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Spectral Analysis of Heart Rate Variability in Student Pilots During Flight Training

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Summary

Spectral analysis techniques are employed to evaluate electrocardiographic signals. The high-frequency (HF) and low-frequency (LF) components of the tachogram reflect autonomic nervous system modulation of heart rate variability (HRV). This study investigated the correlations between successive heartbeats (RR intervals) and these spectral bands to further elucidate autonomic regulation.

Student pilots were monitored with Holter electrocardiograms during training missions. Data were analyzed across three phases: 1) before flight operations on ground (bfo), 2) during flight operations (dfo), and 3) after flight operations on ground.

RR interval analysis identified three trends during dfo compared to bfo: a decrease (45,1% of students), no change (15,7%), and an increase (39,2%). These patterns were associated with alterations in the correlation indices for LF and HF power. Students demonstrating superior performance (D-Students) exhibited the strongest positive correlation coefficient between RR intervals and LF/HF band power (+58,3%). A similar trend was observed for the correlation between LF and HF power in this group (+2,9%).

The findings suggest that a demand for increased cardiac output, typified by decreased RR intervals, is primarily driven by sympathetic activation. A different autonomic state is observed when the heart is already engaged at the onset of a performance challenge. Notably, some individuals establish a new autonomic balance at the start of a demanding task, characterized by increased total spectral power and elevated HRV, indicative of an optimized autonomic state under high-load conditions.

Keywords: Heart rate variability; Spectral analysis; Autonomic nervous system; LF/HF correlation; Flight training; Student pilots.

Analisi Spettrale della Variabilità della Frequenza Cardiaca in Allievi Piloti Durante l'Addestramento all'Attività di Volo

Riassunto

Le tecniche di analisi spettrale vengono impiegate per valutare i segnali elettrocardiografici. Le componenti ad alta frequenza (HF) e a bassa frequenza (LF) del tacogramma riflettono la modulazione della variabilità della frequenza cardiaca (HRV) da parte del sistema nervoso autonomo. Questo studio ha indagato le correlazioni tra battiti cardiaci successivi (intervalli RR) e queste bande spettrali per chiarire ulteriormente la regolazione autonomica.

Gli allievi piloti sono stati monitorati con elettrocardiogrammi Holter durante le missioni di addestramento. I dati sono stati analizzati in tre fasi: 1) prima delle operazioni di volo a terra (bfo), 2) durante le operazioni di volo (dfo) e 3) dopo le operazioni di volo a terra.

L'analisi degli intervalli RR ha identificato tre tendenze durante la fase dfo rispetto alla fase bfo: una diminuzione (45,1% degli allievi), nessuna variazione (15,7%) e un aumento (39,2%). Queste tendenze sono state associate ad alterazioni negli indici di correlazione per la potenza LF e HF. Gli studenti che hanno dimostrato prestazioni superiori (studenti D) hanno mostrato il coefficiente di correlazione positivo più elevato tra intervalli RR e potenza delle bande LF/HF (+58,3%). Un trend simile è stato osservato per la correlazione tra potenza LF e HF in questo gruppo (+2,9%).
I risultati suggeriscono che la richiesta di una maggiore gittata cardiaca, caratterizzata da intervalli RR ridotti, è principalmente guidata dall'attivazione simpatica. Uno stato autonomico diverso si osserva quando il cuore è già impegnato all'inizio di una sfida prestazionale. In particolare, alcuni individui stabiliscono un nuovo equilibrio autonomico all'inizio di un compito impegnativo, caratterizzato da un aumento della potenza spettrale totale e da un'elevata variabilità della frequenza cardiaca (HRV), indicativa di uno stato autonomico ottimizzato in condizioni di carico elevato.

Parole chiave: Variabilità della frequenza cardiaca; Analisi spettrale; Sistema nervoso autonomo; Correlazione LF/HF; Addestramento al volo; Allievi piloti.

1 Introduction

The clinical relevance of Heart Rate Variability (HRV) was first established in 1965 when Hon and Lee (Hon and Lee, 1965; Pam *et al.*, 2021) observed that fetal distress was preceded by alterations in inter-beat intervals prior to any appreciable change in heart rate itself. Subsequently, Sayers and others brought focus to the physiological rhythms inherent within the beat-by-beat heart rate signal (Heart rate variability for risk stratification of life-threatening arrhythmias. American College of Cardiology Cardiovascular Technology Assessment Committee, 1993; Hirsh and Bishop, 1981).

Heart rate (HR), quantified as the number of heart beats per minute (bpm), provides a direct measure of cardiac rhythm. While HR varies in response to physical activity, emotional state, and other external influences, it remains an insufficient parameter for elucidating the underlying mechanisms of its modulation. In contrast, HRV reflects the temporal variation between consecutive heartbeats. It is measured by analyzing fluctuations in successive beat intervals (RR intervals), thereby indicating the capacity of the Autonomic Nervous System (ANS) to regulate cardiac rhythm. Higher HRV generally suggests greater nervous system adaptability and better cardiovascular health. As a diagnostic tool, HRV is highly valuable; it captures the dynamic balance between the sympathetic and parasympathetic nervous systems, offering direct insight into the autonomic regulation of the heart. Reduced HRV may signal stress, cardiovascular pathologies, or other disorders, and has been linked to conditions such as diabetes. Consequently, HRV is considered more informative than HR alone, providing a deeper understanding of cardiac health and ANS function (Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Malik, 1996; Souza *et al.*, 2021). An optimal level of variability within an organism's major regulatory systems is critical for the inherent flexibility, adaptability, and resilience that underpin healthy function. Whereas excessive instability hampers efficient physiological operation, too little variation often indicates pathological exhaustion (Shaffer *et al.*, 2014).

The tachogram, a graphical representation of HRV over time, is a standard tool in research and clinical settings for assessing ANS activity (Janssen *et al.*, 1993; Potter and Kinsner, 2008). The ANS regulates involuntary bodily functions, including heart rate, and the balance between its sympathetic ("fight or flight") and parasympathetic ("rest and digest") branches is reflected in HRV patterns. Tachogram analysis thus offers insights into this autonomic equilibrium, with HRV changes associated with various health conditions, stress levels, and other ANS-modulating factors.

Spectral analysis techniques for examining the electrocardiographic signal have been employed since the late 1960s (Berntson *et al.*, 1997; Kay and Marple, 1981; Sassi *et al.,* 2015). The 1996 Task Force report categorized heart rhythm oscillations into four principal frequency bands: High-Frequency (HF), Low-Frequency (LF), Very-Low-Frequency (VLF), and Ultra-Low-Frequency (ULF). It recommended analysis on discrete 5-minute segments, although other recording durations are common. When alternative durations are used, reporting the precise length is crucial, as it significantly impacts derived HRV values in both frequency and time domains (Hirsh and Bishop, 1981).

The HF band (0.15 to 0.4 Hz) represents the power within each of 288 discrete 5-minute segments over a 24-hour period. This band mirrors parasympathetic (vagal) activity and is commonly termed the respiratory band, as it aligns with heart rate variations linked to the respiratory cycle - a phenomenon known as respiratory sinus arrhythmia. During inhalation, vagal outflow is inhibited, increasing heart rate; during exhalation, vagal outflow is restored, decreasing heart rate via acetylcholine release (Eckberg and Eckberg, 1982). The magnitude of this oscillation is variable but is typically enhanced by slow, deep breathing and can be influenced by stress (Fanning *et al.*, 2020).

The LF band (0.04 to 0.15 Hz), historically called the baroreceptor range, is typically assessed over a minimum of 2 minutes. Under resting conditions, it predominantly reflects baroreceptor activity. LF power can arise from both parasympathetic (PNS) and sympathetic (SNS) nervous systems influencing blood pressure via baroreflexes, though it is primarily driven by parasympathetic activity. The SNS generates rhythms mostly below 0.1 Hz, whereas the parasympathetic system can influence rhythms down to 0.05 Hz (a 20-second period). Thus, at rest, the LF band is considered an indicator of baroreflex activity rather than direct cardiac sympathetic tone. Notably, when respiratory rates are slow (< 8.5 breaths per minute or periods >7 seconds), vagally-mediated oscillations can extend into the LF band, making respiratory influences particularly discernible in this range during deep breaths or sighing (Shaffer and Ginsberg, 2017).

The VLF band (0.0033 to 0.04 Hz), while part of the HRV spectrum, demonstrates stronger associations with all-cause mortality and arrhythmic death compared to LF and HF bands in 24-hour recordings, despite general low HRV being a risk marker (Shah et al., 2013). The ULF band (<0.0033 Hz, or periods >333 seconds) derives primarily from circadian heart rate oscillations, with contributions from other very slow-acting processes like thermoregulation, metabolism, and the renin-angiotensin system (Shaffer et al., 2014; Shaffer and Ginsberg, 2017). This article will not consider the VLF and ULF bands in its analysis.

Our previous experiments demonstrated that flight activity comprises continuous, demanding tasks amenable to HRV analysis. We observed that superior task management under high cardiovascular demand correlates with higher HRV, implicating both LF and HF components in the ANS-mediated modulation required for performance (Li Volsi et al., 2023a, 2023b). To refine this research, we have expanded our cohort of student pilots and introduced the correlation index as an additional analytical tool for HRV assessment during flight training.

2 Materials, Methods, and Data Analysis

Ethical Approval

The study protocol was approved in advance by the Ethics Committee of the Italian Approved Training Organization No. 0043 (IT.ATO.0043). Each subject provided written informed consent before participating. The data were collected non-invasively during training activities that the subjects would have undertaken regardless of the experiment.

Subjects

All subjects provided written informed consent in accordance with the provisions of Article 1, Paragraph 1, of Italian Law No. 219/2017.

Data were collected from a cohort of 21 student pilots (17 males, 4 females) enrolled in a Private Pilot License training course. The anthropometric characteristics of the participants are presented as mean (± standard deviation): age 21.25 years (±3.19), height 1.69 m (±0.21), weight 67.52 kg (±9.81), and body mass index (BMI) 23,81 (±2.71).

Procedure

Flight training was conducted using a Tecnam P2002-JF aircraft: a two-seat, low-wing, fixed-landing-gear airplane equipped with a single engine (Bombardier-Rotax GmbH, model 912 S2). All training flights were performed with a certified Flight Instructor on board.

Training activities were scheduled based on aircraft availability. For each mission, the flight instructor completed a training report documenting the performance quality of individual exercises and providing an overall assessment.

Prior to the experimental session, upon the student's arrival, five non-invasive electrodes were positioned on the thoracic skin and connected to a three-channel Holter monitor (ECG Biomedical, model BI9100). The Holter recorded electrocardiographic data continuously for the entire session. Flight parameters (altitude, speed, track, and time) were recorded using the Viaair hardware and software system[21], enabling precise synchronization of flight activities with the acquired Holter data.

The training mission was divided into three distinct phases.

Before Flight Operations (bfo): A ground phase comprising briefing with the instructor and pre-flight inspection. Specific tasks included Holter setup, weather briefing, mass and balance calculations, aircraft inspection, pre-flight briefing, engine start, post-start procedures, taxiing, and adherence to aerodrome and air traffic control (ATC) procedures, including radio communications.

During Flight Operations (dfo): The in-flight phase, during which the student performed tasks per the training manual. These included ATC compliance and radio procedures, straight and level flight with speed changes, climbs (best rate, climbing turns, level-off), navigation, various manoeuvres, emergency procedures, descents, aerodrome arrival procedures, approach and landing, followed by post-landing ATC and radio procedures.

After Flight Operations (afo): A post-flight ground phase involving engine shutdown, aircraft inspection, debriefing with the instructor, removal of electrodes, and switching off the Holter.

Statistical Analysis

Heart rate variability analysis was performed in compliance with the standards established by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.5 Both time-domain and frequency-domain analyses were employed.22

For the time-domain analysis, the mean of all normal RR intervals (in milliseconds) was calculated for each of the three phases (bfo, dfo, afo). This parameter serves as a fundamental index of HRV, providing insight into autonomic nervous system function and cardiovascular state.

For the frequency-domain analysis, the spectral power of the Low-Frequency (LF) and High-Frequency (HF) bands was quantified (in ms2). Additionally, Pearson's correlation coefficient (CC or "r") was computed to assess the strength and direction of the linear relationship between selected variables, as detailed in the results.

3. Results

Data were collected from a sample of 21 students of both genders (17 men and 4 women), attending the flight training course to obtain the Private Pilot License.

As outlined in the Methods section, RR intervals derived from tachograms were employed to assess the time elapsed between consecutive heartbeats at three distinct time points: before flight operations (bfo), during flight operations (dfo), and after flight operations (afo). Based on the relationship between dfo and bfo values, the sample was stratified into three subgroups: D-students (dfo < bfo), accounting for 43.8% of the cohort; U-students (dfo = bfo), representing 17.2%; and I-students (dfo > bfo), comprising 39.2%. It is worth noting that afo (recovery) patterns exhibited greater complexity compared to those observed in dfo.

Mean RR intervals for D-students were: bfo: 685.43±80.92; dfo: 604.51±64.49; afo: 648.36 ± 80.13. Mean RR intervals for U-students were: bfo: 623.18±127.98; dfo: 623.61±128.39; afo: 707.32±104.23. Mean RR intervals for I-students were: bfo: 607.85±96.97; dfo: 648.41±99.94; afo: 649.36±9.65. Figure 1 depicts a diagram illustrating the trends described above.

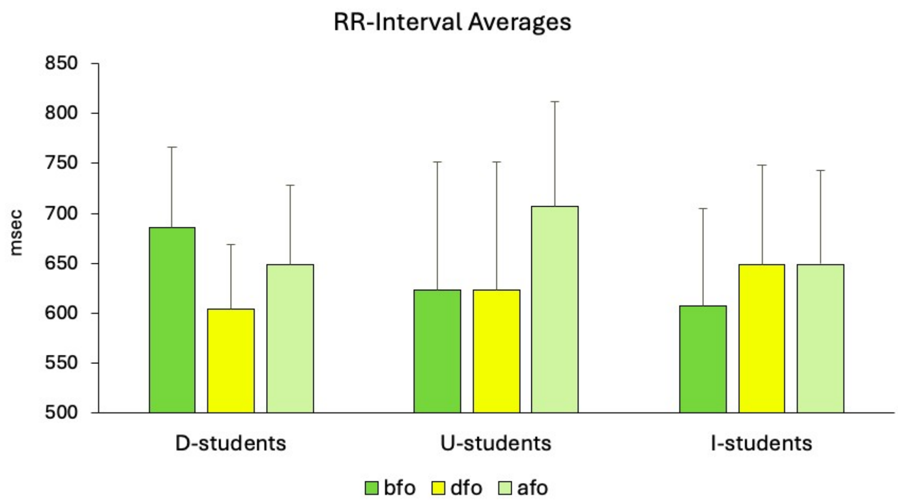

Fig. 1. Mean RR interval duration (ms) before (bfo), during (dfo), and after (afo) flight operations in three student groups: D-students (RR decreased during dfo), U-students (RR unchanged during dfo), and I-students (RR increased during dfo).: bfo: before flight operations on ground; dfo: during flight operations; afo: after flight operations on ground. Error bars: SD.

Subsequently, we quantified the values of LF and HF bands within the three student categories to assess differences in their distribution. Table I provides a concise summary of the numerical data, highlighting the calculated mean values for both LF and HF bands.

FREQUENCY BAND	bfo (mean ± SD)	dfo (mean ± SD)	afo (mean ± SD)
	D-Students		
LF (RR-Int dfo < RR-Int bfo)	1091.12 ± 290.16	589,51 ± 251.33	1032,24 ± 234.14
HF (RR-Int dfo < RR-Int bfo)	294.23 ± 111.24	130.41 ± 68.32	205.46 ± 51.13
	U-Students		
LF (RR-Int dfo = RR-Int bfo)	917.38 ± 345.32	675.23 ± 385.12	1168.43 ± 381.12
HF (RR-Int dfo = RR-Int bfo)	355.67 ± 234.32	217.21 ± 131.43	374.39 ± 114.51
	I-Students		
LF (RR-Int dfo > RR-Int bfo)	902.27 ± 423.12	929.14 ± 446.35	1207.46 ± 390.12
HF (RR-Int dfo > RR-Int bfo)	320.01 ± 171.45	324.92 ± 222.18	354.02 ± 151.34

Tab. 1. LF and HF Band Averages for Student Categories. D-students (RR-Int dfo < bfo); U-Students (RR-Int dfo = bfo); I-students (RR-Int dfo > bfo). LF: Low Frequency; HF: High Frequency.

Figure 2 illustrates the frequency-domain mean values of LF and HF for the three distinct student categories. The left box corresponds to D-students, the central box to U-students, and the right box to I-students.

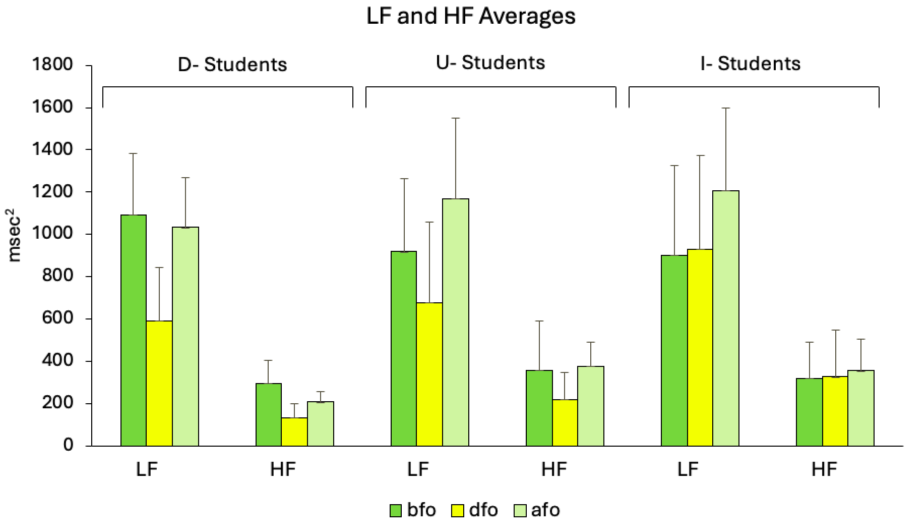

Fig. 2. Frequency domain analysis (average indices) for LF and HF. Left panel histogram. D-students [dfo RR intervals were smaller than bfo ones (dfo < bfo)]. Centre panel histogram: U-students [dfo RR intervals were the same as bfo ones (dfo = bfo)]. Right panel histogram: I-students [dfo RR intervals were greater than bfo ones (dfo > bfo)]. White columns (bfo), heavy grey columns (dfo), light grey columns (afo).

Notably, both D- and U-students exhibited a decrease in LF and HF values under dfo conditions, whereas in I-students both values tended to increase slightly, stabilizing during afo.

To investigate the role played by the two components (sympathetic and parasympathetic) of the ANS in modulating RR intervals across the three student categories, we correlated these data with those of the LF and HF bands. Subsequently, we correlated the two frequency bands with each other. Table II reports the numerical data of the Correlation Coefficients.

Figure 3 depicts diagrams illustrating the trends reported in Table II.

STUDENT CATEGORY	COMPARISON	bfo	dfo		afo	
		CC	CC	% vs. bfo	CC	% vs. bfo
D-students	RR-Int vs. LF	0.36	0.57	+58.33	0.60	+66.69
	RR-Int vs. HF	0.50	0.37	-26.00	0.53	+06.00
U-students	RR-Int vs. LF	0.60	0.53	-11.67	0.36	-40.12
	RR-Int vs. HF	0.69	0.50	-27,54	0.40	-42.03
I-students	RR-Int vs. LF	0.57	0.33	-42.11	0.56	-01.75
	RR-Int vs. HF	0.56	0.34	-39.29	0.57	+01.79
D-students	LF vs. HF	0.54	0.65	+20.68	0.56	+03.70
U-students	LF vs. HF	0.60	0.52	-13.33	0.45	-25.00
I-students	LF vs. HF	0.58	0.51	-12.07	0.53	-07.62

Tab. 2. Correlation Coefficients Between RR Intervals vs. LF and HF Bands and LF vs. HF Bands. CC: Correlation Coefficient; D-students (RR-Int dfo < bfo); U-Students (RR-Int dfo = bfo); I-students (RR-Int dfo > bfo).

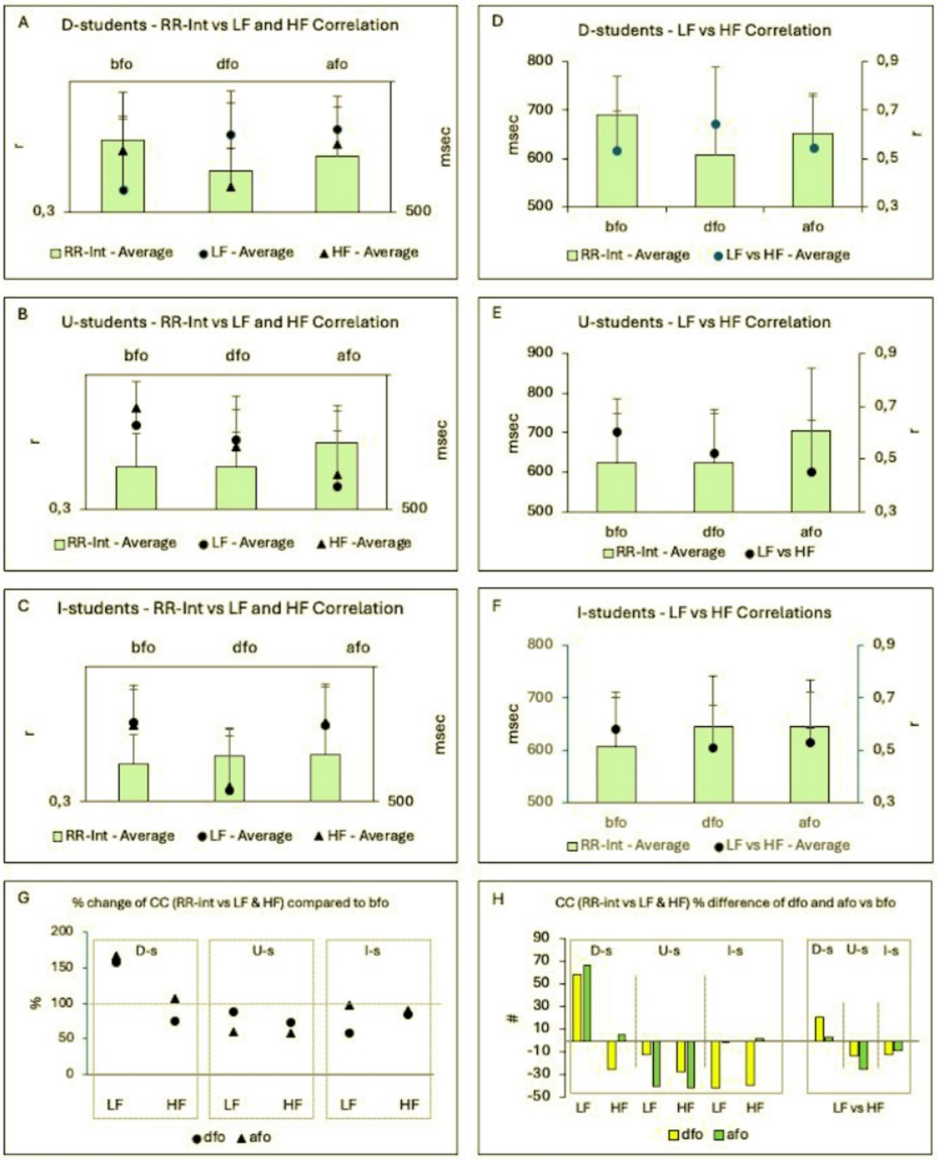

Fig. 3. Correlation coefficient analysis between RR intervals versus LFs and HFs, and LF versus HF. Panel A: Histograms of LF and HF bands of students (D-students) whose RR intervals decrease dfo compared to bfo. RR interval (columns); Black dots and triangles refer, respectively to LF and HF. Panel B: Histograms of LF and HF bands of students (U-students) whose RR intervals did not change dfo compared to bfo. RR interval (columns). Black dots and triangles refer, respectively to LF and HF. Panel C: Histograms of LF and HF bands of students (I-students) whose RR intervals increased dfo compared to bfo. RR interval (columns); Black dots and triangles refer, respectively to LF and HF. Panel D: Correlation coefficients of LF vs HF (black dots) exhibited by D-students. RR intervals (columns). Panel E: Correlation coefficients of LF vs HF (black dots) exhibited by U-students. RR intervals (columns). Panel F: Correlation coefficients of LF vs HF (black dots) exhibited by I-students. RR intervals (columns). Panel G: percentage change of correlation coefficient dfo compared to bfo, and afo compared to

bfo. Panel H: numerical percentage difference (dfo vs bfo (heavy grey column) of the correlation coefficient between LF and HF in D-students, U-students, and I-students. D-s: D-Students; U-s: U-students; I-s: I-students.

4. Discussion

This article describes a more in-depth series of experimental investigations, previously initiated by our research group (Li Volsi *et al.*, 2023a, 2023b) focusing on the study of HRV in student pilots during flight training. For this purpose, we used the tachogram, which is a graphical representation of HRV over time (Janssen et al., 1993; Potter and Kinsner, 2008) commonly used in both research and clinical settings to assess the activity of the ANS (Hon and Lee, 1965; Malik, 1996; Goldberger, 1999). In relation to this analysis, we followed the guidelines outlined in the 1996 Task Force report (Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). As explained in the Introduction, our focus was on the analysis of the time domain, specifically on the LF and HF bands, as we were interested in the role played by baroreceptor activity, as well as the sympathetic and parasympathetic components of the ANS in modulating the RR interval.

In particular, we examined the trend of the interval between two successive RR waves in the electrocardiographic tracings of the students participating in the study in three distinct phases: ground operations before training flights, operations during training flights, and finally, ground operations after training flights. The analysis of the collected data revealed three distinct groups of student pilots: students whose RR intervals decrease during training flights and then rise during ground operations after flights (D-students); students whose RR intervals remain unchanged during training flights and then rise to values higher than those observed during ground operations before flights (U-students); and students whose RR intervals increase during training flights compared to preliminary ground operations before flights and then assume an intermediate trend, tending towards lower values (I-students).

The analysis of the acquired data describes a decrease of both LF and HF bands in D-students as well as U-students; on the contrary, in I-students there is a modest increase in both LF and HF during flight operations.

We proceeded to correlate the three different groups of RR interval values observed with LF and HF band values. In this regard, we observed that in D-students the correlation between RR intervals and LF increases more markedly than that observed for HF. U-students exhibited a different pattern, where the correlation between RR intervals and both LF and HF tends to decrease during flight operations with a more pronounced decrease for high frequencies. Finally, in I-students there is a more pronounced decrease in the correlation between RR intervals and the two considered frequencies. Notably, in this last case, during ground operations after flight, the CC returns to values observed during preliminary ground operations before flight.

We further explored the study of correlations, comparing LF and HF in the three groups of students, and observed that during flight operations, CCs decrease in both U- and I-students while they increase in D-students.

Calculating the percentage variations of these coefficients in the three groups of students revealed that during flight operations, in D-students, the increase in low frequencies is more than 50% compared to pre-flight conditions, while high frequencies decrease by 25%. The trend is different in U-students and I-students, where in both cases there is a decrease in percentage values observed for both LF and HF. In numerical terms, during flight operations, the algebraic difference in the percentage variations of LF compared to HF observed in D-students is 84 [+59-(-25)]. In other words, the decrease in RR interval in this group of students is a direct consequence of the divergence of correlation indices (imbalance) between these two frequency bands. It appears more balanced; however, in the other two groups of students there is a more pronounced balance in I-students. Finally, the percentage variation of correlation indices between LF and HF confirms an inverse correlation in D-students and a decreasing correlation (but of the same sign) in the other two groups of students.

In conclusion, the data we obtained indicate a different role carried by the two components of the ANS where, in cases requiring an increase in cardiac machine performance (equivalent to a decrease in RR intervals), the sympathetic component would have a greater specific weight than the parasympathetic. The condition is different when the required performance increase finds the heart already engaged in preliminary phases. Even more interestingly, the fact that in some subjects, the beginning of a challenging performance initiates the heart towards a new balance in which there is a gain in total power, understood as an increase in HRV. Regarding stress, we emphasize that the three groups of students operated under conditions of eustress and not distress (Lu and Li, 2021; Yoo *et al.*, 2021). This is evidenced by the observed recovery of RR intervals during ground operations after flights (D- and U-students) or even during flight operations (I-students). This could also be closely related to the proficiencies of the flight instructor and the student, as well as the relationship established between the two during the training period. This could also be strongly influenced by the expertise of both the flight instructor and the student, as well as the relationship they build during the training period.

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