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Central Chemoreflex Sensitivity and Parasympathetic Nervous Activity in patients with Heart Failure

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Abstract*— *Although some studies point towards exacerbated central chemoreflex sensitivity (CCS) and reduced parasympathetic nervous activity (PNA) in patients with heart failure (HF), others dispute this finding by indicating their unchanging condition. The aim of this study is to compare CCS and PNA between patients with HF and healthy individuals. Eighteen patients with HF and 14 healthy individuals participated in the study. CCS was assessed through 7% CO_2 rebreathing test for 4 minutes. PNA was determined based on Fast Fourier Transformation using the high-frequency component of heart rate variability. CCS was not different between HF patients [MD: 0.83 (0.49 to 1.54) l.min.mmHg] and healthy individuals [MD: 0.88 (0.16 to 2.56) l.min.mmHg]. PNA in HF patients [MD: 288 (266 to 1188) ms] also did not differ from healthy individuals [MD: 299 (81 to 1099) ms]. In conclusion, HF patients subjected to adequate clinical management may present preservation of CCS and PNA.

Keywords*— *Central chemoreflex sensitivity, vagal modulation of heart rate, heart failure, autonomic control.

I. INTRODUCTION

Heart Failure (HF) is a complex clinical syndrome in which the heart becomes unable to effectively pump blood due to functional and anatomical cardiac impairment [1,2]. Over time, it can lead to several electrophysiological changes, as well as to changes in respiratory and cardiac control reflexes [3] such as chemoreflex sensitivity and parasympathetic nervous activity [4,5].

Exacerbated central chemoreflex sensitivity (CCS) is a pathophysiological change often attributed to HF [4]. It leads to chronic sympathetic nervous system overactivation and to reduced parasympathetic nervous activity (PNA) [5], which favors disease progression and worsened prognosis [6]. Thus, it results in increased cardiac arrhythmia and heart failure-associated mortality rates [7].

Although some studies point towards exacerbated CCS in HF patients [1,7,8,9] others contradict this finding by suggesting that CCS and the parasympathetic nervous activity remain unchanged [10] Based on these divergent findings and on the clinical importance given to these data, the aim of the present study was to compare CCS and parasympathetic nervous activity between HF patients and healthy individuals.

II. METHODS

2.1 Sample

HF patients were screened based on echocardiographic examinations performed at local reference centers in cities located in Rio Grande do Sul State, Brazil, from 2014 to 2018. Eighteen (18) HF patients and 14 healthy individuals, who were matched by sex and age group, participated in the study. Inclusion criteria comprised clinically stable HF patients, who are classified as NYHA functional classes I, II and III, and whose medication had not been changed in the previous 3 months. Patients presenting unstable angina, atrial fibrillation, acute myocardial infarction or recent cardiac surgery (< 6 months), severe obesity, smoking habit, and spirometry-

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assessed chronic obstructive pulmonary disease were excluded from the study [25]. Healthy and physically active individuals reporting smoking habit were also excluded from the study. All participants signed the Informed Consent Form. The study was approved by the local Research Ethics Committee.

2.2 Study Protocol

The CCS and parasympathetic nervous activity assessment protocol was performed in the morning, in a room with controlled temperature (22ºC). All individuals were instructed to fast, to avoid caffeinated and alcoholic beverages for at least 10 hours before the test and to not exercise for at least 48 hours.

CCS was evaluated based on the CO_2 rebreathing technique [26]. After participants were left to rest in sitting position for 15 minutes, they were connected to a system, which consisted of a spirometer coupled to bacteriological and oral filter. The inspiratory pathway was connected to a trachea with three-way valve in order to allow participants to breathe ambient air or gas coming from a 30-liter balloon. Volunteers subjected to the protocol used nasal clip. Initially, 5 basal minutes were recorded and, then, participants inhaled a mixture of CO_2 (7%) and O_2 (93%), for 4 minutes. CCS was based on the ratio between minute ventilation (MV) and partial end-tidal CO_2 pressure ($PetCO_2$), which was calculated through linear regression analysis and expressed in liters per minute per mmHg (l.min.mmHg).

PNA was evaluated after participants had rested in supine position for 15 minutes. Individuals were connected to a system composed of a spirometer coupled to bacteriological and oral filter. They used nasal clip and were instructed to perform 15 controlled breathings per minute, which were guided by the sound of a metronome for 10 minutes. PNA was obtained through spectral analysis by applying the *Fast Fourier Transform* algorithm over 5-minute segments. The low frequency component (0.04 - 0.15 Hz), representative of sympathetic and parasympathetic nervous activity and the high frequency component (0.15 -0.45 Hz), representative of parasympathetic nervous activity, were expressed in ms [27].

2.3 Variables

Respiratory flow was assessed in a spirometer (FE141 spirometer, ADInstruments, Sydney Australia, 1000-liter flow head) calibrated with a 3-liter syringe. Respiratory rate (RR) and tidal volume (TV) were determined through the respiratory flow channel. Minute ventilation was calculated in additional channel by multiplying the RR (breathing/min) by the tidal volume [28]. HR was noninvasively measured beat by beat (ADInstruments, bioamp ML132, Australia). $PetCO_2$ was measured in a capnograph (CO_2 gas Analyzer-17630, Vacumed, Silver Edition, USA). Systolic (SBP) and diastolic (DBP) blood pressures were measured in mercury sphygmomanometer (Unitec®, Brazil), which was placed on participants' dominant arm along with a stethoscope (Rappaport Premium). Arterial oxygen saturation ($SatO_2$) was measured with a pulse oximeter (CONTEC CMS50C) positioned on participants' middle finger. The PowerLab system [Powerlab / 16SP ML880, AD Instruments (ADI) A, USA] was used to collect data, which were analyzed in the LabChart Pro V.8 software, ADInstruments.

2.4 Statistical analysis

Data presenting normal distribution were expressed as mean and standard deviation. Student's t-test for independent samples was used to compare normal distribution variables between groups. CCS and PNA data did not present normal distribution, so they were expressed as median and confidence interval. Mann-Whitney test was used to compare these two variables between groups. Two-way analysis of variance (ANOVA) was used to evaluate respiratory and hemodynamic responses during CCS assessment, since they recorded normal distribution. $P \leq 0.05$ was considered significant.

III. RESULTS

Table 1 shows the characteristics of healthy individuals and HF patients. One HF patient was classified as NYHA functional class III (moderate symptoms), eight HF patients were classified as functional class II (mild symptoms) and nine of them were at functional class I (no symptoms) [11]. The low and the high frequency component of HR variability in HF patients did not differ from healthy individuals.

HF patients presented CCS: 0.83 (0.49 to 1.54) l.min.mmHg, whereas healthy individuals presented CCS: 0.88 (0.16 to 2.56) l.min.mmHg; there was not significant difference between groups (P = 1.00).

Table 1. Participants' characteristics

	HF (n=18)	HS (n=14)	P
Sex	12 men / 6 women	9 men / 5 women	-
Age (years)	57 ± 7	57 ± 6	0.95
Weight (kg)	80 ± 15	72 ±10	0.41

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Height (m)	1.99 ±0.06	1.80 ± 0.17	0.76
BMI (kg/cm^2)	27.6 ± 3.9	25.03 ± 2.55	0.15
Beta-blockers (%)	88.8	-	-
ACE-I (%)	50	-	-
Diuretics (%)	72.2	-	-
Anticoagulants (%)	72.2	-	-
LVEF (%)	35.8 ± 8.9	-	-
Ischemic HF (%)	77	-	
NYHA (I/II/III)	9/8/1	-	-
CCS (l.min.mmHg)	0.83 (0.49 - 1.54)	0.88 (0.16 - 2.56)	1.00
LF (ms)	288 (266 - 1188)	299 (81- 1099)	0.96
HF (ms)	1063 (416 - 5257)	357 (195 - 1685)	0.32

Normally distributed data were expressed as mean and standard deviation. Non-normally distributed data were expressed as median and confidence interval. HF: heart failure; HS: healthy subjects; CCS: central chemoreflex sensitivity; LVEF: left ventricular ejection fraction; LF: low frequency component of heart rate variability. HF: high frequency component of heart rate variability; BMI: body mass index; ACE-I: angiotensin-converting-enzyme inhibitors; NYHA: New York Heart Association.

Table 2 shows that the central chemoreflex progressive increases MV in HF patients and healthy subjects. $PetCO_2$ presented similar increase between HF patients and healthy subjects at all times in comparison to baseline values. HR increase at the 3rd and 4th minutes was similar in both groups in comparison to baseline values. HR increase was similar in both groups from the 2nd minute on. SBP and $SatO_2$ increase in all CO_2 administration minutes, and DBP increase from the 2nd minute on, were similar in both groups in comparison to baseline values (Table 2).

Table 2- Responses during CCS assessment.

	Group	Baseline	1 min	2 Min	3 min	4 min
MV	HF	24 ±12	29 ± 11*	35 ± 13*	37 ± 16*	40 ± 18*
	HS	22 ±10	26 ± 12*	30 ± 14*	34 ± 16*	38 ± 18 *
$PetCO_2$	HF	48 ± 6	52 ± 7*	55 ± 7*	55 ± 8*	55 ± 9*
	HS	52 ± 7	58 ± 7*	60 ± 9*	62 ± 9*	63 ± 10*
RR	HF	17 ± 4	17 ± 4	17 ± 4	18 ± 5*	18 ± 5*
	HS	17 ± 3	16 ± 4	18 ± 4	17 ± 4*	18 ± 5*
HR	HF	72 ± 7	73 ±8	75 ± 8*	77 ± 9*	77 ± 11*
	HS	67 ±10	67 ± 10	67 ± 11*	68 ± 12*	70 ± 13*
SBP	HF	127 ±18	129 ± 17*	132 ± 18*	136 ± 21*	134 ± 17*
	HS	127 ±12	135 ± 16*	138 ± 13*	141 ± 15*	147 ± 15*
DBP	HF	84 ± 9	88 ± 12	90 ± 15*	93 ±17*	93 ±18*
	HS	87 ± 8	91 ± 10	92 ± 13*	97 ± 16*	94 ±17*
$SatO_2$	HF	98 ± 2	99 ± 1*	99 ± 1*	99 ± 0*	99 ± 0*
	HS	97 ± 2	98 ± 2*	99 ± 0*	99 ± 0*	99 ± 0*

Data expressed as mean and standard deviation.

CCS: central chemoreflex sensitivity; HF: heart failure; HS: healthy subjects. ANOVA: MV (minute ventilation): time = 0.001; group = 0.549; time*group = 0.855; $PetCO_2$ (partial end-tidal CO_2 pressure): time = 0.001; group = 0.038; time*group = 0.573; RR (respiratory rate): time = 0.003; group = 0.187; time*group = 0.574; HR (Heart Rate): time = 0.001; group = 0.071; time*group = 0.224; SBP (systolic blood pressure): time = 0.001; group = 0.326; time*group = 0.056; DBP (diastolic blood pressure): time = 0.001; group = 0.540; time*group = 0.969; $SatO_2$ (peripheral oxygen saturation): time = 0.001; group = 0.303; time*group = 0.149.

* $p < 0.05$ in comparison to baseline value.

IV. DISCUSSION

The current study has shown that the CCS of HF patients does not differ from that of healthy individuals.

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CCS preservation in HF patients could explain the preserved PNA recorded in our study, a fact that could contribute to improve the survival prognosis of these individuals.

Our findings about CCS preservation in HF patients in comparison to such preservation in healthy individuals are in compliance with previous studies conducted by Paleczny *et al.* (2017) [10] and Contini *et al.* (2013) [12]. According to these studies, patients presented low-severity symptoms and most of them were classified as NYHA I and II. The low CCS severity recorded for HF patients assessed in the aforementioned studies, and in the present research, could explain the CCS preservation [10,12].

In addition, the use of beta-blocker drugs could influence the chemoreflex sensitivity response. Paleczny *et al.* (2017) [10] found unchanged CCS in HF patients treated with beta-blockers and ACE-I. These findings may explain, at least in part, the findings in the present study, since 88% of the assessed individuals used beta-blockers. According to Contini *et al.* (2013) [12], beta-blockers with different pharmacological characteristics (drug-blocked receptor type) have different effects on CCS - Carvedilol is the most effective drug in reducing CCS and peripheral chemoreflex sensitivity, since it improves ventilation efficiency during exercise sessions. According to Toledo *et al.* (2017) [13], the use of propranolol (beta-blocker) in animal models also eliminated the deleterious effects of CCS overactivation, such as autonomic dysfunction and cardiac arrhythmia.

The use of beta-blockers could also explain the preserved PNA found in HF patients than in healthy individuals. These drugs presented antagonistic action to sympathetic activation, restored cardiac and circulatory reflex control, attenuated vasoconstrictor neurohumoral systems and improved myocardial performance by reducing individuals' heart rate and oxygen demand [14,15]

Although some studies have recorded exacerbated CCS, the prevalence of this finding was not high in all studies. Mirizzi *et al.* (2016) [8] have found CCS exacerbation in 56% of patients. However, these patients were older, presented lower LVEF, larger right ventricular diameter and worse ventilatory efficiency than the ones assessed in the present study. Giannoni *et al.* (2008) [9] found increased CCS in only 20% of patients who presented the worst clinical severity. On the other hand, Giannoni *et al.* (2009) [7] found CCS exacerbation in 23% of HF patients who have presented low functional capacity, low parasympathetic nervous activity, as well as high prevalence of paroxysmal atrial fibrillation and ventricular tachycardia.

Accordingly, findings in the present study may have been influenced by the exclusion of patients with atrial fibrillation, since previous studies have shown that patients with chemoreflex exacerbation have higher prevalence of atrial fibrillation [16,17], as well as that atrial fibrillation in HF patients may be associated with reduced vagal modulation of HR [16,18], which leads to increased cardiac arrhythmic events [19] and heart failure-associated mortality rates [20].

According to the meta-analysis conducted by Pearson & Smart (2018) [21], PNA was predominant or improved in HF patients who underwent, or started, physical exercises of several modalities such as weight training, aerobics, inspiratory muscle training and yoga, among others. Prospective long-term cohort study with 256 HF patients reinforces that the severity of inspiratory muscle weakness, and shorter walking distance, by 6-minutes walk distance test, proportionally increases mortality risk, but this outcome is more accurately discriminated by the maximal inspiratory pressure [22].

However, patients assessed in the present study were physically inactive, and it corroborates the idea that adequate clinical management based on the use of beta-blockers, and the clinical stability provided by these drugs, represent a protective effect according to the vagal modulation perspective.

The CCS and PNA preservation in HF patients assessed in the present study suggests better prognosis for them, since increased chemoreflex sensitivity can cause peripheral vasoconstriction and lead to clinical HF worsening [16] exacerbate dyspnea symptoms and exercise-related fatigue [23] as well as increase the risk of cardiac events with reduced survival rates [9].

Thus, the adequate clinical management based on beta-blockers, the exclusion of individuals with atrial fibrillation, the low disease severity and the clinical stability of HF patients assessed in the present study may have contributed to preserve both CCS and the PNA.

According to a recent study [24], the preservation, or not, of the PNA in optimally treated HF patients did not show differences in survival rates in a 5-year follow-up. Although CCS preservation did not show prognostic survival implications in a previous study with a 15-month follow-up [10] it is necessary conducting a long-term research on the prognostic and clinical implications on the chemoreflex sensitivity of patients subjected to optimal clinical management.

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V. CONCLUSION

Heart failure patients may present central chemoreflex sensitivity preservation, and even increased parasympathetic nervous activity, due to appropriate clinical management. The impact of these findings on patients' survival should be investigated in long-term cohort studies.

REFERENCES

[1] Departamento de Insuficiência Cardíaca (DEIC) & Sociedade Brasileira de Cardiologia (SBC) (2018). Diretriz Brasileira de *Insuficiência Cardíaca Crônica e Aguda*. *Arq Bras Cardiol. 111*(3):436-539.

[2] Yancy CW, Jessup M, Bozkurt B, Butler J, Casey Jr DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJV, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WHW, Tsai EJ, Wilkoff BL, American College of Cardiology Foundation & American Heart Association Task Force on Practice Guidelines. (2013). 2013 ACCF/AHA Guideline for the Management of Heart Failure. *J Am Coll Cardiol. 62*(16):e147-239.

[3] Ribeiro JP, Chiappa GR & Callegaro CC. (2012). The contribution of inspiratory muscles function to exercise limitation in heart failure: pathophysiological mechanisms. *Rev Bras Fisioter;16* (4):261-67.

[4] Callegaro CC, Martinez D, Ribeiro PA, Brod M & Ribeiro JP. (2010). Augmented peripheral chemoreflex in patients with heart failure and inspiratory muscle weakness. *Respir Physiol Neurobiol.171*(1):31-35.

[5] Trombetta IC, Maki-Nunes C, Toschi-Dias E, Alves MJNN, Rondon MUPB, Cepeda FX, Drager LF, Braga AMFW, Lorenzi-Filho G & Negrao CE. (2013). Obstructive sleep apnea is associated with increased chemoreflex sensitivity in patients with metabolic syndrome. *Sleep. 36*(1):41-9.

[6] Skalska A, Wizner B, Więcek A, Zdrojewski T, Chudek J, Klich-Rączka A, Piotrowicz K, Błędowski P, Mossakowska M, Michel JP & Grodzicki T. (2014). Reduced functionality in everyday activities of patients with self-reported heart failure hospitalization - Population-based study results. *Int J Cardiol.176*(2):423-29.

[7] Giannoni A, Emdin M, Bramanti F, Iudice G, Francis DP, Barsotti A, Piepoli M & Passino C. (2009). Combined increased chemosensitivity to hypoxia and hypercapnia as a prognosticator in heart failure. *J Am Coll Cardiol. 53*(21):1975-80.

[8] Mirizzi G, Giannoni A, Ripoli A, Iudice G, Bramanti F, Emdin M & Passino C. (2016). Prediction of the Chemoreflex Gain by Common Clinical Variables in Heart Failure. *PLoS One.11*(4):e0153510.

[9] Giannoni A, Emdin M, Poletti R, Bramanti F, Prontera C, Piepoli M & Passino C. (2008). Clinical significance of chemosensitivity in chronic heart failure: influence on neurohormonal derangement, Cheyne-Stokes respiration and arrhythmias. *Clin Sci (Lond).114*(7):489-97.

[10] Paleczny B, Olesińska M, Siennicka A, Niewiński P, Jankowska EA, Ponikowska B, Banasiak W, Von Haehling S, Anker SD, & Ponikowski P. (2017). Central Chemoreceptor Sensitivity Is Not Enhanced in Contemporary Patients With Chronic Systolic Heart Failure Receiving Optimal Treatment. *J Card Fail.23*(1):83-87.

[11] Lang RM, Badano LP, Mor-Avi V, et al. Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W & Voigt JU. (2016). Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr. 17*(4):412.

[12] Contini M, Apostolo A, Cattadori G, Paolillo S, Iorio A, Bertella E, Salvioni E, Alimento M, Farina S, Palermo P, Loguercio M, Mantegazza V, Karsten M, Sciomer S, Magrì D, Fiorentini C & Agostonie P. (2013). Multiparametric comparison of CARvedilol, vs. NEbivolol, vs. BIsoprolol in moderate heart failure: the CARNEBI trial. *Int J Cardiol. 168*(3):2134-40.

[13] Toledo C, Andrade DC, Lucero C, Arce-Alvarez A, Díaz HS, Aliaga V, Schultz HD, Marcus NJ, Manríquez M, Faúndez M & Del Rio R. (2017). Cardiac diastolic and autonomic dysfunction are aggravated by central chemoreflex activation in heart failure with preserved ejection fraction rats. *J Physiol. 595*(8):2479-95.

[14] Adamson PB & Gilbert EM. (2006). Reducing the risk of sudden death in heart failure with beta-blockers. *J Card Fail.12*(9):734-46.

[15] Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G & Butler J. (2009). The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications. *J Am Coll Cardiol.54*(19):1747-62.

[16] Niewinski P, Engelman ZJ, Fudim M, Tubek T, Paleczny B, Jankowska EA, Banasiak W, Sobotka PA & Ponikowskiet P. (2013). Clinical predictors and hemodynamic consequences of elevated peripheral chemosensitivity in optimally treated men with chronic systolic heart failure. *J Card Fail.19*(6):408-15.

[17] Budeus M, Hennersdorf M, Perings C & Strauer BE. (2004). The prediction of atrial fibrillation recurrence after electrical cardioversion with the chemoreflex sensitivity. *Z Kardiol.93*(4):295-9.

[18] Drexel T, Eickholt C, Mühlsteff J, Ritz A, Siekiera M, Kirmanoglou K, Schulze V, Shin D-I, Balzer J, Rassaf T, Kelm M & Meyeret C. (2013). Vagal heart rate control in patients with atrial fibrillation: impact of tonic activation of peripheral chemosensory function in heart failure. *Adv Exp Med Biol.755*:287-97.

[19] Farrell TG, Bashir Y, Cripps T, Malik M, Poloniecki J, Bennett ED, Ward DE, & Camm AJ. (1991). Risk stratification for arrhythmic events in postinfarction patients based on heart rate variability, ambulatory

https://dx.doi.org/10.22161/ijaers.78.19 *ISSN: 2349-6495(P) | 2456-1908(O)*

electrocardiographic variables and the signal-averaged electrocardiogram. *J Am Coll Cardiol.18*(3):687-97.

[20] Odemuyiwa O, Poloniecki J, Malik M, T. Farrell, Xia R, Staunton A, Kulakowski P, Ward D & Camm J. (1994). Temporal influences on the prediction of postinfarction mortality by heart rate variability: a comparison with the left ventricular ejection fraction. *Br Heart J.71*(6):521-7.

[21] Pearson MJ & Smart NA. (2018). Exercise therapy and autonomic function in heart failure patients: a systematic review and meta-analysis. *Heart Fail Rev.23*(1):91-108.

[22] Ramalho SHR, Cipriano Junior G, Vieira PJC, Nakano EY, Winkelmann ER, Callegaro CC & Chiappa GR. (2019). Inspiratory muscle strength and sixminute walking distance in heart failure: Prognostic utility in a 10 years follow up cohort study. *PLoS ONE. 14*(8): e0220638.

[23] Ciarka A, Cuylits N, Vachiery JL, Lamotte M, Degaute J-P, Naeije R & Bornee P. (2006). Increased peripheral chemoreceptors sensitivity and exercise ventilation in heart transplant recipients. *Circulation.113*(2):252-57.

[24] Paleczny B, Olesińska-Mader M, Siennicka A, Niewiński P, Nowak K, Buldańczyk A, Jankowska EA, Banasiak W, Haehling S, Ponikowska B, Anker SD & Ponikowski P. (2019). Assessment of baroreflex sensitivity has no prognostic value in contemporary, optimally managed patients with mild-to-moderate heart failure with reduced ejection fraction: a retrospective analysis of 5-year survival. *Eur J Heart Fail. 21*(1):50-58.

[25] American Thoracic Society & European Respiratory Society. (2002). Statement on Respiratory Muscle Testing. *Am J Respir Crit Care Med.166*(4):518-624.

[26] Chua TP & Coats AJ. (1995). The reproducibility and comparability of tests of the peripheral chemoreflex: comparing the transient hypoxic ventilatory drive test and the single-breath carbon dioxide response test in healthy subjects. *Eur J Clin Invest. 25*(12):887-92.

[27] Callegaro CC, Moraes RS, Negrão CE, C Trombetta, Rondon MU, Teixeira MS, Silva SC, Ferlin EL, Krieger EM & J P Ribeiro JP. (2007). Acute water ingestion increases arterial blood pressure in hypertensive and normotensive subjects. *J Hum Hypertens. 21*(7):564-7.

[28] MacKay CM, Skow RJ, Tymko MM, Boulet LM, Davenport MH, Steinback CD, Ainslie PN, Lemieux CCM & Day TA. (2016). Central respiratory chemosensitivity and cerebrovascular CO2 reactivity: a rebreathing demonstration illustrating integrative human physiology. *Adv Physiol Educ. 40*(1):79-92.