
SVI, ml/m^2	54 ± 2	41 ± 2	–13 ± 1	**0.04**
CO, l/min	6.9 ± 0.1	6.8 ± 0.3	–0.1 ± 0.2	0.68
CI, l/min·m^2	3.4 ± 0.1	3.4 ± 0.2	–0.1 ± 0.1	0.68
SVR, dyn·sec/cm^5	988 ± 48	1109 ± 98	121 ± 81	0.68
SVRI, dyn·sec·m^2/cm^5	2019 ± 148	2271 ± 238	252 ± 163	0.68
HRV, time domain measures				
SDNN, msec	48 ± 2	40 ± 2	–8 ± 1	**0.04**
RMSSD, msec	52 ± 4	30 ± 4	–22 ± 5	**0.04**
NN50, msec	39 ± 4	14 ± 3	–25 ± 6	**0.04**
pNN50, %	15 ± 2	6 ± 2	–9 ± 2	**0.04**
HRV, frequency domain measures				
LF, msec^2	0.20 ± 0.04	0.71 ± 0.24	0.51 ± 0.25	**0.04**
HF, msec^2	0.33 ± 0.04	0.34 ± 0.02	0.01 ± 0.05	0.68
Baroreflex sensitivity				
BRS, msec/mm Hg	18.6 ± 2.1	6.5 ± 0.8	–12.1 ± 1.7	**0.04**

Footnotes: mRR, mean duration of the R–R intervals; SPB, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SV, stroke volume; SVI, stroke volume index; CO, cardiac output; CI, cardiac index; SVR, systemic vascular resistance; SVRI, systemic vascular resistance index; SDNN, standard deviation of the averaged R–R intervals; RMSSD, square root of the mean of the sum of the squares of differences between adjacent R–R intervals; NN50, number of interval differences between successive R–R intervals greater than 50 msec; pNN50, proportion derived by dividing NN50 by the total number of R–R intervals; LF, low- frequency component; HF, high-frequency component of the HRV spectrum, and BRS, arterial baroreflex sensitivity calculated using the sequence method. Cases with $P < 0.05$ are shown in bold.

DISCUSSION

We have compared different measures of the integrated cardiovascular and ventilatory reflex regulation in young healthy men examined in the horizontal vs. vertical body positions. Importantly, the measurements were performed in the stationary modes; the intervals between testings in different positions were sufficient to neutralize the effect of an orthostatic reaction related to the position change.

In our study, the CO, as well as SBP, did not differ from each other between the examined positions. This observation probably reflects the existence of efficient reflex mechanisms enabling the maintenance of constant blood perfusion in the whole body in both positions. It can be achieved by adequate modulation of the HR, SV, and BRS, which was observed in our tests and also by other authors [4, 14, 15].

Table 3. Averaged Values of Minute Ventilation During the Subsequent Stages of the Protocol for ERGO Assessment and ERGO Values in the Vertical vs. Horizontal Positions in the Examined Goupr

Таблиця 3. Усереднені значення однохвилинної вентиляції, л/хв, в межах послідовних стадій протоколу оцінки м'язової ергорефлекторної активності, а також значення останнього показника у вертикальному та горизонтальному положеннях тіла тестованих осіб

Variables	Supine position	Vertical position	Difference (vertical – supine)	*P* value
Minute ventilation, l/min				
At rest	6.8 ± 0.4	7.4 ± 0.5	0.6 ± 0.4	0.11
1st + 2nd min of the exercise	8.5 ± 0.4	9.2 ± 0.5	0.7 ± 0.3	**0.03**
3rd +4th + + 5th min of the exercise	9.5 ± 0.8	10.4 ± 0.7	0.9 ± 0.4	**0.03**
Post-exercise recovery with PCO	11.8 ± 1.0	11.6 ± 0.5	–0.2 ± 0.9	0.99
Post-exercise recovery without PCO	8.0 ± 0.5	10.2 ± 1.3	2.2 ± 1.4	0.11
Muscle ergoreflex activity/sensitivity, l/min	3.8 ± 1.0	1.4 ± 1.6	–2.4 ± 1.3	**0.03**

Footnotes: PCO, posthandgrip circulatory occlusion. Cases with $P < 0.05$ are shown in bold.

In our study, all HRV time domains decreased, while HRV-LF increased in the vertical position as compared to the horizontal one. This indicates the increased sympathetic drive. The autonomic balance is known to be sensitive to postural changes; the available data on the pattern of HRV differences between horizontal vs. vertical positions are, however, equivocal [16–19].

Along with changes in HRV, the BRS was also reduced in the vertical position, which is consistent with previous findings [14].

The pattern of differences in minute ventilation recorded in the vertical and horizontal positions has not been comprehensively investigated earlier. In our study, we found that VE did not differ significantly between the two examined body positions at rest. This fact might reflect the effectiveness of the mechanisms maintaining the stability of ventilation, regardless of the body position, which was observed previously [20–22]. However, the response of VE to exercise was greater in the vertical position. This might suggest that the same level of exercise reveals different responses of ventilation, depending on the body position, when the assessed stimulus interacts with muscle metaboreceptors. Interestingly, although there was a greater ventilatory response to exercise in the vertical position, we found that ERGO values decreased in this situation. In other words, we have observed that the same exercise elicited lower ventilatory responses in the vertical position as compared to those in the horizontal one.

There is no simple explanation regarding decreased ERGO in the vertical position. It is known that the muscle ergoreflex is responsible for the adjustment of ventilation and blood perfusion to maintain the adequate oxygen supply with respect to the metabolic needs of working muscles [3, 8, 9]. These differences in ERGO may result from the fact that the vertical position is related to changes in the local arterial and venous pressures in the forearm muscles [9, 23, 24]. While the forearm muscles in the above positions are mostly placed below the heart level, the local arterial pressure is slightly higher, as compared to that in the horizontal position, due to the posture-related influences on the blood pressure (hydrostatic pressure) [23, 25]. Additionally, during repetitive muscle contractions, the pressure in intramuscular veins becomes equal to zero [26]. When the body is in the vertical position, both arterial and perfusion pressures increase within the intervals between muscle contractions. Hence, we assume that, under our experimental conditions, the forearm muscle perfusion pressure was related mainly to the arterial pressure (as the venous pressure was nearly zero); and the arterial pressure was higher in the vertical position [23, 26]. Moreover, a higher perfusion pressure facilitating oxygen supply of the body and extremities in the vertical position contributes to a decreased acidification of the exercising muscles. Accumulation of H^+ ions (originating from lactic acid) in contracting skeletal muscles is a well-established metabolic stimulus eliciting the integrated reflex cardiovascular and ventilatory reactions, including the response of ERGO [27]. Additionally, the premature acidosis occurring during intense exercise is considered to trigger the excessive ergoreflex activity in patients with heart failure [3]. Thus, we hypothesize that the higher perfusion pressure in the working forearm muscles contributes to the reduced local acidification and decreased ergoreceptor stimulation, which is reflected by diminished ERGO activity in the vertical position as compared to that in the horizontal position. Our observations have to be verified in further studies.

In sum, we have shown that, the body position noticeably affects both reflex regulation of the cardiovascular system and autonomic balance. These observations have to be taken into account during physiological testing, including routine clinical ones.

The study was performed in accordance with the guidelines of the Declaration of Helsinki and all existing ethical standards for tests on humans. The study was approved by the local Ethics Committee. All subjects gave their written informed consent.

The authors of this communication, A. Siennicka, M. Tkaczyszyn, K. Ludwikowska, W. Banasiak, M. Piepoli, P. Ponikowski, and E. A. Jankowska, confirm the absence of any conflict related to commercial or financial interests, to interrelations with organizations or persons in any way involved in the research, and to interrelations of the co-authors.

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М'ЯЗОВА ЕРГОРЕФЛЕКТОРНА АКТИВНІСТЬ ТА АВТОНОМНИЙ БАЛАНС У ВЕРТИКАЛЬНОМУ ТА ГОРИЗОНТАЛЬНОМУ ПОЛОЖЕННЯХ ТІЛА У МОЛОДИХ ЗДОРОВИХ ЧОЛОВІКІВ

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Резюме

Ми досліджували, чи розрізняються показники рефлекторної регуляції серцево-судинної системи, а саме м'язова ергорефлекторна активність (ERGO) та характеристики автономного балансу, зареєстровані у вертикальному та горизонтальному положеннях тіла у молодих здорових чоловіків. Були обстежені 10 чоловіків (середній вік 28 ± 1 рік, індекс маси тіла 23.6 ± 0.8 кг/м^2). У кожного тестованого вимірювали значення ERGO (вентиляторної відповіді на серію контрольних силових стискань кисті з 50 % максимальної сили), барорефлекторну чутливість (використано послідовну методику), варіабельність серцевого ритму (ВРС) та низку параметрів гемодинаміки в двох положеннях тіла – горизонтальному та вертикальному. В останній позиції частота серцевих скорочень, а також діастолічні та середні значення тиску крові були вищими, а пульсовий викид меншим (в усіх випадках $P < 0.05$); різниць між значеннями систолічного тиску та системного судинного опору не спостерігалось (усі $P > 0.2$). У вертикальній позиції порівняно з горизонтальною часові показники ВРС, а також барорефлекторна чутливість були зменшеними, тоді як ВРС-низькочастотний компонент збільшувався ($P < 0.05$). Різниць між іншими параметрами ВРС у двох положеннях не спостерігалося ($P > 0.2$). У вертикальному положенні величина однохвилинної вентиляції при тест-зусиллях зростала, в той час як значення ERGO зменшувалося. Отже, положення тіла помітно впливає і на рефлекторну регуляцію серцево-судинної системи, і на автономний баланс. Ці спостереження треба брати до уваги в перебігу фізіологічного тестування.

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