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Authorship contribution

1. Conceptualization: MESH, ELG, OQM and JCGN

2. Data curation: RCE, AAH, MESH

3. Formal analysis: RCE, GABM

4. Acquisition of funds: ELG, JCGN, OQM

5. Research: ELG, AAH

6. Methodology: RCE, GABM, FAAT

7. Project administration: ELG, JCGN, OQM

8. Resources: JCGN, ELG, OQM

9. Software: RCE, GABM, FAAT

10. Supervision: MESH, JCGN, OQM

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Abstract

Purpose: The aim of the present study was to investigate the role of occlusion time in dynamic changes of autonomic activation during reactive hyperemia.

Methods: Healthy subjects (n = 30) in the age range of 18–25 years participated in this study. Vascular reactivity was assessed by measuring the dynamic changes in finger pulse volume amplitude (PVA) and pulse transit time relative to the RR intervals in the test (occluded arm) and control arm (contralateral non-occluded arm) during 1, 3 and 5 minute of occlusion using two separate Photoplethysmographic sensors. Heart Rate Variability was computed from a simultaneously acquired ECG signal to monitor the dynamic changes in cardiac autonomic nervous activity. Time-varying analysis of all signals were shown every 1 second in average response graphs.

Results: Time-varying analysis of vascular and autonomic response during reactive hyperemia demonstrated the presence of a characteristic response pattern with an increase in the Sympathetic index and a decrease in Parasympathetic index at 8 to 10 seconds, an increase in heart rate at 20 seconds and a progressive increase in PVA during the first 60 seconds after occlusion regardless of the time spent in the procedure. Moreover, a decrease in pulse transits time relative to RR intervals, followed by an increase regardless of the occlusion time was evidenced.

Conclusions: Early cardiovascular sympathetic activation is independent of occlusion time during reactive hyperemia, which suggests this is a vascular autonomic reflex response involved in the generation of the physiological phenomenon of reactive hyperemia.

Keywords Reactive hyperemia · Autonomic nervous system · Photoplethysmography · Heart rate variability

Abbreviations

ANS Autonomic nervous system
ECG Electrocardiogram
FMD Flow-mediated dilatation
HRV Heart rate variability
PPG Photoplethysmography
PVA Pulse volume amplitude
RH Reactive hyperemia

Introduction

Reactive hyperemia (RH) is a well-stablished technique characterized by a rapid and exaggerated reperfusion to an organ or tissue when the circulation is restored after a period of complete circulatory arrest. Previous works have found a direct relationship between time of occlusion and the magnitude of the hyperemic response [1–3]. Peripheral vaso-regulation during RH is mediated, in part, by the vascular endothelium [4]. Endothelial dysfunction can predict cardiovascular events and it is considered a key alteration in the development of atherogenesis and in the pathophysiology of coronary atherosclerosis. One of its functional consequences is the deterioration in the release of the relaxing factor derived from the endothelium (nitric oxide), which has important antiatherogenic properties [5,6].

In order to evaluate endothelial function, techniques such as angiography have been employed. Invasiveness and need of expert operators [7] are among the main difficulties of these approaches. On the other hand, non-invasive techniques have also been used to measure endothelial function, which vasodilation mediated by the flow of the arteries of the arm (FMD) as the most widespread. This technique measures the ability of the arteries to respond to the release of nitric oxide (NO) during reactive hyperemia test, but this method is difficult to use, needs an expert operator and does not allow detecting changes at the level of the microvasculature [3,8,9]. Measuring endothelial function with peripheral arterial tonometry has gained greater attention and an appropriate device has been developed to measure pulsatile arterial volumetric changes independent of the observer with photoplethysmography (PPG) through the finger pulse volume amplitude (PVA) [3,8,10].

NO, derived from the vascular endothelium, is one of the major factors contributing to vasodilatation caused by reactive hyperemia. However, several studies have shown an association between autonomic nervous system activity and RH [11–14]. The forearm clamping procedure to induce reactive hyperemia possibly affects autonomic nervous system activity. The circulatory arrest during the phase of occlusion may by itself, inherently lead to increase the sympathetic activity. Such activation participates in the regulation of vascular tone [11,15]. Moreover, this sympathetic activation possibly plays an important role in mediating the inter-subject variability of vascular responses [15] and in shear rate changes [16] during reactive hyperemia.

Previous authors have shown that dynamic exercise results in a release of metabolic byproducts which increase the sympathetic neural activity to the systemic vasculature through muscle metaboreflex. The muscle metaboreflex is a very powerful blood pressure-raising reflex. Group III and IV muscle afferents of this reflex are stimulated by metabolites (e.g. protons, lactate, potassium, and diprotonated phosphate), which accumulate within underperfused active skeletal muscle. Muscle metaboreflex activation results in a reflex

increase in sympathetic outflow. During submaximal dynamic exercise, metaboreflex activation markedly increases heart rate [17].

Bade et al. have shown that a similar response, through chemosensitive nerve fibers activated by metabolites freed by the ischemic tissue which tend to accumulate due to circulatory arrest in forearm, may be associated with RH and can lead to sympathetic activation. Moreover, it has been indicated that opposing influences of endothelium-dependent vasodilation and sympathetically mediated vasoconstriction possibly determine the resultant PVA response in the test arm during RH [15]. However, the sympathetic activation during reactive hyperemia remains incompletely understood. The present study investigated the role of the occlusion time in dynamic changes of autonomic activation during reactive hyperemia. We hypothesized that the reflex nature of the sympathetic activation during reactive hyperemia and the temporary sequence of this phenomenon could be evidenced applying time-frequency methods.

Methods

Selection of subjects

Thirty healthy volunteers in the age range of 18–25 years (Mean=20.3 ± 1.7) participated in the present study. None of the subjects had any history of cardiovascular, respiratory, endocrine, or neural diseases, and were normotensive (resting blood pressure: systolic 101±11.2 mm Hg / diastolic 63.6 ± 8.5 mm Hg), non-obese (body mass index= 21.9 ± 2.6 kg/m^2), non-smokers, and free from intake of any medications. Subjects were asked to avoid ingest of food for 2 hours and abstain from intake of tea, coffee, alcohol and strenuous physical activity for 24 hours before the tests.

Ethics statement

The experimental protocol was approved by the Research and Ethics Committee for research in human subjects of Universidad de Ciencias Médicas de Santiago de Cuba. Written informed consent was obtained from all the subjects before enrollment in the study.

Physiological records

All recordings were done in controlled ambient temperature (24-27 °C) and luminance (dim light). Baseline blood pressure was measured after 15 min of supine rest. The sphygmomanometer cuff was kept fastened to the arm for subsequent use when arterial occlusion had to be produced. ECG signal was recorded in the bipolar limb lead II mode using disposable Ag – AgCl electrodes. The PPG waveform was recorded employing an infrared emitter (LED, 850 nm) and a photosensor (photodiode), placed on the middle finger of both upper limbs. The ECG and PPG signal of the AngioECG equipment [18] with a bandpass filter (0.5-30 Hz) was digitized at a sampling rate of 1 kHz.

State of rest: at the beginning of the session, the patients rested in bed under the aforementioned environmental conditions until a better adaptation to the conditions of the room was achieved and then the blood pressure was measured. A control electrocardiogram was

recorded in order to detect any alteration of the heart rhythm and then the ECG and PPG were simultaneously measured for 5 minutes.

Reactive hyperemia test: the sphygmomanometer placed on the right arm was inflated 50 mmHg above the baseline systolic blood pressure, during 1, 3 and 5 minutes of occlusion with ECG and PPG recording.

Time-varying analysis of heart rate variability

Time-varying analysis of heart rate variability (HRV) at rest and during reactive hyperemia test (1, 3 and 5 minute of occlusion) were performed using the Kubios HRV Premium Professional program (Kubios Ltd, Finland), which allowed the pre-processing of the RR interval series and the calculation of time-frequency indicators. In the pre-processing of the RR interval series an Automatic correction filter was used to detect ectopic beats. The discrimination of the R peaks of the ECG signal and the calculation of the RR intervals were performed using a robust detection method [(19)]. The replacement of the ectopic intervals was performed employing polytomic cubic interpolation once the low frequency baseline drift was removed smooth. The time- frequency values of the heart rate, sympathetic index (SNS index) and parasympathetic index (PNS index) interpolated at 1 Hz were exported to archives * .mat for processing in Octave software.

Time-varying analysis of pulse volume amplitude

For the construction of the time-varying hyperemia curve, the procedure recommended by the experts was employed [(20)]. Firstly, the average pulse volume amplitude of a record at rest in the upper right limb was obtained. Pulse volume amplitude of a post-occlusive record of the assessed limb was obtained, as well as the hyperemic curve of the post-occlusive and rest record ratio. Pulse volume amplitude (onset to peak) for the construction of the hyperemic response curve was measured. For determining the clinical interest points of the pulse wave the procedure recommended by Carrazana et al. was employed [(21)]. Time-varying analysis of the temporal sequence of pulse volume amplitudes were interpolated at 1 Hz and exported to *.mat files for better statistical management in Octave software.

Time-varying analysis of Relative Pulse Transit Time

Pulse Transit Time (PTT) is defined as the time between the R-peak of the ECG and any critical point of the pulse wave in the PPG signal. The systolic peaks were chosen as the critical points in this study. Relative PTT is defined as the ratio of PTT and the correspondent RR interval assuming that the PTT is within this RR interval. Time-varying analysis of temporal sequence of Relative Pulse Transit Time were interpolated at 1 Hz and exported to * .mat files for better statistical management in Octave software.

Statistical analysis

Using the Octave software 2019 version 5.1.0 (https://www.gnu.org/software/octave/download.html), the graphs of average values in time-

frequency were constructed, from the matrices (* .mat) of the values obtained from the time-varying analysis of heart rate variability and pulse volume amplitude interpolated at 1 Hz.

Results

According to the dynamic changes in time during reactive hyperemia as show in Fig. 1, an increase in heart rate was observed 20 seconds after cuff deflate independently of the occlusion time. The response becomes more evident after three and five minutes of occlusion.

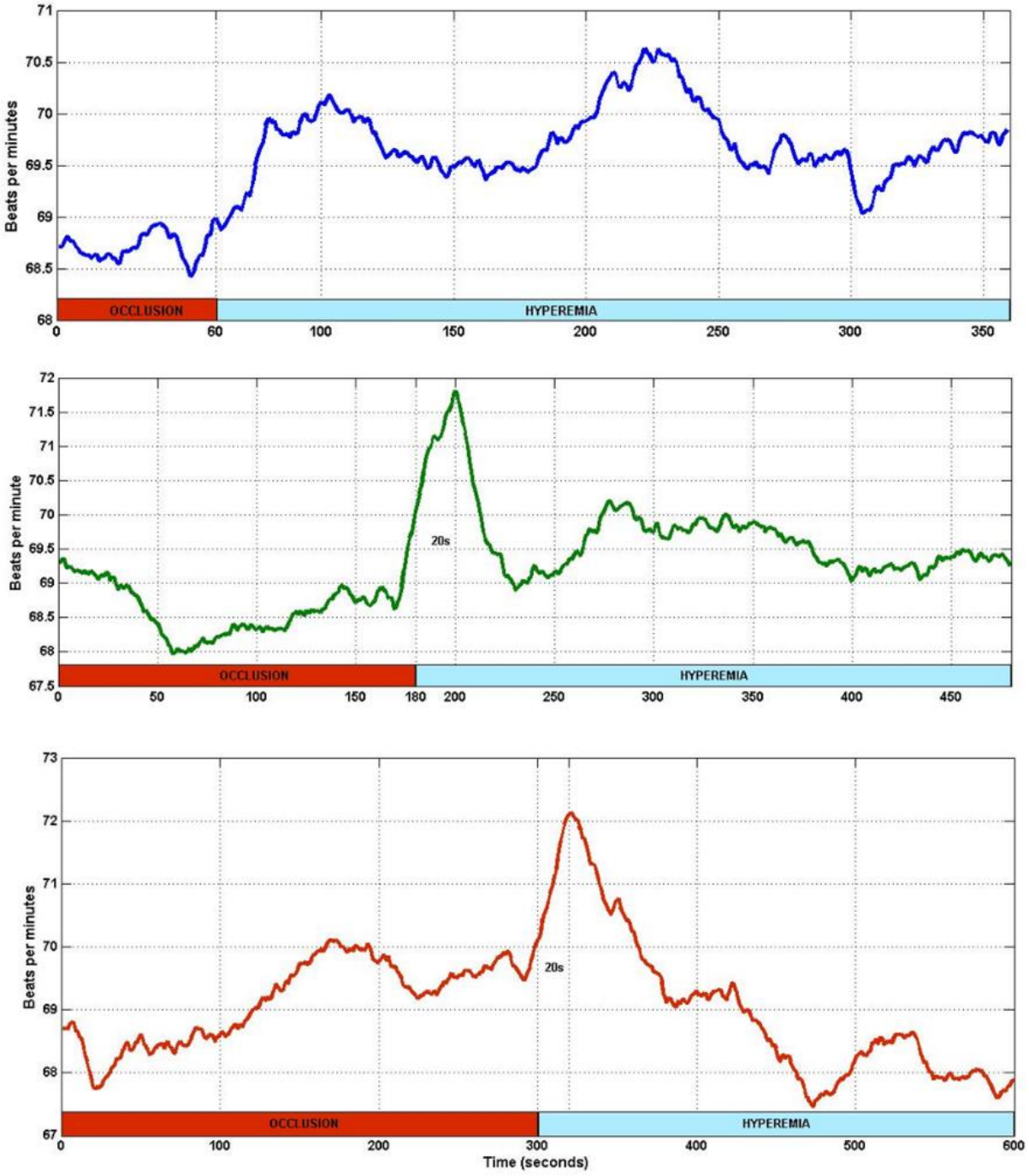

Fig. 1 Dynamic changes in heart rate during reactive hyperemia test.

Fig. 2 shows the temporal evolution of the sympathetic component of the cardiac autonomic regulation during the test of reactive hyperemia with different occlusion times. This maneuver produces an increase in the activity of the sympathetic nervous system (SNS), which reaches its maximum peak around eight seconds after cuff deflate independently of the occlusion time.

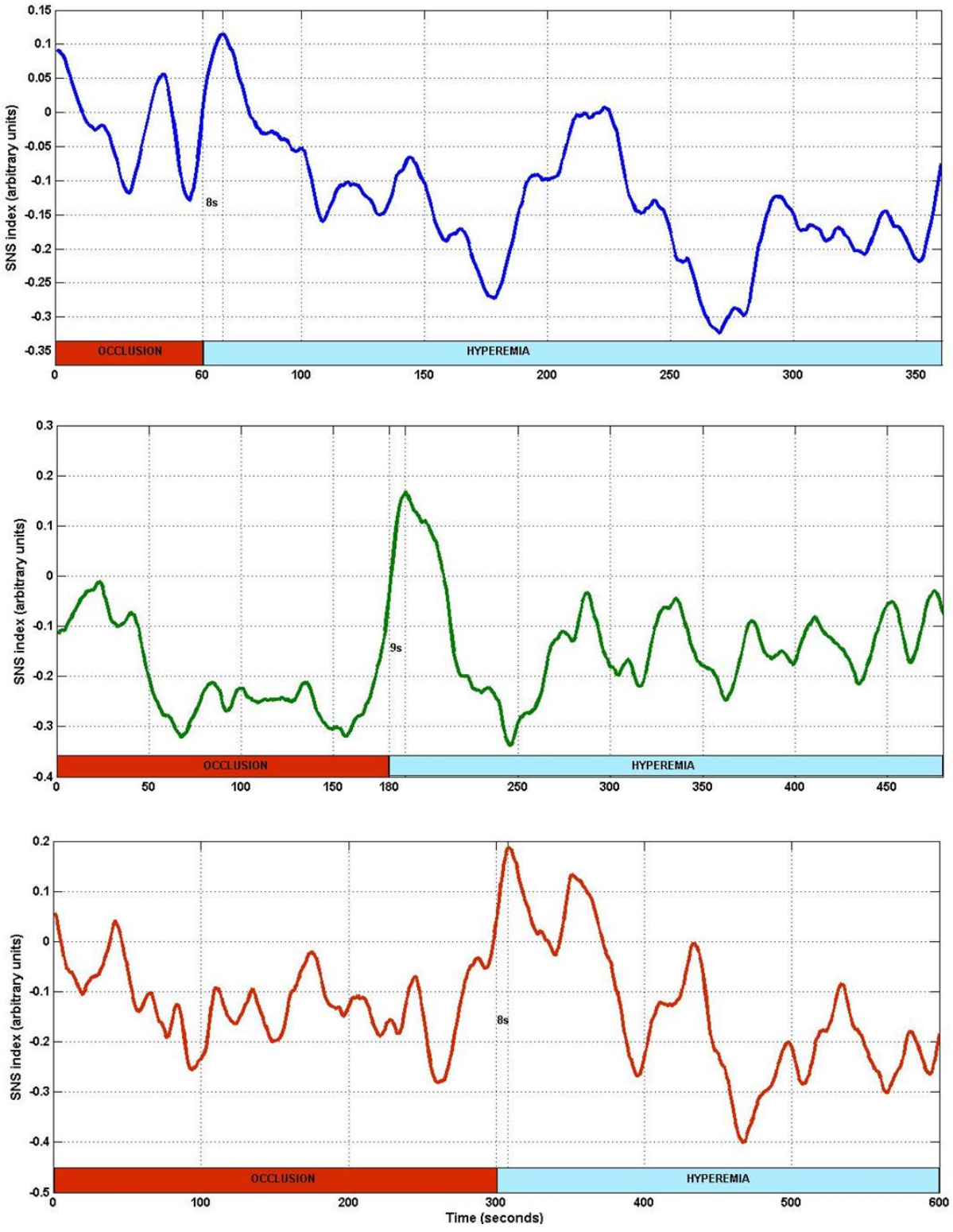

Fig. 2 Dynamics of the sympathetic component of the cardiovascular autonomic regulation measured by the SNS index during reactive hyperemia test.

Fig. 3 shows the temporal evolution of the parasympathetic component of the cardiac autonomic regulation during the test of reactive hyperemia at different times of occlusion, where it is evidenced how there is a decrease in parasympathetic nervous system (PNS) activity seconds after cuff deflate regardless of the occlusion time used.

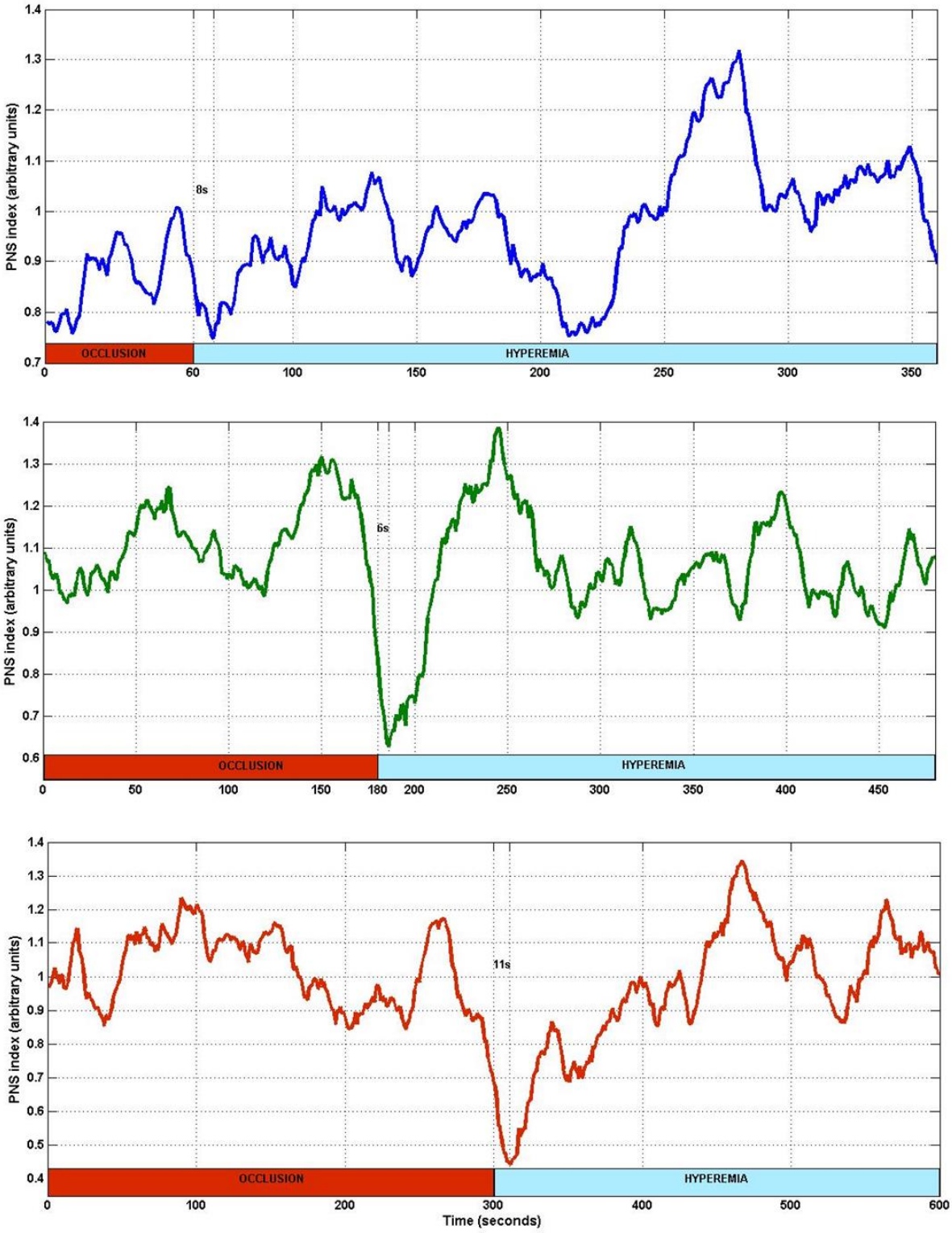

Fig. 3 Dynamics of the parasympathetic component of the cardiovascular autonomic regulation measured by the PNS index during reactive hyperemia test.

Fig. 4 shows the temporal evolution of the pulse transit time relative to PP intervals (PTT/PP) in contralateral arm during the test of reactive hyperemia at different times of occlusion, where

it is evidenced how there is a decrease in PTT/PP and then an increase regardless of the occlusion time used.

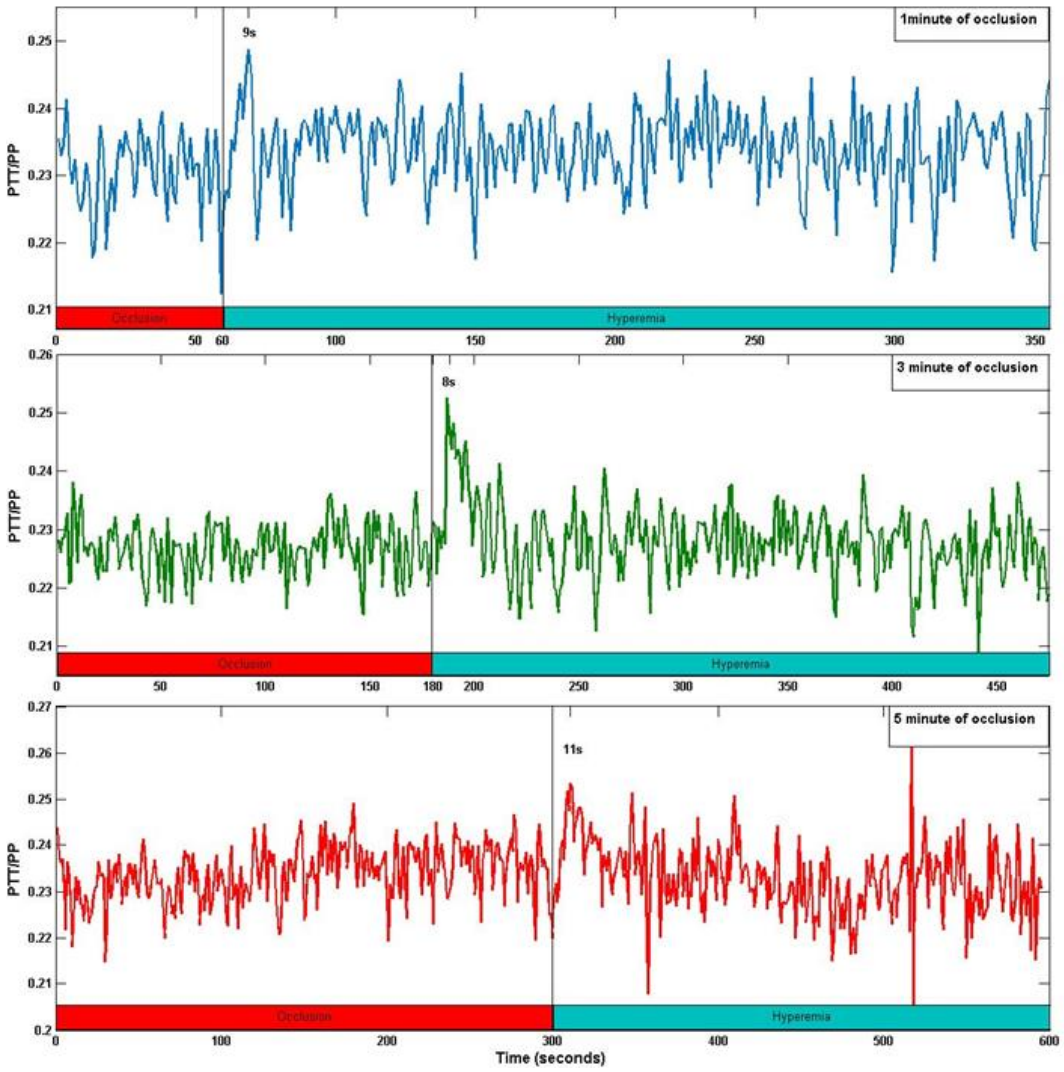

Fig. 4 Dynamics of the pulse transit time relative to PP intervals in contralateral arm.

Fig. 5 shows how there is a direct relationship between the time of occlusion and the magnitude of the hyperemic response, causing the greater the time of occlusion there is a greater in magnitude and the time of the vascular response measured with the PVA after cuff deflate.

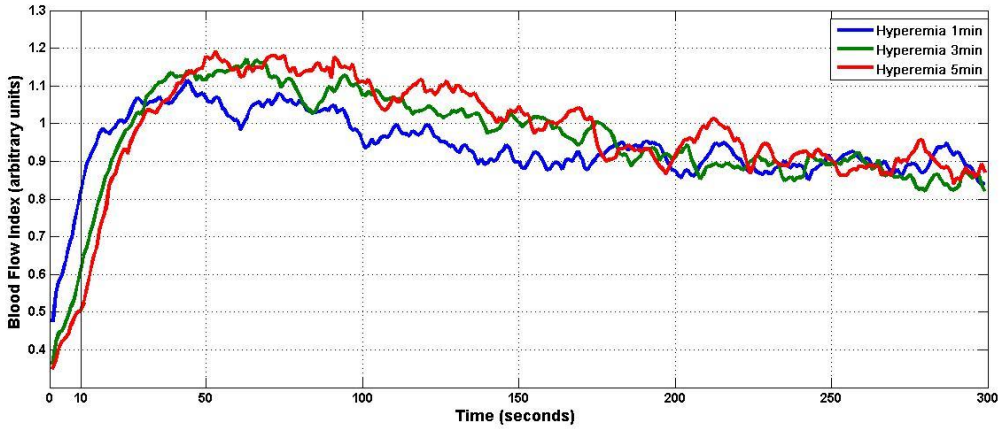

Fig. 5 Vascular response measured with photoplethysmographic finger pulse volume amplitude in the hyperemia with 1, 3 and 5 minutes of occlusion.

Discussion

At rest the predominance of the PNS activity guarantees that the HR is the possible minim to satisfy the metabolic requests of the organism. Therefore, in the first moments of the occlusion there is a predominance of the parasympathetic activity, which begins to diminish at the same time that an increment of the SNS tone happens, probably owed to metaboreflex response. This response is more evident right after the three and five minutes of occlusion, which suggests that one minute of occlusion is not enough to produce a specific activation from the sympathetic nervous system. Probably, in this period of time the concentration of different metabolite is not enough for triggering a support metaboreflex response[11].

In the present investigation, a marked increase has been observed in SNS activity immediately when the cuff was released, that reaches it maximum peak around the 8 s after the cuff is released. These findings are consistent with the results of Bade et al. (2019)[15] in which, there was an increase in the total and LF power of HRV during RH when compared to baseline. Moreover, with five minutes of occlusion the sympathetic response was bigger, probably for the higher concentration of freed metabolites for the ischemic tissues like adenosine. Boushel (2010)[22] concluded that adenosine does not activate the muscle metaboreflex, but it increases the sympathetic nerve activity with a latency corresponding to the circulatory time for activation of either central chemoreceptors.

When analyzing the evolution of the parasympathetic component it was observed a decrease of the activity of the PNS after the cuff is deflated. This effect, is reciprocated with an increment of the SNS activity, which agrees with the phenomenon of reciprocal inhibition between both systems [23]. Next, the parasympathetic nervous system activity was increased compensating the central mechanism and eliminating the vasoconstrictor effect of the sympathetic component

of the cardiovascular autonomic regulation. This, reduces the negative influence on the vascular endothelium of the SNS and endothelium increases it production and liberation of NO due to the shear stress. Hijmering et al. (2002)[12] found that the sympathetic stimulation could impair flow-mediated endothelial-dependent dilatation through a mechanisms caused by adrenergic receptors of type α and concluded that released transmitters from nerve endings are important modulators of endothelial function and myogenic response. Moreover, existed a direct relation between the time of occlusion and the magnitude of the hyperemic response. However, for longer time of occlusion the elevation latency of the bloodstream, is bigger and more prolonged in the time. This is due to the bigger period of vasoconstriction generated by the SNS activity s which slows down the increment of the bloodstream.

A significant reduction in the PVA in the control arm during RH has been observed, which is suggestive of sympathetically mediated vasoconstriction. However, appearance of vasoconstriction in the control arm as early as in the first minute of occlusion contradicts the hypothesis based on muscle metaboreflex and leaves the questions on the exact cause and origin of sympathetically mediated vasoconstriction [15]. In the present study, the effects of ANS activation are evidenced in the contralateral arm during the test of reactive hyperemia at different times of occlusion. It generates an initial vasoconstriction, evidenced by a decrease in PTT/PP followed by a vasodilation with an increase in PTT/PP at times regardless of the occlusion time used. In other study the Pulse Transit Time response reflects the myogenic components in the early part of RH [24]. All these changes are produced as a reflected activity to occlusion during reactive hyperemia test, which reinforces the hypothesis of the metaboreflex.

The analysis of the autonomic nervous system dynamics measured with time frequency methods of the HRV allows determining the temporary association of the different physiological events implicated during reactive hyperemia. It can suggest that the SNS activity takes part in the period of myogenic contraction that precedes the phase of vasodilatation. In addition, this is in close relation with the shear stress, increasing the power of the force of shearing of the blood on the vascular endothelium, twisting the endothelial cells and provoking the release of NO right after of the cuff occlusion ends. NO, along to different vasodilator metabolites liberated by the ischemic tissues it is responsible for the increment of the bloodstream that characterizes the reactive hyperemia.

Conclusion

Early cardiovascular sympathetic activation is independent of occlusion time during reactive hyperemia, suggesting this is a vascular autonomic reflex response that intervenes in the generation of the physiological phenomenon of reactive hyperemia.

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References

1. Leeson P, Thorne S, Donald A, Mullen M, Clarkson P, Deanfield J. Non-invasive measurement of endothelial function: effect on brachial artery dilatation of graded endothelial dependent and independent stimuli. Heart. 1997;78(1):22–7.

2. Jasperse JL, Shoemaker JK, Gray EJ, Clifford PS. Positional differences in reactive hyperemia provide insight into initial phase of exercise hyperemia. Journal of applied physiology. 2015;119(5):569–75.

3. Rosenberry R, Nelson MD. Reactive hyperemia: a review of methods, mechanisms, and considerations. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2020 Feb 5;318(3):R605–18.

4. Mohiaddin RH, Gatehouse PD, Moon JC, Youssuffidin M, Yang GZ, Firmin DN, et al. Assessment of reactive hyperaemia using real time zonal echo-planar flow imaging. Journal of cardiovascular magnetic resonance. 2002;4(2):283–7.

5. Sidhu JS, Newey VR, Nassiri DK, Kaski JC. A rapid and reproducible on line automated technique to determine endothelial function. Heart. 2002;88(3):289–92.

6. Reriani M, Sara JD, Flammer A, Gulati R, Li MJ, Rihal C, et al. Coronary endothelial function testing provides superior discrimination compared to standard clinical risk scoring in prediction of cardiovascular events. Coronary artery disease. 2016;27(3):213.

7. Lenkey Z, Illyés M, Böcskei R, Husznai R, Sárszegi Z, Meiszterics Z, et al. Comparison of arterial stiffness parameters in patients with coronary artery disease and diabetes mellitus using Arteriograph. Physiological research. 2014;63(4).

8. Zahedi E, Jaafar R, Ali MM, Mohamed AL, Maskon O. Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. Physiological measurement. 2008;29(5):625.

9. Onkelinx S, Cornelissen V, Goetschalckx K, Thomaes T, Verhamme P, Vanhees L. Reproducibility of different methods to measure the endothelial function. Vascular Medicine. 2012;17(2):79–84.

10. Kandhai-Ragunath JJ, Jørstad HT, de Man F, Peters RJG, Von Birgelen C. Approaches for non-invasive assessment of endothelial function: focus on peripheral arterial tonometry. Netherlands heart journal. 2013;21(5):214–8.

11. Tomiyama H, Yoshida M, Higashi Y, Takase B, Furumoto T, Kario K, et al. Autonomic nervous activation triggered during induction of reactive hyperemia exerts a greater influence on the measured reactive hyperemia index by peripheral arterial tonometry than on flow-mediated vasodilatation of the brachial artery in patients with hypertension. Hypertension Research. 2014;37(10):914–8.

12. Hijmering ML, Stroes ES, Olijhoek J, Hutten BA, Blankestijn PJ, Rabelink TJ. Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. Journal of the American College of Cardiology. 2002;39(4):683–8.

13. Padilla J, Young CN, Simmons GH, Deo SH, Newcomer SC, Sullivan JP, et al. Increased muscle sympathetic nerve activity acutely alters conduit artery shear rate patterns. American Journal of Physiology-Heart and Circulatory Physiology. 2010;298(4):H1128–35.

14. Yoshida M, Tomiyama H, Shiina K, Odaira M, Yamashina A. The difference of the influence of autonomic nervous activation caused by reactive hyperemia on two different endothelial function tests. European Heart Journal. 2013;34(suppl_1).

15. Bade G, Chandran DS, Jaryal AK, Talwar A, Deepak KK. Contribution of systemic vascular reactivity to variability in pulse volume amplitude response during reactive hyperemia. European journal of applied physiology. 2019;119(3):753–60.

16. Thijssen DH, Atkinson CL, Ono K, Sprung VS, Spence AL, Pugh CJ, et al. Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation. Journal of Applied Physiology. 2014;116(10):1300–7.

17. Spranger MD, Kaur J, Sala-Mercado JA, Machado TM, Krishnan AC, Alvarez A, et al. Attenuated muscle metaboreflex-induced pressor response during postexercise muscle ischemia in renovascular hypertension. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2015;308(7):R650–8.

18. Cuadra Sanz M, Esteban R, Delisle Rodriguez D, Mantaras MC, Perrone M, Fainstein D, et al. SISTEMA PARA EL REGISTRO SIMULTÁNEO DE LA ONDA DE PULSO Y EL ELECTROCARDIOGRAMA ORIENTADO AL ESTUDIO DE LA REGULACIÓN AUTONÓMICA. 4th Int In Conf FIE'08 [Internet]. 2008 Jan 1; Available from: https://www.researchgate.net/publication/259557317_SISTEMA_PARA_EL_REGISTRO_SIMULTANEO_DE_LA_ONDA_DE_PULSO_Y_EL_ELECTROCARDIOGRAMA_ORIENTADO_AL_ESTUDIO_DE_LA_REGULACION_AUTONOMICA

19. Manikandan MS, Soman KP. A novel method for detecting R-peaks in electrocardiogram (ECG) signal. Biomedical Signal Processing and Control. 2012 Mar 1;7(2):118–28.

20. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. American Journal of Physiology-Heart and Circulatory Physiology. 2011;300(1):H2–12.

21. Carrazana-Escalona R, Ricardo-Ferro BT, Fernández-de la Vara Prieto R, Sánchez-Hechavarría ME. Algoritmo para la detección de puntos clínicos de interés de la onda de pulso arterial. Revista Cubana de Informática Médica. 2018;10(1):40–8.

22. Boushel R. Muscle metaboreflex control of the circulation during exercise. Acta physiologica. 2010;199(4):367–83.

23. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Frontiers in psychology. 2014;5:1040.

24. Selvaraj N, Jaryal AK, Santhosh J, Anand S, Deepak KK. Monitoring of reactive hyperemia using photoplethysmographic pulse amplitude and transit time. Journal of clinical monitoring and computing. 2009;23(5):315–22.