

Heart Rate Variability Indices of Student Pilots Undergo Modifications During Flight Training

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- INTRODUCTION:** Heart rate variability (HRV) indicates the temporal fluctuation of the intervals between adjacent beats. HRV expresses neuro-cardiac activity and is generated by heart-brain interactions and dynamics related to the function of the autonomous nervous system and other components. To investigate this issue, we started a series of experiments by coupling the flight tasks of student pilots and their HRV.
- METHODS:** Before each experimental session, the participating student was fitted with a five-electrode, three-channel Holter electrocardiogram monitor. We defined three time-phases for each training mission: before flight operations on the ground, during flight operations, and after flight operations on the ground. The HRV analysis was performed by quantifying some indices of the time domain and the frequency domain.
- RESULTS:** The analysis of RR-wave intervals revealed two types of trends: 1) students whose RR intervals decreased during flight operations compared to before flight ground operations; and 2) students whose RR intervals increased during flight operations compared to before flight ground operations. These differences found in the RR intervals produced changes in the indices of both the time and frequency domains of the two students' samples.
- DISCUSSION:** Flight training involves regular and advanced tasks and/or emergency situations. When this happens, the total power of the heart decreases because the RR intervals are forced toward low values. Flight activity involves continuous demanding tasks that can be potentially read by an analysis of the HRV; a high HRV ensures better management of tasks that require a greater commitment of cardiovascular function.
- KEYWORDS:** cardiac function, heart rate variability, flight training, eustress.

Li Volsi G, Monte IP, Aruta A, Gulizzi A, Libra A, Mirulla S, Panebianco G, Patti G, Quattrocchi F, Bellantone V, Castorina W, Arcifa S, Papale F. Heart rate variability indices of student pilots undergo modifications during flight training. *Aerosp Med Hum Perform.* 2023; 94(11):835–842.

Although the heart rate is an important parameter for understanding myocardial activity levels, it is not enough for an understanding of the mechanisms underlying its modulation. For this purpose, it is much more useful to evaluate the instantaneous variation of the intervals between two consecutive R waves (RR interval) of the electrocardiogram. Therefore, the study of heart rate variability (HRV) can provide more detailed information on the modulation exerted by the autonomic nervous system (ANS) in cases where an organism leaves its resting conditions to devote itself to changing psychophysical activities. Within certain limits, this fluctuation is not a malfunction, but rather a physiological behavior given the conditions of dynamic equilibrium that distinguish the function of complex biological systems.

The HRV is therefore the result of interdependent regulatory systems that operate at different time scales on the heart. It

reflects a balanced action influenced by the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), as well as blood pressure, vascular tone, gas exchange, the intestine, and perhaps even the facial muscles.^{6,9}

A stress condition implies a long-term detrimental imbalance between an individual's resources and environmental demands. From a physiological point of view, a stressful situation triggers a cascade of hormones linked to a particular

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This manuscript was received for review in May 2023. It was accepted for publication in August 2023.

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DOI: <https://doi.org/10.3357/AMHP.6305.2023>

condition, as well as an acceleration of the ANS which gives rise to muscle tension and an increase in performance.²⁷ Subsequently, the concept of stress has been deepened and now the scientific community prefers to speak in terms referring to the framework of the stress system. Stress is now defined as a state of homeostasis that is challenged, which includes both systemic stress and local stress. A specific stressor can elicit a specific local stress response, while stress intensity beyond a certain threshold can activate the hypothalamic-pituitary-adrenal axis and elicit a systemic stress response, also influenced by the amount of cortisol released by the adrenal cortex.¹⁸

The stress system includes three types: *sustress* (inadequate stress), *eustress* (good stress), and *distress* (bad stress). Both *sustress* and *distress* could impair normal physiological functions and even lead to pathological conditions, while *eustress* can benefit health through hormesis-induced optimization of homeostasis. Therefore, an optimal stress level is essential to build biological shields to ensure normal life processes. In other words, people may stress themselves unnecessarily, too much, or optimally. Stress is a form of homeostasis that is mildly challenged by moderate levels of stressors, which may induce a mild stress response, improve the buffering capacity of homeostasis, and benefit overall health.¹⁷

HRV can be used as a stress indicator during a sport activity,⁷ to assess learning in the classroom,⁸ or during a flying activity, even if only simulated.⁵ The success of a learning process that occurs under conditions of extreme individual variability will depend on several concomitant factors. In pilot training, the learning process can be optimized if a flight instructor has information acquired based on the analysis of some physiological parameters of the student pilot (student). In this context, our Aviation Biophysics and Medicine Research Unit is carrying out a series of experimental procedures with the aim of quantifying the variations of some HRV indices in students who are applying to obtain a private pilot license, as well as in pilots who already hold such a license and are continuing their studies in order to acquire a commercial pilot license. Our preliminary results have been promising.^{15,16} With information gained from these experimental sessions, we have made changes to the training protocol and further deepened the investigations reported above. This will allow us to obtain a sample of students as homogeneous as possible in relation to operations before, during, and after each training flight mission.

METHODS

Since the experimental protocol had to be applied to students during the flight mission, it was submitted and subsequently approved by the National Civil Aviation Authority (Aeromedical Section) of the Italian Republic.

Subjects

Participation in this project by students of both sexes took place on a voluntary basis with a guarantee of confidentiality for the purposes of processing personal data. Each subject signed the

informed consent document, in accordance with the provisions of the first paragraph of Article 1 of Law 219/2017 of the Italian Republic.

Procedure

Before the experimental session and immediately after arrival, the student was equipped with five noninvasive electrodes, positioned in areas of the thoracic skin, and connected to a three-channel Holter (ECG Biomedical, BI9100) that recorded data until completion of the experimental session. The flight track (altitude, speed, track, and time) of the aircraft, obtained by using Viaair Hardware and Software²⁶ for the entire duration of the flight, allowed us to correlate the times at which the flight activities scheduled by the instructors took place with the Holter recordings. The training aircraft used was the Tecnam P2002JF.²³

The training mission consisted of three phases: a ground phase that included a briefing with the flight instructor and an aircraft inspection [before ground flight operations (bfo)], an in-flight phase in which the student was asked to perform tasks scheduled by the instructor [in-flight operations (dfo)], and a ground debriefing that was scheduled at the end of the mission [postflight ground operations (afo)].

The tasks for the bfo phase were as follows: position Holter electrodes and turn Holter on; obtain weather forecast; perform mass and balance calculations; inspect airplane; briefing with flight instructor; boarding; engine starting and after-starting procedures; taxiing and aerodrome procedures; and air traffic control compliance and radio/telecommunication procedures. The tasks for the dfo phase were as follows: air traffic control compliance and radio/telecommunication procedures; straight and level flight, with speed changes, climbing (best rate of climb, climbing turns, leveling off), navigation, and maneuvers; any emergency procedures; descending; aerodrome arrival procedures; approach to landing; and air traffic control compliance and radio/telecommunication procedures. The tasks for the afo phase were as follows: engine shut down; airplane inspection; debriefing with flight instructor; and removing electrodes and turning of the Holter off.

Statistical Analysis

The HRV was quantified according to the guidelines of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).²² We assessed the HRV data based on the time domain and frequency domain analysis.⁹

In the time domain, we quantified the mean of all RR-wave intervals and their averages before, during, and after various tasks. In the frequency domain, we quantified total power (TP) that mainly reflected the level of the autonomic nervous activities (parasympathetic and sympathetic nervous system) and high-frequency normalized units (HF, nu) versus low-frequency normalized units (LF, nu) to emphasize the balance between the sympathetic and parasympathetic arms of the ANS.

With the time domain, we computed the mean of the RR intervals (RR-int, ms), the standard deviation of all the mean normal-to-normal (NN) intervals (SDNN, ms), the square root of the mean of the sum of squares of the differences between adjacent intervals (RMSSD, ms), and the percentage of adjacent NN intervals that differed from each other by more than 50 ms (pNN50). In the frequency domain, we computed the total power (TP, ms^2), very low frequency (VLF, ms^2), low frequency (LF, ms^2), high frequency (HF, ms^2), LF normalized units and HF normalized units, and LF/HF ratio.

RESULTS

The data collected refer to a sample of 14 students of both sexes (12 men and 2 women). We report averages (\pm SD) of anthropometric-related factors of the subjects: age 21.64 yr (\pm 3.13), height 1.72 m (\pm 0.06), weight 70.93 kg (\pm 9.57), and BMI 26.93 (\pm 2.53). As described in Methods, we used time-domain indices to evaluate the modulation exerted by ANS, blood pressure, vascular tone, gas exchange, and hypothalamic-pituitary-adrenal axis.¹⁸ Specifically, SDNN index is correlated with ANS activities and hormonal influences¹²; RMSSD index is a marker of parasympathetic modulation regulation of the heart;²¹ and pNN50 is closely correlated with PNS activity.²⁴ Regarding the frequency domain, similarly to what happens in the electroencephalogram, we used this parameter to separate HRV into its VLF, LF, HF, LF, and HF normalized units, and LF/HF ratio components to describe the power spectrum density distribution.²⁰ The indices just described allowed us to quantify the contribution of the ANS into its sympathetic and parasympathetic components, as well as the component dependent on baroreceptor activity. An initial analysis of RR intervals revealed two types of trends: 1) nine students whose RR intervals decreased dfo compared to bfo (D-students), and 2) five students whose RR intervals increased dfo compared to bfo (I-students).

Fig. 1 (histograms A–D) depicts time-domain analyses of the two samples of students. Averages of RR intervals, SDNNs, RMSSDs, and pNN50s of both D-students (white columns) and I-students (gray columns) are shown, with dfo compared to bfo. In both samples, afo values are also reported to determine recovery. Panel A shows RR interval averages of D-students (dfo = 597.59 ms \pm 35.36 compared to bfo = 675.00 ms \pm 75.28) and I-students (dfo = 599.69 ms \pm 33.85 compared to bfo = 551.63 ms \pm 44.66). Panel B shows SDNN averages of D-students (dfo = 37.34 ms \pm 35.36 compared to bfo = 52.65 ms \pm 75.28) and I-students (dfo = 41.58 ms \pm 10.38 compared to bfo = 42.48 ms \pm 16.11). Panel C shows RMSSDs of D-students (dfo = 25.56 ms \pm 09.00 compared to bfo = 34.36 ms \pm 15.51) and I-students (dfo = 28.56 ms \pm 10.12 compared to bfo = 28.75 ms \pm 13.91). Panel D shows pNN50s of D-students (dfo = 04.11 ms \pm 03.99 compared to bfo = 07.86 ms \pm 05.98) and I-students (dfo = 08.05 ms \pm 04.12 compared to bfo = 06.60 ms \pm 06.02). Panel E shows percentage changes of RR intervals, SDNNs, RMSSDs, and pNN50s dfo in the two samples (D-students and I-students).

Fig. 2 depicts the frequency-domain analyses of the two samples of students. The left column histograms (A–D) show data of D-students dfo compared to bfo. Afo average values are also reported for recovery estimation. Panel A is box and whisker plots of total power averages; a decrease of 46.56% was observed dfo (969 $\text{ms}^2 \pm$ 540.74) compared to bfo (1814.19 $\text{ms}^2 \pm$ 1064.52). Panel B is a histogram of VLF, LF, and HF averages showing a decrease of all frequency values dfo. Panel C's histogram depicts data reported in Histogram B, calculated as a percentage to compare the magnitude of each frequency. As can be seen in the bfo column, the largest component is represented by LFs (55.13%), followed by VLFs (28.14%) and HFs (16.73%). All frequency bands dfo undergo a decrease that is more marked for HFs [7.99% (-52.25%)], followed by VLFs [14.95% (-46.88%)] and LFs [31.39% (-43.05%)]. In Panel D, in order to magnify the balance between sympathetic and parasympathetic arms of the ANS, we computed both LFs and HFs as normalized units. Values are reported as insets.

The right column histograms (E–H) show data of I-students dfo compared to bfo. Afo averages are also reported for recovery estimation. In Panel E, box and whisker plots show total power averages; an increase of 8.30% was observed dfo (1272.04 $\text{ms}^2 \pm$ 555.27) compared to bfo (1174.60 $\text{ms}^2 \pm$ 791.09). In Panel F, histograms of VLF, LF, and HF averages show a more complex trend: an increase of LFs and HFs, and a decrease of VLFs. In Panel G, the histogram depicts data reported in Histogram F, calculated as a percentage to compare the magnitude of each frequency. As can be seen in the bfo column, the largest component is represented by LFs (34.26%), followed by VLFs (16.90%) and HFs (13.58%). VLFs only undergo a decrease [14.37% (-14.98%)] dfo, while an increase was observed for HFs [16.76% (+23.40%)] and LFs [34.26% (+13.79%)]. In Panel H, in order to magnify the balance between sympathetic and parasympathetic arms of the ANS, we computed both LFs and HFs as normalized units. Values are reported as insets. The histogram at the bottom left summarizes data related to the ratio of LFs to HFs average values related to both D-students and I-students. Finally, the histogram at the bottom right contains the same data related to LFs/HFs but calculated as percent variation dfo. Note the increasing trend of the ratio in D-student dfo.

DISCUSSION

It is well known that the heart is an electromechanical pump that propels oxygenated blood in proportion to demand and receives deoxygenated blood from the whole body,¹¹ and that this activity depends upon the myocytes of the heart (Frank-Starling Law). The heart's function can be influenced by age, lifestyle, physical activity, lack of exercise, metabolic disorders, nutritional status, physiological changes, pathological conditions, and so on.⁴ The heart works actively, constantly, and is influenced by areas of the brain, both directly and indirectly.²⁵ During the first studies of this topic, Hans Selye¹⁹ defined stress as the nonspecific response of the body to any request for change.

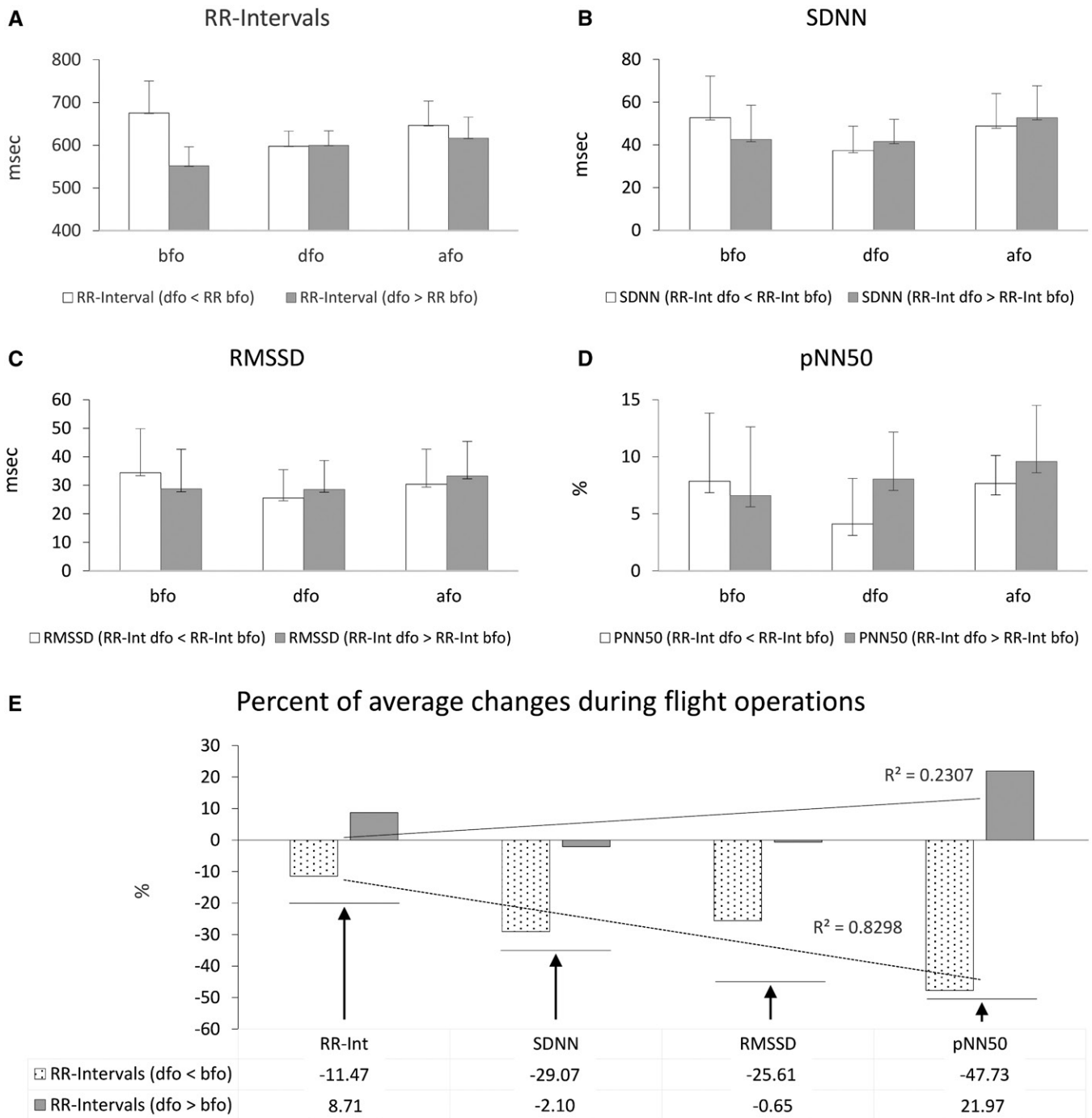


Fig. 1. Time-domain analysis (average indices). White columns refer to D-students; gray columns refer to I-students. A) RR intervals; B) SDNNs; C) RMSSDs; D) pNN50s; E) percentages of RR intervals, SDNNs, RMSSDs, and pNN50 during flight operations of both D-students and I-students computed as percent of changes. Error bars: SD.

HRV is a commonly used indicator of ANS activity that is strictly linked to alert/stress conditions and stress scenarios. In our case, the quantification of its indices (time domain and frequency domain) has given us the opportunity to gain a deeper understanding of some aspects concerning the flight training of students at their first approach to an airplane. In this regard, we report that all the student pilots participating in the research had never had any flight experience before, had been informed

in advance about the aims of the research, and were advised that participation was on a voluntary basis covered by privacy. In particular, this study has also allowed us to extrapolate information about a) the human-machine relationship and b) any changes that could improve the flight training process.

A learning process, whether theoretical or practical, presupposes a commitment of various components of the nervous system, as well as other organs and systems, that can achieve

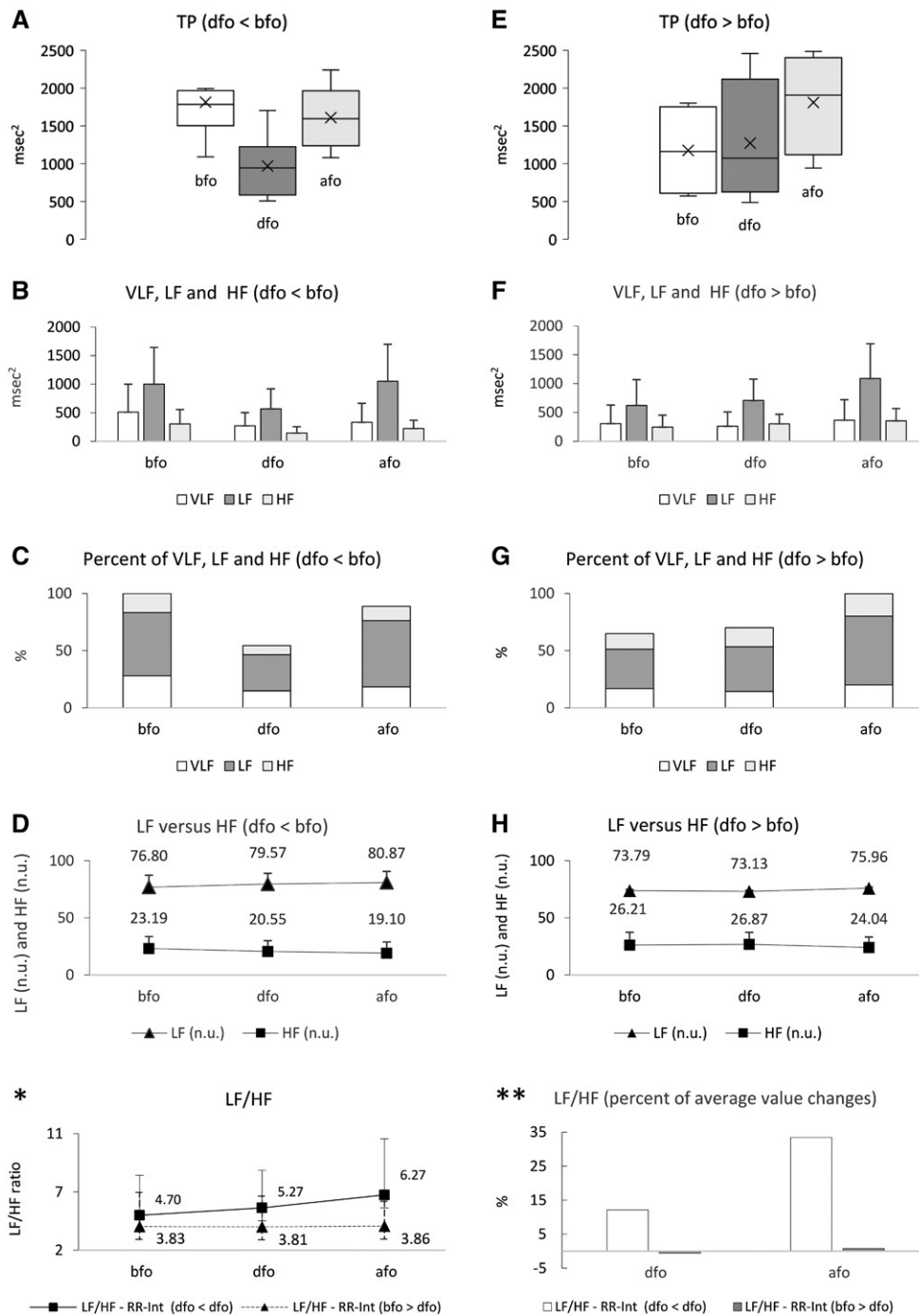


Fig. 2. Frequency-domain analysis (average indices). Left column (histograms from A–D): data related to D-students. A) Box and whisker plots of total power averages. White box bfo, heavy gray box dfo, light gray box afo. B) Histogram of VLF (white columns), LF (heavy gray columns), and HF (light gray columns) bfo, dfo, and afo. C) Same data reported in B but calculated as a percentage to compare the magnitude of each frequency. D) LF versus HF normalized units. Values are reported as insets. Right column (histograms from E to H): data related to I-students, dfo compared to bfo. E) Box and whisker plots of total power averages. White box bfo, heavy gray box dfo, light gray box afo. F) Histogram of VLF (white columns), LF (heavy gray columns), and HF (light gray columns) bfo, dfo and afo. G) Same data reported in F but calculated as a percentage to compare the magnitude of each frequency. H) LF versus HF normalized units. Values are reported as insets. *Ratio of LFs to HFs averages. Black squares show LFs/HFs ratio of D-students, whereas black triangles show LFs/HFs ratio of I-students. **LFs/HFs of averages changes dfo and afo compared to bfo. White columns report LFs/HFs ratio of D-students, while gray columns show LFs/HFs ratio of I-students. Error bars: SD.

the final goal: learning a concept, scenario, sound, etc., or an action/task. Leaving aside the concepts of flight theory, and assuming that the students have already acquired the necessary knowledge, we will deal with what is needed to fly an airplane.

Familiarization with the airplane and its flight controls is an essential prerequisite. Therefore, all motor acts foreseen for a given maneuver (e.g., a takeoff, a climbing turn, or a slow flight) must be acquired, planned, implemented, verified, corrected,

and improved on the basis of what was learnt during the classroom lessons. All this will take place in a mental context originating from the character and personality of the student and his/her attraction for flight. Finally, it is important to note that flight operations take place in a three-dimensional environment in which weather conditions play an important role.

As mentioned above, HRV can be assessed with various analytical approaches, although the most commonly used are frequency domain, power spectral density, and time-domain analysis. Time-domain indices of HRV quantify the variability of the interbeat interval, which is the time period between successive heart beats,²⁰ hence the first indications, according to which some students face alert conditions dfo, while others are already alerted in the preliminary phases bfo. This alert/stress condition would be expressed by the decrease of the RR interval values.

The histogram shown in Fig. 1E compares the trend of the indices relating to the RR intervals, SDNNs, RMSSDs, and pNN50s in the two samples of students, and the columns indicate the percentage variation of the indices. The literature on this topic^{20,21} indicates that the SDNN index refers to the activity of the ANS as a whole, while RMSSD and pNN50 indices are mainly correlated with the parasympathetic branch of the ANS. One can see that the magnitude of the indices is more marked in D-students dfo (see R² values in both cases). The maximum variation is reached by the pNN50 index, whose sharp decrease expresses a consistent decrease in HRV. In the opposite case with I-students, the recovery is more moderate. The data suggest an unbalanced management of HRV, which can be interpreted as a condition in which PNS and SNS would have different specific weights.

Table I shows the numerical data relating to the graphs of Fig. 1 (A, B, C, D). Note that the values of the RR intervals of both samples of students dfo are almost identical (values in bold with an asterisk), while they are quite different bfo and afo. Indeed, they are greater in one case and less in the other. This leads us to assume that HRV has its own standard dfo, i.e., that an already evident alert status condition bfo expresses lower HRV values than dfo, while higher HRV conditions bfo express a higher alert status (HRV decrease) dfo. Now, we can imagine the RR intervals dfo values of both samples of students as expressing eustress, just as the values found afo (>dfo) indicate an increase in HRV. Furthermore, data depicted in Histogram E of Fig. 1 supports the idea that HRV is quantitatively more controlled by the PNS than that exercised by the SNS, since the percentage variations of the different indices in the two samples are not superimposable. Finally, the values of

the RR intervals, in one case higher than those found dfo, and in the other case lower than those found dfo, indicate that some students exhibit normal operating conditions in the bfo, while others could already be in conditions of eustress caused by situations not included in the bfo and/or caused by the bfo.

Frequency-domain indices estimate the power distribution as a function of frequency bands.^{5,20} Box and whisker plots A and E of Fig. 2 depict the total power (TP) of the two samples of students; note that TP averages of D-students bfo (1814.19 ms²) is almost equal to that of I-students afo (1809.95 ms²). The data observed on TP in both samples of students lead to a preliminary conclusion. It is possible to identify a common (and maximum) mean value of TP bfo in D-students. Conversely, it is possible to observe the same value afo in I-students dfo. In this case, a greater size of the boxes is also observed, reflecting a greater dispersion (variability) of the TP, perhaps generated by a sustress or distress that starts to become eustress. More generally, we could imagine a system stress, as described by Lu.¹⁷

Histograms C and G of the same figure depict, in both samples of students, the contribution of VLFs, LFs, and HF bands, as it is known, operate within different frequency ranges. A datum common in both cases is the conspicuous presence of LFs bfo, dfo, and afo. Considering data of D-students dfo, the three frequency bands also decreased dfo, to recover afo, even if not entirely (Histogram C). Conversely, in I-students, LFs and HF bands increased, while VLFs did not. Finally, the recovery was total afo (Histogram G). The literature in this regard^{13,20,22} associates the aforementioned frequency bands with various components that refer to the effects exerted by branches of the ANS, the aortic and carotid baroreceptors, the pressure regulated by baroreceptive reflexes, and reflexes related to the respiratory cycle.

As mentioned above, in both samples of students there is a conspicuous presence of LFs (0.04–0.15 Hz) bfo, dfo, and afo. LF power can be produced by both SNS and PNS^{3,14,22} as well as blood pressure regulation via baroreceptors,¹⁰ primarily by the PNS. LFs decrease in D-students dfo.

Bearing in mind that the HF bands or respiratory band (0.15–0.4 Hz) is primarily produced by the PNS and is highly correlated with the pNN50 and RMSSD indices,¹³ it is reasonable to observe its almost halving in the sample of D-students dfo. In other words, the HRV would be proportional to the parasympathetic input. For this reason, it would be reasonable to observe an increase, albeit modest, of HF bands in I-students. The trend of the VLF band (0.0033–0.04 Hz) was more complex. The intracardiac nervous system is involved in the genesis of the VLF rhythm, and, furthermore, the SNS influences the amplitude and frequency of its oscillations.²¹ In addition to

Table I. RR Intervals, SDNN, RMSSD, and pNN50 Averages.

TIME DOMAIN	LOWER RR INTERVALS (dfo COMPARED TO bfo)			HIGHER RR INTERVALS (dfo COMPARED TO bfo)		
	bfo	dfo	afo	bfo	dfo	afo
RR-Int	675.00	597.59*	646.15	551.63	599.69*	616.50
SDNN	52.65	37.34	48.78	42.48	41.58	52.70
RMSSD	34.36	25.56	30.37	28.75	28.56	33.29
pNN50	7.86	4.11	7.66	6.60	8.05	9.59

*The values of the RR intervals of both samples of students dfo are almost identical.

this are physical² and thermoregulatory activity, the renin-angiotensin system, and the influences of the endothelium on the heart.¹ As reported by Shaffer and Ginsberg,²⁰ experimental evidence suggests that the heart intrinsically generates the VLF rhythm and efferent SNS activity due to physical activity; a stress response modulates its amplitude and frequency. In both samples of students, VLFs values decrease dfo, although this drop is barely noticeable in I-students. They recover 99.7% of TP (compared to the 100% of the TP quantified in the other sample) afo and with slightly more difference in HFs and LFs, but less in VLFs. Therefore, we can imagine that the ANS acts on the HRV continuously with a wide-range dynamic balance. The hearts of D-students are less able to be modulated in dfo. The balance is dynamic because it would not be a simple scale, but instead a variable one in which the equilibrium point has moment arms of different lengths, together with the application of different weights (see Fig. 1E indices). This happens in other biological fields, for example: genesis of the membrane potential of cells as the result of the unequal ion distribution between the inner and outer layers of their membranes.

In conclusion, as mentioned above,¹⁶ the amount of HRV is an energy store for better cardiac performance in eustress activities. Flight training engages regular and advanced tasks and/or emergency situations. When this happens, the total power of the heart decreases because the RR intervals are forced toward low values; this would cause a decrease in the capability of being modulated by the numerous controlling mechanisms. Flight activity involves continuous demanding tasks that can be potentially read by analysis of the HRV; a high HRV ensures better management of tasks that require a greater commitment of the cardiovascular function. Furthermore, this experimental protocol can also be useful to flight instructors for the purpose of revision during the training protocols of our students.

ACKNOWLEDGMENTS

We are grateful to Professor Emeritus David Tracey and to Doctor Leslie Bentz for linguistic revision of the article, respectively, before and after revision. We wish to thank all the staff of the Aero Club/IT.ATO.0043 for the valuable contributions they make in both administrative and technical activities of our organization.

Financial Disclosure Statement: The authors have no competing interests to declare.

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