Does Cardiovascular Autonomic Dysfunction Contribute to Fatigue in Myasthenia Gravis?

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Summary

Myasthenia gravis (MG) is an autoimmune disease characterized by fatigable muscle weakness. Despite full spontaneous or pharmacological remission some MG patients still complain of physical and mental fatigue. Fatigue has been related to autonomic dysregulation. The aim of this study was to assess autonomic responses in a group of MG patients in complete remission but complaining of persistent fatigue. Seventeen well-regulated but persistently fatigued MG patients and 17 individually matched controls underwent echocardiography assessing systolic and diastolic heart function. Beat to beat cardiovascular responses at rest and to 30° head-up tilt, tilt-back, and 2-min static handgrip contraction were recorded. Fatigued MG patients had a statistically significant higher resting HR than their matched controls (p=0.03). The difference in resting heart rate between MG patients not using acetylcholine esterase inhibitors (AChEi) and their matched controls was even more pronounced (p=0.007). The autonomic cardiovascular adjustments to head-up tilt, tilt-back and handgrip contraction were not statistically significant different between patients and controls. We found a higher resting heart rate in all well-regulated but fatigued MG patients compared with controls. The difference was more pronounced between patients not taking AChEi compared to their matched controls. This finding may reflect a disturbed resting sympathovagal balance and this might be a contributing factor to the fatigue symptoms.

Key words

Myasthenia gravis • Fatigue • Autonomic disturbance • Cardiovascular response • Acetylcholine esterase inhibitors

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Introduction

Myasthenia gravis (MG) is an autoimmune disorder with specific antibodies directed mainly against nicotinic acetylcholine receptors (AChR) of skeletal muscles leading to muscle weakness and reduced muscular endurance. Decrement in muscle performance assessed by patient history, clinical and neurophysiological examination is the main criteria for the diagnosis of MG [1,2].

We have observed that some well-regulated MG patients complain of subjective physical and mental exhaustion (fatigue) despite absence of any myasthenic symptoms or signs in repeated clinical examinations. Chronic fatigue is a known phenomenon in many neurological and non-neurological diseases independent of muscle weakness [3,4]. This complaint is highly prevalent and represents disabling symptom [5-7] among MG patients; even those who are in spontaneous or pharmacological remission [8]. Although it is currently

widely accepted that MG is a disease of voluntary muscles, the phenomenon of fatigue in MG is too complex to be explained merely by the lack neuromuscular efficiency.

Lack of direct association between subjective fatigue and neurophysiological proven muscle fatigue has been previously reported [9]. In addition to physical fatigue, MG patients have been found to experience cognitive fatigue significantly more often than the normal population [10].

Previous studies using orthostatic stress and isometric muscle contraction as a challenge found that fatigue could be related to autonomic dysregulation [11,12]. Low-grade (30) head-up tilt, tilt-back and handgrip contraction have been proven to be useful for identifying patients with chronic fatigue syndrome [12,13].

Rapid change in posture (head-up tilt and tilt-back to supine position), causes the displacement of blood by gravity and changes venous return which in turn, results in a change in central blood volume and diastolic filling of the heart leading to a change in cardiac stroke volume (explained by the Frank-Starling relationship) followed by a change in mean arterial blood pressure (MAP) [14]. Normally these changes are compensated for by a number of complex cardiovascular responses. A change in baroreceptor activity, central modulation of vasomotor networks and an increase in local vasomotor activity act together to maintain arterial blood pressure and cardiac output [15]. Sustained handgrip contraction activates both the autonomic nervous exercise pressor reflex and a central command.

The exercise pressor reflex is activated due to the increased intramuscular pressure during contraction and compromises arterial blood flow, leading to ischemia in the contracting muscles. This activates mechanically (muscle mechanoreflex) and chemically (muscle metaboreflex) sensitive skeletal muscle receptors. Activation of these receptors and their associated afferent fibres reflexively adjust sympathetic and parasympathetic nerve activity [16].

Several case reports have ascertained the coexistence of autonomic disorders such as bladder dysfunction and intestinal pseudo-obstruction in patients with MG [17,18]. Cardiac muscle has been proven to be a target for the autoimmune process in MG, especially in the presence of a thymoma [19,20]. Cardiac involvement in MG has previously been reported [21,22] and ranged from asymptomatic ECG changes [23], arrhythmia [21], to conduction disturbances [24] and myocarditis[20]. Pathological changes in autonomic cholinergic peripheral nerves has been described in MG patient without any clinical signs of dysautonomia [25]. MG is an inflammatory disease and the relationship between inflammation and autonomic disorder has been assumed [26].

Dysautonomia in MG has to some extent been studied and both sympathetic and parasympathetic dysfunction has been shown [21,27-34]. The main findings have been a decrease in parasympathetic control, suggesting parasympathetic deficiency and disturbed sympathovagal balance with sympathetic hyper-reactivity [21,29].

However, none of these studies linked signs of impaired autonomic nervous system to physical and mental fatigue in MG. We have previously shown that fatigue score, independent of MG severity, were in linear association with score of symptoms related to autonomic dysfunction [8].

The aim of the present study was to assess cardiovascular responses in well-regulated MG patients with documented chronic fatigue in order to identify possible autonomic mechanisms for fatigue in this patient group. Cardiovascular response was provoked by mild head-up tilt, tilt-back, and 2-min handgrip contraction and results were compared to those of a carefully matched healthy control group.

Methods

Subjects

Seventeen well-regulated ethnic Norwegian MG patients followed regularly in our department were included. These patients also participated in a previous study concerning fatigue in MG [8]. The diagnosis was based on typical clinical features, and the fulfilment of two of the following three criteria: the presence of acetylcholine receptor antibodies in serum, a positive edrophonium test, and neurophysiological findings consistent with MG (decrement >10 % at 3 Hz repetitive motor nerve stimulation, increased jitter on single-fiber electromyogram, or both). Inclusion criteria in this study were; well established diagnosis, none or trivial ocular muscle weakness, chronic fatigue at least 6 months and absence of all co-morbidity that could be related to cardiovascular or other autonomic disorders. Sustained mild ocular symptoms, defined as degree 1 according to classification developed by the Task Force of the Medical

Scientific Advisory Board of the Myasthenia Gravis Foundation of America [35], were also accepted. At inclusion 36 patients fulfilled criteria for fatigue caseness; 20 of them without any symptoms or signs of muscular weakness but symptoms of physical and mental fatigue over at least 6 months, were invited to participate.

By history, clinical examination and echocardiography examination three patients were excluded due to mild aortic stenosis, chronic obstructive lung disease and Wolff-Parkinson-White syndrome. Blood sample were tested regarding anemia and thyroid dysfunction.

All patients had previously suffered from generalized MG, and 15 of them had undergone thymectomy. Thymus histology showed thymoma in two patients, follicular lymphoid hyperplasia in 10 and 3 of them had a normal or atrophic thymus. Seven patients had attained complete spontaneous remission and the remainder had achieved pharmacological remission more than six months prior to the study start. Seven patients used small doses of pyridostigmine bromide; with maximum dose of 120 mg daily, seven used prednisolone; with maximum dose of 15 mg every other day, and in two cases azathioprine (Table 1).

Table 1. Patient and control characteristics and patients' medication.

	Patient no.	Gender	Age (years)	Height (cm)	Weight (kg)	Duration* (years)	MGFA** score	Medication**		
								PY***	Pred***	Az***
	1	F	52	173	70	6	1	120	15 mg	-
	2	F	46	176	73	23	1	120	5 mg	-
	3	F	24	154	46	1.5	0	-	-	-
	4	F	49	158	52	21	0	-	-	-
	5	F	48	168	75	11	1	60	-	-
	6	F	55	172	81	33	1	80	5 mg	150 mg
	7	F	28	173	85	8	1	80	-	-
	8	M	28	188	90	1.5	0	-	-	-
	9	M	43	180	86	23	0	-	10 mg	-
	10	F	19	167	55	1.5	0	-	-	-
	11	F	51	159	48	15	0	-	5 mg	-
	12	M	69	182	84	4	0	-	7.5 mg	-
	13	M	50	181	90	4	1	-	-	-
	14	F	41	160	55	11	0	-	-	-
	15	M	37	177	103	2	1	120	-	150 mg
	16	M	61	179	77	2	0	120	12.5 mg	
	17	M	66	178	88	10	0	-	-	-
Patients	N=17	F/M(10/7)	45 (14)	172(12)	74 (17)	13(11)				
Controls	N=17	F/M(10/7)	45(14)	176(10)	72 (12)					

* Duration since diagnosis. ** According to the quantitative MG score for disease severity, Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation (MGFA); 0: asymptomatic; 1: very mild and intermitting exclusively ocular symptoms. *** PY: pyridostigmine daily; Pred: prednisolone every other day; Az: azathioprine daily.

Seventeen volunteers matched individually for age, gender, height and weight (Table 1) were recruited from hospital staff. All controls were healthy subjects. They were in reasonable physical shape but did not follow any strength training program. None of them were taking any medication.

None of the included patients or controls had any additional co morbidity. All subjects underwent echocardiography without pathological findings. The whole group of patients were compared to their carefully matched controls. In addition, the group of 10 patients not using pyridostigmine was separately compared to their controls, since pyridostigmine affects sympatho-vagal balance and slower heart rate [36].

Written informed consent was obtained from all participants. All experiments conformed to the Helsinki

Declaration and the experimental protocol was approved in advance by the relevant regional committee for medical research ethics in Norway.

Echocardiography

Echocardiography was performed in a separate session by one of the authors (KR) with the patients in the left recumbent position, using a General Electric Vivid 7 echocardiograph (GE Healthcare, Horten, Norway) with a 2.5-3.5 MHz transducer. Left ventricular volumes were measured from apical four-chamber and apical long-axis views and the left ventricular ejection fraction was calculated by the modified Simpson's biplane formula. Left ventricular filling was recorded with pulsed-wave Doppler at the mitral tip in an apical four-chamber view. The peak velocities of the early (E) and atrial (A) waves were measured. Tissue Doppler imaging (TDI) was recorded from an apical four-chamber view in a narrow sector including the interventricular septum. Offline measurements were made with EchoPac-PC® (GE Vingmed Ultrasound). The region of interest was positioned in the basal third of the respective walls at the beginning of the QRS complex. Tissue velocities were analyzed at systole (S'), early (E') and late (A') diastole. Global strain was calculated in two-chamber, four-chamber and apical long-axis views using speckle tracking echocardiography (frame rate 67 (5±7) s^{-1}).

Cardiovascular responses

For patients using pyridostigmine the medication was taken one hour before the experiment to allow for maximal drug effect and the experiment was conducted in the prednisolone-free day for patients using prednisolone.

To minimize cardiovascular variations induced by digestion, all participants were asked to eat a light breakfast no later than 2 h before the trial. All subjects were lightly dressed, and the ambient temperature was maintained at 20-24 °C. The subjects were familiarized with the test situation in several trail runs, and none of them reported any discomfort during the tests. To minimize any muscular activity, the subject was stabilized supine on the tilt-table bed by a vacuum mattress preformed to the body. Sessions lasting about 2 h and consisting of two experimental runs of head-up tilt and handgrip were conducted for each subject.

Head-up tilt

Experiments were carried out using a manually driven tilt-table bed. The gravitational stress consisted of rotating the table and thus the subject about the pitch (y) axis from supine to a 30° head-up position in <2 s. This position was maintained for 120 s, and the subject was then tilted back to a supine position in <2 s. The tilt was repeated once, and a 4-min rest was allowed for recovery between the two tilting sessions. Cardiovascular variables were continuously recorded during the 12 min. it took to complete the test, as shown in the bottom panel of Figure 1. Great care was taken to ensure that the fingers of the left hand, where arterial pressure was recorded, remained exactly level with the right atrium (at the 4th intercostal space, 5 cm below the sternal angle) throughout the tilting procedure.

Handgrip contraction

A static contraction of the forearm muscles of constant magnitude was obtained by gripping with the right hand around two vertical rods that were pressed towards each other (All patients and controls are right-handed). The applied force was measured and presented on a digital display (Gripit; AB Detector, Gothenburg, Sweden). The test subject continuously observed the force and adjusted the strength of the handgrip using this visual feedback. The subjects were asked to exert a force corresponding to 30 % of their individual maximal voluntary contraction force. The maximal voluntary contraction force was determined prior to the experiment by asking the subjects to press the rods together using maximal force for 10 s. They were instructed and directly observed to avoid the Valsalva maneuver and recruitment of accessory muscle mass [37]. The mean of the maximal force exerted during three sessions was calculated and used as the maximal voluntary contraction force given in Newton. The recording began 2 min before the onset of the 2-min handgrip contraction, which was followed by a 4-min recovery interval and a second 2-min contraction (i.e. over a 12-min period), as shown in Figure 1.

Instrumentation

Beat-to-beat stroke volume was recorded by the ultrasound Doppler method. A bidirectional ultrasound Doppler velocimeter (model SD-100, GE Vingmed Ultrasound, Horten, Norway) was operated in pulsed mode at 2 MHz with a hand-held transducer, as previously described [38]. The ultrasound Doppler transducer was hand-held in the suprasternal notch by one of the authors (AE), who sat beside the subject and followed the movements of the tilt table during tilt-up and tilt-back. Blood flow velocity in the ascending aorta was thus continuously recorded before, during and after tilting or handgrip contraction.

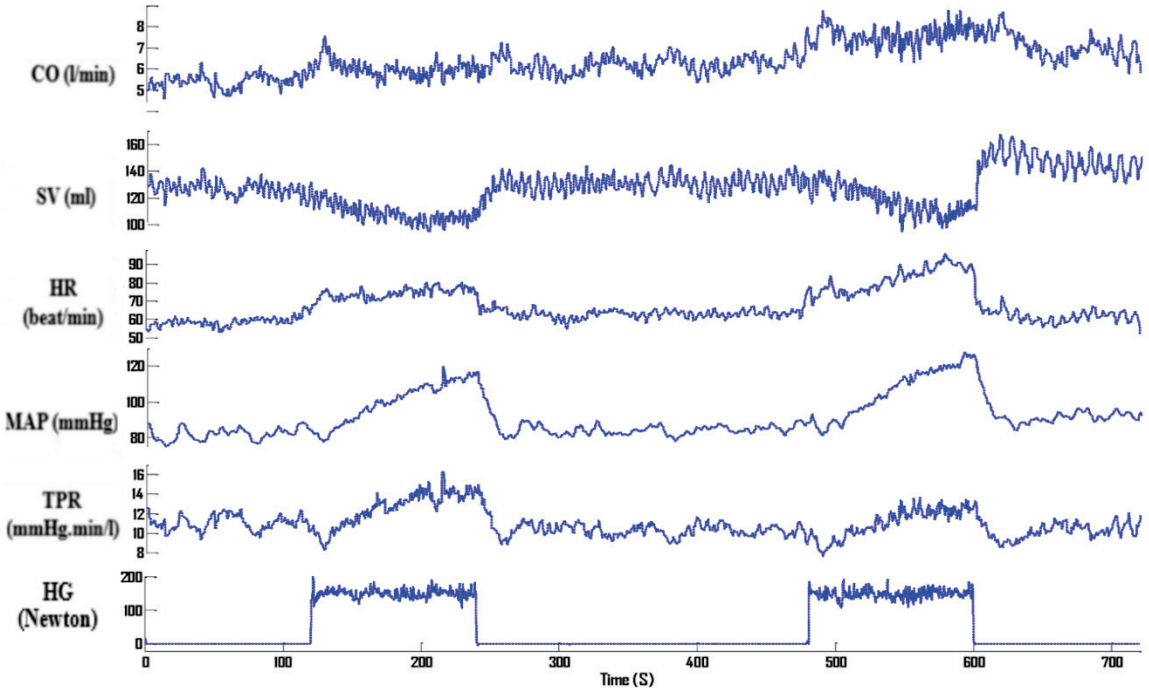

Fig. 1. A complete recording of the cardiovascular responses to handgrip contraction in MG patient no. 8 during one typical experiment. Primary recordings of cardiovascular variables (from top to bottom). CO: cardiac output, SV: stroke volume, HR: heart rate, MAP: mean arterial blood pressure, TPR: total peripheral resistance and HG: handgrip contraction force.

Instantaneous heart rate (HR) was obtained from each R-R interval in the electrocardiogram signal, and beat-to-beat cardiac output (CO) was calculated from the corresponding heart rate and stroke volume (SV) values (CO = SV*HR). Finger arterial pressure was recorded continuously from the third finger of the left hand (model 2300 Finapres blood pressure monitor, Ohmeda, Madison, WI), which was supported throughout by a board held at heart level. Instantaneous pressure output was transferred online to the recording computer, and beat-to-beat mean arterial pressure (MAP) was calculated by numerical integration. Arterial pressure obtained by this method has been shown to be in accordance with central intra-arterial pressure in a number of different situations [39,40]. Total peripheral resistance (TPR) was calculated from beat to beat by dividing mean arterial pressure by cardiac output assuming that mean central venous pressure is zero.

Data analysis

The recordings were processed by a MATLAB program (MathWorks®) specially designed for the purpose. Because the original beat-by-beat sampling was irregularly sampled, all recorded variables were first converted into a 4 Hz-sampled signals by interpolation. The automatic calibration of Finapres was not turned off during recording. The calibration signals were identified and removed offline, and linear interpolation was performed from the successful beat previous to calibration to the first successful beat after calibration. After filtration the program calculated CO (HR × SV) and TRP (MAP/CO).

There was considerable beat-to-beat variation in the recorded variables throughout the registration period, as shown in Figure 1. These variations have been reported previously and are primarily due to the influence of respiration [41]. Variations in the recorded variables unrelated to the onset of posture changes or handgrip contraction were eliminated to some extent by coherent averaging of the responses from a number of identical experimental runs in each subject (Fig. 2). This average response was calculated as the median of each synchronous sample for each 0.25 s time step [42]. The median was used because of the small number (2-4) of individual experimental runs in each subject. All the 17 individual averaged curves from patients and controls respectively were then pooled for calculation of the interindividual average responses by finding the mean value in each set of synchronous samples for each time step (Figs 3 and 4).

The time-averaged cardiovascular responses (HR, MAP and SV) during 5 different intervals of 30 s duration were calculated and analyzed (Table 3). The 5 time-intervals were:

- Resting values was calculated over the period from 60 s to 90 s.

- Steady state after head-up tilt over period from 185 s to 215 s.

- At the end of handgrip contraction over the period from 210 s to 240 s.

- Resting values after tilt back and after the release of handgrip contraction i.e. the period from 250 s to 280 s.

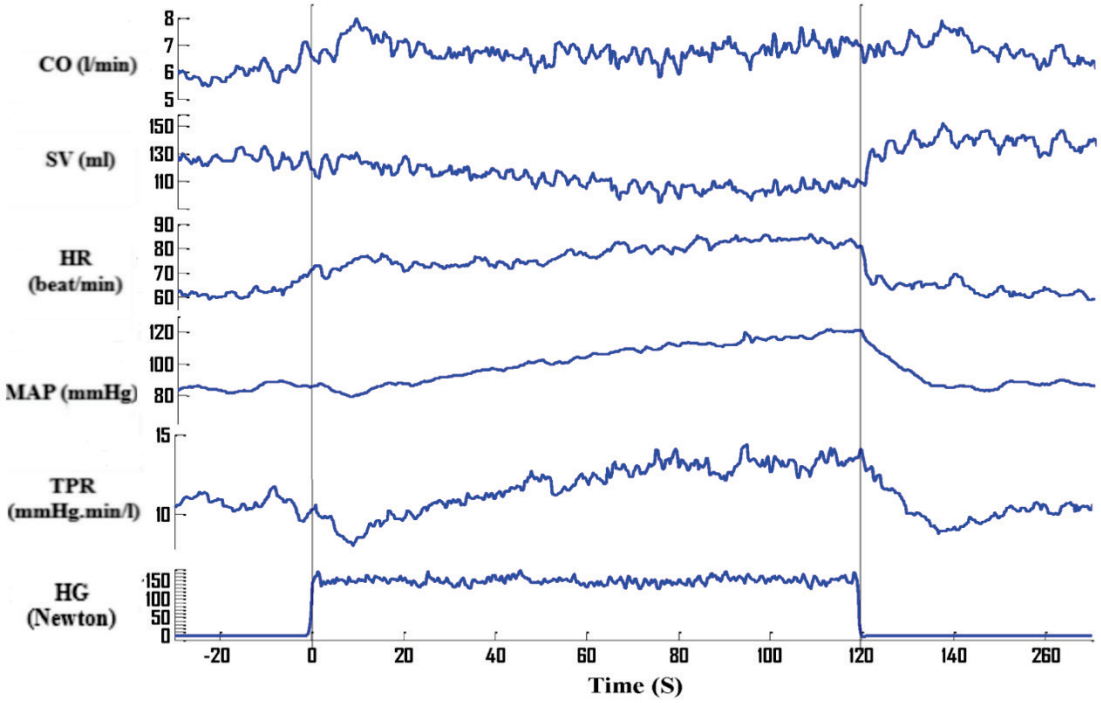

Fig. 2. Median cardiovascular responses to isometric muscle contraction in the same patient as in Figure 1. Median values of cardiovascular variables (from top to bottom). CO: cardiac output, SV: stroke volume, HR: heart rate, MAP: mean arterial blood pressure, TPR: total peripheral resistance and HG: handgrip contraction force.

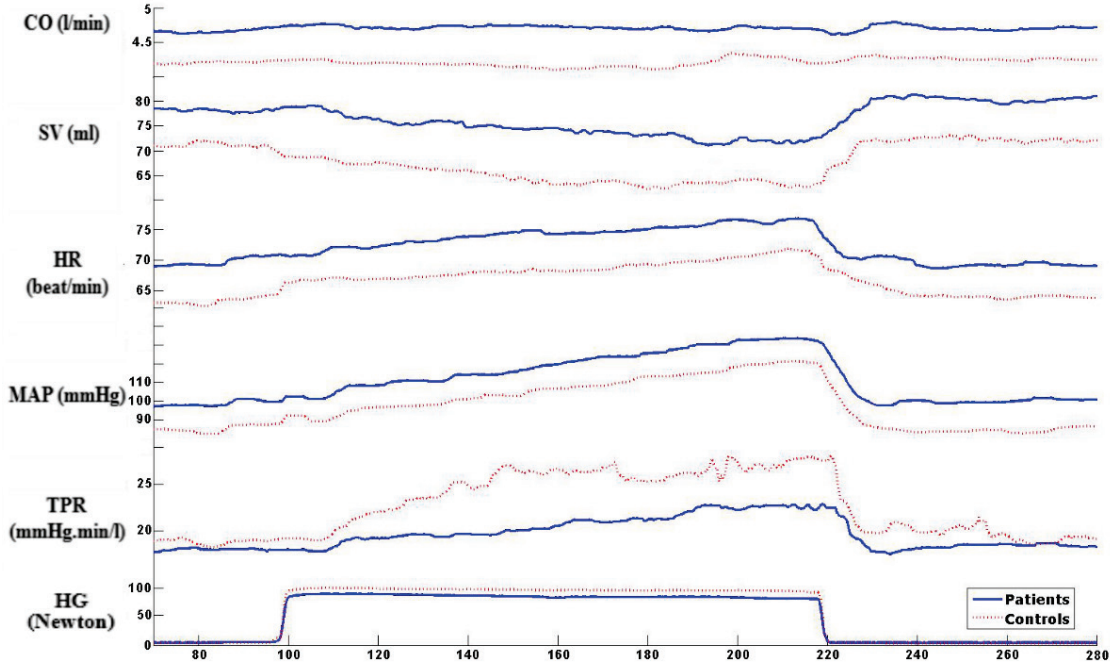

Fig. 3. Mean cardiovascular variables based on the 17 patients' median responses to isometric handgrip contraction compared with those of controls. Recorded mean of variables (from top to bottom). CO: cardiac output, SV: stroke volume, HR: heart rate, MAP: mean arterial blood pressure, TPR: total peripheral resistance and HG: handgrip contraction force.

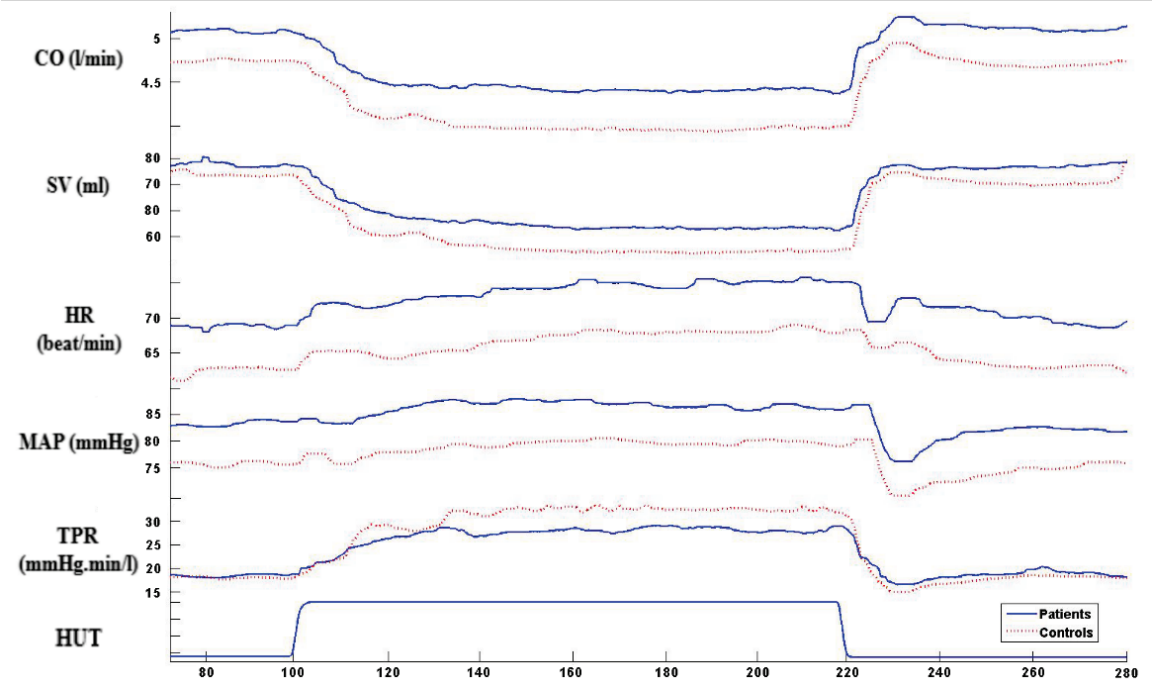

Fig. 4. Mean cardiovascular variables based on the 17 patients' median responses to head-up tilt and tilt-back to supine position compared with those of controls. Recorded median of variables (from top to bottom). CO: cardiac output, SV: stroke volume, HR: heart rate, MAP: mean arterial blood pressure, TPR: total peripheral resistance and HUT: markers for tilt-up and tilt-back to supine position.

Statistical analysis

Descriptive statistical analysis of data was performed with SPSS Statistical software and continuous data was expressed in both mean ± SD and median (min – max). Normality of data distribution was visually inspected by plotting. Data were near to a normal distribution but with some outliers and we therefore used nonparametric unpaired Mann-Whitney statistical tests of differences, which were considered significant if $p<0.05$.

Results

17 patients were included, 7 of them were using pyridostigmine.

Echocardiography

All subjects included had no sign of ischemic or valvular heart diseases. There were no significant differences in either systolic or diastolic values between patients and controls. TDI measurements could not be performed for two patients and two controls due to angle dependency or poor image quality.

At rest

Mean resting HR in MG patients was higher (69±1 beat/min) than in controls (63±9 beats/min) ($p=0.034$). The difference was more pronounced between the group of patients not using pyridostigmine (n=10) and their matched controls (72±13 and 60±7 beats/min respectively, ($p=0.007$). Patients also had a higher mean SV compared to controls (78±31 ml) vs. (72±19 ml), and a higher MAP (84±14 vs. 77±14 mm Hg, but these differences were not statistically significant. The mean TPR was almost the same in patients (18±6 mm Hg.min/l) and controls (19±6 mm Hg.min/l) (Table 2).

Isometric handgrip contraction

The maximal force of handgrip contraction was not significantly different between patients (279±87 Newton) and controls (300±112 Newton) ($p=0.9$). Both patients and controls were able to maintain the contraction force at 30 % of previously registered maximal force approximately constant during the 2-min contraction by using the visual feedback from the digital display.

The average of the 17 patients' responses before, during and after handgrip contraction together with those of the controls are presented in Table 3 and Figure 3.

During isometric muscle contraction, the mean of MAP increased linearly and similarly in both patients and controls from 84 and 77 to 100 and 94, respectively (Fig. 3). During handgrip contraction, an increase in heart rate and a concomitant decrease in stroke volume resulted

in an essentially unchanged cardiac output. However, there must have been continuous peripheral vasoconstriction, since TPR showed a continuous increase. The increment in TPR was more pronounced in controls compared to patients but the difference was not statistically significant. The heart rate increased similarly in patients and controls. Thus, patients maintained their higher HR during HG, with the same difference as in the resting situation.

After release of handgrip contraction for 120 s, MAP, SV, HR and TPR returned to baseline within 1 min.

Table 2. Cardiovascular variables at rest in all patients and also separately in the group of ten patients not using Acetyl choline Esterase inhibitors (AChEi), compared to those in their matched controls.

Variable (unit)		All patients			Patients not using AChEi		
		Patients (n=17)	Controls (n=17)	p-value	Patients (n=10)	Controls (n=10)	p-value
HR (beats/min)	Mean	69	63	0.03	72	60	0.007
	(SD)	(13)	(9)		(13)	(7)	
SV (ml)	Mean	78	72	ns	80	74	ns
	(SD)	(31)	(19)		(37)	(22)	
MAP (mm Hg)	Mean	84	77	ns	89	76	ns
	(SD)	(14)	(14)		(14)	(17)	
TRP (mm Hg.min/l)	Mean	18	19	ns	18	19	ns
	(SD)	(6)	(6)		(6)	(7)	

HR=Heart rate, SV=Stroke volume, MAP=Mean arterial pressure, TPR=Total peripheral resistance.

Table 3. The average of cardiovascular responses to head- up tilt and isometric handgrip contraction in the 17 patients' compared with those of controls.

Variable (unit)		At rest		Head-up tilt		At rest after tilt-back		Handgrip contraction		At rest after handgrip	
		Patients N=17	Controls N=17	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
HR	Mean	69	63	75	68	70	63	76	71	69	64
(beats/min)	(SD)	(13)	(9)	(16)	(10)	(14)	(10)	(15)	(12)	(14)	(11)
SV	Mean	78	72	53	44	77	70	72	65	80	72
(ml)	(SD)	(31)	(19)	(30)	(16)	(35)	(14)	(31)	(21)	(36)	(20)
MAP	Mean	84	77	86	79	82	75	100	94	85	77
(mm Hg)	(SD)	(14)	(14)	(17)	(14)	(15)	(15)	(15)	(18)	(13)	(14)
TPR	Mean	18	19	28	32	19	18	22	28	18	19
(mm Hg.min/l)	(SD)	(6)	(6)	(13)	(16)	(8)	(6)	(9)	(17)	(7)	(9)

HR=Heart rate, SV=Stroke volume, MAP=Mean arterial pressure, TPR=Total peripheral resistance.

Head-up tilt

Table 3 and Figure 4 show the average of patients' and the controls' responses before, during head-up tilt, and after tilt-back. Both in patients and in controls, there was a clear asymmetry between the time course of cardiovascular changes during head-up tilt and tilt-back (Fig. 4). Adjustments generally took up to 30 s after head-up tilt, but most changes were completed more rapidly (~10 s) after tilt-back to a supine position. Changes in heart rate were equally rapid in patients and controls both in head-up tilt and tilt-back. Some initial fluctuations in HR after the tilt back were observed in both groups. Heart rate decreased to baseline level during the few seconds following tilt-back. During head-up tilt,

mean MAP increased equally in patients and controls from (84) and (77) to (86) and (79) mm Hg, respectively. MAP began to decrease rapidly after tilt-back, taking 3-5 s in both patients and controls. During head-up tilt, stroke volume decreased steadily in all subjects during the initial 30 s and stabilized at a level significantly lower than its pre-tilt value. However, the decrease in stroke volume during head-up tilt was smaller in the patients. After tilt-back, stroke volume increased rapidly to its pre-tilt value in 10 s in both groups. Cardiac output (the product of SV × HR) followed the same pattern. TPR began to increase after head-up tilt and reached a stable level after 30 s from (18) and (19) to (28) and (32) in patients and controls respectively, although the increase was greater in the controls. During tilt-back, TPR decreased rapidly in both groups, however the decline was slower in patients. All the variables returned to baseline (pre-tilt) levels after tilt-back in both groups. No statistically significant differences between responses in patients and controls were observed.

Discussion

In the present study we analyzed cardiovascular response in well-regulated MG patients who experienced fatigue, in order to identify possible mechanisms for fatigue symptoms in this otherwise asymptomatic patient group. Firstly, the main finding is that these patients had a higher resting HR than their matched controls. Secondly, the difference was more pronounced in patients not using AChEi compared to their individually matched controls. Thirdly, patients seemed to keep a higher HR during both HG and HUT/TB. Finally, the MG patients had similar cardiovascular responses to orthostatic stress and to a two-minute isometric muscle contraction as their matched controls.

To our knowledge, this is the first study to focus on autonomic regulatory responses as a possible mechanism for fatigue in otherwise asymptomatic MG patients. We minimized the effect of a myasthenic muscle weakness by including only patients in spontaneous or pharmacological complete remission at least six months, who still complained of persistent fatigue. Absence of muscle weakness in these patients was also demonstrated when MG patients were able to maintain handgrip contraction for the whole 2-min period like the controls.

There is a limited number of earlier studies evaluating autonomic control in MG patients describing variable changes in resting heart rate compared to control subjects, and studies of heart rate variability has also been inconclusive.

Earlier investigations of autonomic nervous integrity in MG patients have shown signs of autonomic dysfunction suggesting both deficiencies in sympathetic and parasympathetic nervous system [21,27-31,34]. In two studies, signs of sympathetic deficiency in MG patients were observed by analyzing noradrenaline and adrenaline in response to stress [28,30].

Another study, using head up tilt test and isometric handgrip test, suggested sympathetic hyper responsiveness in MG patients [29]. They found a somewhat higher heart rate at rest (not significant), and a significant rise in heart rate and blood pressure both under orthostatic test and isometric hand grip test. However, the majority of patients in that study had a more severe myasthenia, and 22 % of them were admitted with a myasthenic crisis during current hospitalization, which could be a contributing factor for the abnormal hyperreactivity to stress. They also used a head up tilt to 70 degrees, making the stress more challenging.

Autonomic function tests were performed in a group of MG patients and these revealed significant changes in heart rate variability, suggesting parasympathetic dysfunction as well as a shift of the sympathovagal balance towards raised sympathetic tone [29]. With regards to conventional autonomic function tests, there was statistically significant decrease in values of heart rate-based tests as well as blood pressure-based test (isometric handgrip test) in the study group compared with controls, concluding that in MG, cholinergic transmission is affected more diffusely than previously thought [34]. Another study showed increased systolic and diastolic blood pressure at rest and during tilt in patients with MG [31]. These results suggest disturbed sympathovagal balance in favor of sympathetic tone and that parasympathetic insufficiency has become more prominent. A very recent study characterized MG patients by reduced baroreflex sensitivity (BRS) and increase in sympathovagal ratio of HRV compared to healthy controls indicating parasympathetic deficiency with a shift of sympathovagal balance toward sympathetic predominance [21].

None of these studies linked signs of impaired autonomic nervous system to physical and mental fatigue in MG. We have previously shown that fatigue score, independent of MG severity, were in linear association

with score of symptoms related to autonomic dysfunction [8]. Fatigue has previously been related to autonomic dysregulation in patients with chronic fatigue syndrome using the same stressors as in the present study. [11,12]. Patients had higher heart rate, diastolic blood pressure, plasma norepinephrine ($p<0.01$), mean blood pressure and plasma epinephrine ($p<0.05$) at rest compared to controls. Sympathetic nervous activity was suggested as a part of the underlying pathophysiology of fatigue [13].

The MG patients in the present study showed similar autonomic response as the control group during our interventions, indicating a preserved dynamic autonomic control similar to findings in previous studies on young healthy individuals [43,44]. However, we found that well-regulated MG patients with fatigue had a higher resting heart rate than controls. This increased resting HR may indicate a change in static autonomic balance, with a lower vagal tone and possibly an increase in sympathetic tone, which in turn may contribute to fatigue symptoms. The difference was significantly higher ($p=0.007$) in patients who were not taking pyridostigmine despite the small number (N=10).

The moderate increase in resting HR may explain why this has not been discovered by clinical examination in previous studies [29,30]. We excluded all co-morbidity among these patients including anemia and thyroid abnormality that could alter HR.

In accordance with all previous studies [27-30], mean resting HR in our MG patients was lower than 100 bpm. When HR is below 100 bpm, any changes are mainly suggesting an increase or decrease in firing frequency in the parasympathetic nerves rather than change in sympathetic nerve signals to the sinoatrial node [14]. We therefore hypothesize that the increase in resting heart rate is caused by diminished vagal activity. This might also be combined with a compensatory slight increase in the circulating adrenalin previously shown in MG patients [28,30] and in patients with chronic fatigue syndrome [12].

MG patients not taking AChEi seem to have a lower exercise tolerance than matched controls, while the opposite was true of patients on AChEi [45]. A higher heart rate may indicate a subclinical alteration in autonomic function, with a constantly decreased vagal tone.

The limitation of the study is a small number of patients and that we operated with head-up tilt to 30° that may be too weak challenge to cardiovascular stress and a greater angle of tilt may be needed. However, this angel allows us to register stroke volume beat for beat by ultrasound probe in suprasternal notch. Moreover, it has been shown that this method is useful when investigating dysautonomia in young patients with chronic fatigue syndrome [13]. Further studies on autonomic function in MG patients are needed. It would be interesting to apply the method in a larger number and more seriously affected MG patients.

Conclusions

Well-regulated MG patients complaining of fatigue have higher resting heart rate compared to matched healthy controls. This finding was more pronounced in patients not using acetylcholine esterase inhibitors. We suggest that the higher heart rate may reflect a lower resting vagal tone, and that this may have been a contributing factor to their fatigue symptoms. Since the vast majority of MG patients use AChEi, such a decrease in basic vagal tone may have been masked and thus not reported previously. We found no alteration in dynamic autonomic cardiovascular control. This finding should be further investigated in future studies in order to explain the phenomenon more thoroughly.

Conflict of Interest

There is no conflict of interest.

Abbreviations

AChEi – Acetylcholine esterase inhibitor, AChR – Acetylcholine receptor, BMI – Body mass index, BPM – Beat per minute, CNS – Central nervous system, CO – Cardiac output (CO = SV*HR), HG – Handgrip, HR – Heart rate, HUT/TB – Head up tilt/Tilt back, MAP – Mean arterial blood pressure, MG – Myasthenia gravis, SV – Stroke volume, TPR – Total peripheral resistance (TRP = MAP/CO).

References

1. Hughes BW, Moro De Casillas ML, Kaminski HJ. Pathophysiology of myasthenia gravis. Semin Neurol 2004;24:21-30. https://doi.org/10.1055/s-2004-829585
2. Keesey JC. AAEE Minimonograph #33: electrodiagnostic approach to defects of neuromuscular transmission. Muscle Nerve 1989;12:613-626. https://doi.org/10.1002/mus.880120802

3. Di Vico IA, Cirillo G, Tessitore A, Siciliano M, Venturelli M, Falup-Pecurariu C, Tedeschi G, Morgante F, Tinazzi M. Fatigue in hypokinetic, hyperkinetic, and functional movement disorders. Parkinsonism Relat Disord 2021;86:114-123. https://doi.org/10.1016/j.parkreldis.2021.03.018
4. Grongstad A, Vøllestad NK, Oldervoll LM, Spruit MA, Edvardsen A. The effects of high- versus moderate-intensity exercise on fatigue in sarcoidosis. J Clin Med 2019;8:460. https://doi.org/10.3390/jcm8040460
5. Hoffmann S, Ramm J, Grittner U, Kohler S, Siedler J, Meisel A. Fatigue in myasthenia gravis: Risk factors and impact on quality of life. Brain Behav 2016;6:e00538. https://doi.org/10.1002/brb3.538
6. Alekseeva TM, Gavrilov YV, Kreis OA, Valko PO, Weber KP, Valko Y. Fatigue in patients with myasthenia gravis. J Neurol 2018;265:2312-2321. https://doi.org/10.1007/s00415-018-8995-4
7. Ruiter AM, Verschuuren J, Tannemaat MR. Fatigue in patients with myasthenia gravis. A systematic review of the literature. Neuromuscul Disord 2020;30:631-639. https://doi.org/10.1016/j.nmd.2020.06.010
8. Elsais A, Wyller VB, Loge JH, Kerty E. Fatigue in myasthenia gravis: is it more than muscular weakness? BMC Neurol 2013;13:132. https://doi.org/10.1186/1471-2377-13-132
9. Symonette CJ, Watson BV, Koopman WJ, Nicolle MW, Doherty TJ. Muscle strength and fatigue in patients with generalized myasthenia gravis. Muscle Nerve 2010;41:362-369. https://doi.org/10.1002/mus.21493
10. Paul RH, Cohen RA, Goldstein JM, Gilchrist JM. Fatigue and its impact on patients with myasthenia gravis. Muscle Nerve 2000;23:1402-1406. https://doi.org/10.1002/1097-4598(200009)23:9<1402::aid-mus11>3.0.co;2-b
11. Stewart JM. Autonomic nervous system dysfunction in adolescents with postural orthostatic tachycardia syndrome and chronic fatigue syndrome is characterized by attenuated vagal baroreflex and potentiated sympathetic vasomotion. Pediatr Res 2000;48:218-226. https://doi.org/10.1203/00006450-200008000-00016
12. Wyller VB, Saul JP, Walløe L, Thaulow E. Sympathetic cardiovascular control during orthostatic stress and isometric exercise in adolescent chronic fatigue syndrome. Eur J Appl Physiol 2008;102:623-632. https://doi.org/10.1007/s00421-007-0634-1
13. Wyller VB, Due R, Saul JP, Amlie JP, Thaulow E. Usefulness of an abnormal cardiovascular response during low-grade head-up tilt-test for discriminating adolescents with chronic fatigue from healthy controls. Am J Cardiol 2007;99:997-1001. https://doi.org/10.1016/j.amjcard.2006.10.067
14. Rowell LB. *Human Cardiovascular Control*. Oxford University Press, 1993, 516 p.
15. Low PA, Benarroch EE. *Clinical Autonomic Disorders*. Lippincott Williams & Wilkins, 2008, 768 p.
16. Smith SA, Mitchell JH, Garry MG. The mammalian exercise pressor reflex in health and disease. Exp Physiol 2006;91:89-102. https://doi.org/10.1113/expphysiol.2005.032367
17. Musthafa CP, Moosa A, Chandrashekharan PA, Nandakumar R, Narayanan AV, Balakrishnan V. Intestinal pseudo-obstruction as initial presentation of thymoma. Indian J Gastroenterol 2006;25:264-265.
18. Pande R, Leis AA. Myasthenia gravis, thymoma, intestinal pseudo-obstruction, and neuronal nicotinic acetylcholine receptor antibody. Muscle Nerve 1999;22:1600-1602. https://doi.org/10.1002/(sici)1097-4598(199911)22:11<1600::aid-mus19>3.0.co;2-3
19. Mygland A, Tysnes OB, Matre R, Aarli JA, Gilhus NE. Anti-cardiac ryanodine receptor antibodies in thymoma-associated myasthenia gravis. Autoimmunity 1994;17:327-331. https://doi.org/10.3109/08916939409010673
20. Suzuki S, Utsugisawa K, Yoshikawa H, Motomura M, Matsubara S, Yokoyama K, Nagane Y, Maruta T, Satoh T, Sato H, Kuwana M, Suzuki N. Autoimmune targets of heart and skeletal muscles in myasthenia gravis. Arch Neurol 2009;66:1334-1338. https://doi.org/10.1001/archneurol.2009.229
21. Rzepiński Ł, Zawadka-Kunikowska M, Newton JL, Zalewski P. Cardiac Autonomic Dysfunction in Myasthenia Gravis and Relapsing-Remitting Multiple Sclerosis-A Pilot Study. J Clin Med 2021;10:2173. https://doi.org/10.3390/jcm10102173
22. Shivamurthy P, Parker MW. Cardiac manifestations of myasthenia gravis: A systematic review. IJC Metab Endocr 2014;5:3-6. https://doi.org/https://doi.org/10.1016/j.ijcme.2014.08.003
23. Kato T, Hirose S, Kumagai S, Ozaki A, Matsumoto S, Inoko M. Electrocardiography as the first step for the further examination of cardiac involvement in myasthenia gravis. BioMed Res Int 2016;2016:8058946. https://doi.org/10.1155/2016/8058946
24. Călin C, Savu O, Dumitru D, Ghiorghiu I, Călin A, Capraru C, Popescu BA, Croitoru M, Vîlciu C, Ginghină C. Cardiac involvement in myasthenia gravis--is there a specific pattern? Rom J Intern Med 2009;47:179-189.

25. Kimura S, Nezu A. Peripheral nerve involvement in myasthenia gravis. Brain Dev 1989;11:429-432. https://doi.org/10.1016/s0387-7604(89)80030-0
26. Zila I, Mokra D, Kopincova J, Kolomaznik M, Javorka M, Calkovska A. Vagal-immune interactions involved in cholinergic anti-inflammatory pathway. Physiol Res 2017;66(Suppl 2):S139-S145. https://doi.org/10.33549/physiolres.933671
27. Douchet MP, Quiring E, Bronner F, Vi-Fane R, Messier M, Chauvin M, Warter JM. Paradoxal lowering of parasympathetic indices in myasthenic patients. (Article in French) Arch Mal Coeur Vaiss 1999;92:711-717.
28. Lechin F, van der Dijs B, Pardey-Maldonado B, John E, Jimenez V, Orozco B, Baez S, Lechin ME. Enhancement of noradrenergic neural transmission: An effective therapy of myasthenia gravis: A report on 52 consecutive patients. J Med 2000;31:333-361.
29. Shukla G, Gupta S, Goyal V, Singh S, Srivastava A, Behari M. Abnormal sympathetic hyper-reactivity in patients with myasthenia gravis: a prospective study. Clin Neurol Neurosurg 2013;115:179-186. https://doi.org/10.1016/j.clineuro.2012.05.013
30. Stoica E, Enulescu O. Deficiency of sympathetic nervous system function in myasthenia. J Auton Nerv Syst 1992;38:69-76. https://doi.org/10.1016/0165-1838(92)90218-6
31. Kocabas ZU, Kizilay F, Basarici I, Uysal H. Evaluation of cardiac autonomic functions in myasthenia gravis. Neurol Res 2018;40:405-412. https://doi.org/10.1080/01616412.2018.1446690
32. Nikolić A, Perić S, Nišić T, Popović S, Ilić M, Rakočević Stojanović V, Lavrnić D. The presence of dysautonomia in different subgroups of myasthenia gravis patients. J Neurol 2014;261:2119-2127. https://doi.org/10.1007/s00415-014-7465-x
33. Benjamin RN, Aaron S, Sivadasan A, Devasahayam S, Sebastin A, Alexander M. The spectrum of autonomic dysfunction in myasthenic crisis. Ann Indian Acad Neurol 2018;21:42-48. https://doi.org/10.4103/aian.AIAN_270_17
34. Puneeth CS, Chandra SR, Yadav R, Sathyaprabha TN, Chandran S. Heart rate and blood pressure variability in patients with myasthenia gravis. Ann Indian Acad Neurol 2013;16:329-332. https://doi.org/10.4103/0972-2327.116912
35. Jaretzki A 3rd, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, Sanders DB. Myasthenia gravis: Recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Ann Thorac Surg 2000;70:327-334. https://doi.org/10.1016/s0003-4975(00)01595-2
36. Villacorta AS, Villacorta H, Caldas JA, Precht BC, Porto PB, Rodrigues L, Neves M, Xavier AR, Kanaan S, Mesquita CT, da Nóbrega ACL. Effects of Heart Rate Reduction With Either Pyridostigmine or Ivabradine in Patients With Heart Failure: A Randomized, Double-Blind Study. J Cardiovasc Pharmacol Ther 2019;24:139-145. https://doi.org/10.1177/1074248418799364
37. Hisdal J, Toska K, Flatebø T, Waaler B, Walløe L. Regulation of arterial blood pressure in humans during isometric muscle contraction and lower body negative pressure. Eur J Appl Physiol 2004;91:336-341. https://doi.org/10.1007/s00421-003-0982-4
38. Eriksen M, Walløe L. Improved method for cardiac output determination in man using ultrasound Doppler technique. Med Biol Eng Comput 1990;28:555-560. https://doi.org/10.1007/bf02442607
39. Imholz BP, Settels JJ, van der Meiracker AH, Wesseling KH, Wieling W. Non-invasive continuous finger blood pressure measurement during orthostatic stress compared to intra-arterial pressure. Cardiovasc Res 1990;24:214-221. https://doi.org/10.1093/cvr/24.3.214
40. Parati G, Casadei R, Groppelli A, Di Rienzo M, Mancia G. Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. Hypertension 1989;13:647-655. https://doi.org/10.1161/01.hyp.13.6.647
41. Toska K, Eriksen M. Respiration-synchronous fluctuations in stroke volume, heart rate and arterial pressure in humans. J Physiol 1993;472:501-512. https://doi.org/10.1113/jphysiol.1993.sp019958
42. Toska K, Eriksen M. Peripheral vasoconstriction shortly after onset of moderate exercise in humans. J Appl Physiol (1985) 1994;77:1519-1525. https://doi.org/10.1152/jappl.1994.77.3.1519

43. Toska K. Handgrip contraction induces a linear increase in arterial pressure by peripheral vasoconstriction, increased heart rate and a decrease in stroke volume. Acta Physiol (Oxf) 2010;200:211-221. https://doi.org/10.1111/j.1748-1716.2010.02144.x
44. Toska K, Walløe L. Dynamic time course of hemodynamic responses after passive head-up tilt and tilt back to supine position. J Appl Physiol (1985) 2002;92:1671-1676. https://doi.org/10.1152/japplphysiol.00465.2000
45. Elsais A, Johansen B, Kerty E. Airway limitation and exercise intolerance in well-regulated myasthenia gravis patients. Acta Neurol Scand 2010;190(Suppl):12-17. https://doi.org/10.1111/j.1600-0404.2010.01369.x