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**ORIGINAL**

# Temporal inequality of RR intervals like a new psychophysiological indicator of mental stress

## Desigualdad temporal de los intervalos RR como un nuevo indicador psicofisiológico de estrés mental

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**ABSTRACT**

**Introduction:** Gini coefficient (Gini index or Gini ratio) is a parameter that is normally used in economy to measure the income distribution in a country or in the whole wide world, but it can be used to measure any kind of distribution. In the present study it is exposed an innovative proposal of application of the Gini coefficient to Heart Rate Variability (HRV) like a psychophysiological indicator of mental stress.
**Objective:** to assess the application of the Gini coefficient as a psychophysiological indicator of mental stress.
**Methods:** a non-observational crossover study, carried out in the biomedical laboratory of the Medical University of Santiago de Cuba. The involved participants are 13 healthy individuals (age 19 ± 1,5 years). Heart rate was continuously recorded at rest (5 minutes) and during a mental stress (5 minutes). Linear and nonlinear methods of heart rate variability were assessed, and 2 new indicators (Sequential and Non-Sequential Gini) were calculated and proposed to measure HRV differences between states.
**Results:** when comparing rest and mental stress conditions, a sensible decrease of the traditional indicators of the HRV was founded (p<0,05), an increase of the heart rate (p=0,004) and of the Sequential Gini (p=0,004) and Non-Sequential Gini (p=0,04).
**Conclusions:** the results suggest that temporary inequality of the RR intervals analyzed from the Gini coefficient could be an adequate indicator of sympathetic activity present during the mental stress.

**Keywords:** Heart Rate Variability; Mental Stress; Gini coefficient; Autonomic Nervous System; Mental Arithmetic Test

**RESUMEN**

**Introducción:** el coeficiente de Gini o índice de Gini es un parámetro que normalmente se usa en economía

para medir la distribución del ingreso en un país o en todo el mundo, pero puede usarse para medir cualquier tipo de distribución. En el presente estudio se expone una propuesta innovadora de aplicación del coeficiente de Gini a la Variabilidad de la Frecuencia Cardíaca (VFC) como indicador psicofisiológico del estrés mental.
**Objetivo:** evaluar la aplicación del coeficiente de Gini como indicador psicofisiológico de estrés mental.
**Métodos:** estudio no observacional de corte transversal, llevado a cabo en el laboratorio de estudios biomédicos de la Universidad Médica de Santiago Cuba. Los participantes involucrados fueron 13 individuos sanos (edad 19 ± 1,5 años). La frecuencia cardíaca se registró continuamente en reposo (5 minutos) y durante un estrés mental (5 minutos). Se evaluaron métodos lineales y no lineales de variabilidad de la frecuencia cardíaca, y se calcularon y propusieron 2 nuevos indicadores (Gini secuencial y no secuencial) para medir las diferencias de VFC entre estados.
**Resultados:** al comparar las condiciones de reposo y estrés mental, se encontró una sensible disminución de los indicadores tradicionales de la VFC (p<0,05), un aumento de la frecuencia cardiaca (p=0,004) y del Gini Secuencial (p=0,004) y Gini no secuencial (p=0,04).
**Conclusiones:** Los resultados sugieren que la desigualdad temporal de los intervalos RR analizados a partir del coeficiente de Gini podría ser un indicador adecuado de la actividad simpática presente durante el estrés mental.

**Palabras clave:** Variabilidad de la Frecuencia Cardiaca; Estrés mental; Coeficiente Gini; Sistema Nervioso Autónomo; Prueba de Aritmética Mental.

## INTRODUCTION

Stress has a huge impact on physical and psychological functions, because it affects several physiological processes in the human body. The stress of psychological origin begins with an emotional reaction whose intensity on the organism is linked to various psychosocial factors to which the human being is vulnerable, having the ability to elicit a response that has been called «stress response».[1]

Acute psychological stress is known to cause neural response with the release of hormonal and humoral factors activated by the autonomic nervous system (ANS) in the hypothalamus. It is known that it is associated with developmental and psychopathological disorders,[2] as well as it constitutes a risk factor to many chronic non-communicable diseases.[3] Responses to stressors have adaptive functions developed within the evolution, from which both adverse and positive consequences can be listed. Therefore, stress interventions are useful to understand how healthy individuals respond to stressful stimulus.[4,5]

Acute mental stress can be assessed with various methods available in the medical literature, such as circadian rhythm dysregulations,[6] Stroop test [7] and arithmetic test.[8, 9, 10] Results from previous studies have shown a physiological coherence between stress and physical and cognitive performance in young individuals.[11] However, acute stress and maladaptive responses have been related with physiological changes during stress like endocrine, autonomous and neural responses, with cortisol release under an induced psychological stressor, brain activity changes and heart rate adaptive modifications.[12,13]

Heart rate variability (HRV) is defined as the time intervals variation among serial heart beats. Other terms have been used in the literature; for instance: cycle longitude variability, heart period variability and R-R variability. However, the term of HRV is the one that has had greater acceptance.[14] The autonomic regulation of the heart beats has been widely used to assess the functioning of the autonomic nervous system; methods include time and frequency domain HRV analysis for short and long term recordings.[15]

The analysis of fluctuations of a signal scale with the number of samples of that signal, is applicable to HRV for the RR interval time series analysis, and it found applications in many different research fields, like psychophysiological studies of the autonomic modulation of heart functioning.[16,17]

Acute stress models have shown results on HRV analysis, regarding the different populations over it has been applied. In young adults findings show that it correlates with a diminution of the irregularity of the intervals, as well as entropy and multiscale entropy withdrawal.[10] The stress states have been also associated with changes in HRV, like increased sympathetic activity and parasympathetic lessening.[8]

The authors have found in a spectral analysis of HRV, the inequality of the distribution of slow fluctuations of heart rate under mental stress, with better sensibility than spectral density methods traditionally used in HRV analysis.[18] Gini coefficient is a parameter that normally is used in the economy to measure the inequality in the income, in a country, but can be used to measure any kind of distribution. The application of the Gini coefficient to biological phenomena has recently been explored. The applications of this coefficient include the analysis of electroencephalogram (EEG) traces,[19] functional magnetic resonance imaging,[20] gene expression analysis[21] and the application to measure inequality in power spectral density of RR intervals was recently assessed.[18] However, the application of the Gini coefficient to the analysis in time domain of the inequality of the distribution of RR intervals has not been applied previously.

In this sense, the hypothesis was that the temporal inequality of RR intervals can be associated with autonomic changes in HRV during stress, more effectively than the traditional HRV indexes. It could be really valuable in the evaluation of the adaptive and maladaptive response of the autonomic cardiac modulation to stressful situations, as an adequate indicator of autonomic modifications during mental stress. Therefore, this investigation aimed to assess the application of the Gini coefficient as a psychophysiological indicator of mental stress.

## METHODS

### *Participants*

A non-observational crossover study, carried out in the biomedical laboratory of the Medical University of Santiago de Cuba. The involved participants are thirteen healthy individuals, 6 males and 7 females (age: 19±1,5 years; BMI 22,3 ± 1,3 kg/m$^2$). Database: https://www.researchgate.net/publication/338657618_RR_intervals_of_13_healthy_subjects_in_rest_and_mental_stress_Open_Primary_Database. The participants were non-smokers and without history of heart disease, systemic hypertension or other diseases which could potentially influence HRV changes. The individuals were not under medication and they were required to be satiated and not to have taken any caffeine, drugs or alcohol in the 12 hours preceding the experiment.

### *Measurements*

After signing informed consent, the participants were cited to the specific day and hour when measurements were going to be carried out. The sessions were conducted between 9:00 a.m. and 12:30 p.m. in a dimly lit room with controlled environmental noise and humidity, and a temperature range between 24 and 27 degrees Celsius, with no interactions or distractions among the volunteers. They were allowed to rest for 15 minutes to adapt to local conditions. There was not circulation of people inside of that environment. The participants were asked for substance ingestion and physical activities before starting the recording sessions; they were also required to be satiated (having had breakfast about 1 hour before) and not having taken any product with caffeine.

### *Psychophysiological Data Acquisition and Processing*

The PowerLab Acquisition System 8® (ADInstruments) was used to collect the electrocardiographic (ECG) recordings, with a sampling rate of 1000 Hz for data collection. A standard Lead II was used for ECG measurement. After the skin was cleaned with alcohol wipe, ECG electrodes (Ag/AgCl) were attached to the participant's right midclavicle and the lowest left rib (left wrist as the ground) and subjects were allowed to relax for 10 min to adapt to local conditions. Recordings at rest and during spontaneous breathing were performed for 5 minutes in a seated position; immediately after, subjects performed the arithmetic calculation test for others 5 minutes. An algorithm[22] was used to detect the QRS-complexes in the ECG-signal from which we could determine the RR-intervals.

The Heart rate variability analysis software (HRVAS) https://sourceforge.net/projects/hrvas Copyright© 2015 by John T. Ramshur, [23] was used for preliminary processing of the RR interval series. The standard deviation (SD) filter with SD=3 and percentage filter with value of 20 % from the previous interval were used to detect ectopic intervals.[24] Cubic Spline Replacement was employed to replace ectopic intervals using cubic spline interpolation.[23] Traditional time domain indexes of Heart Rate Variability were calculated, keeping of HRV recommendations.[14] Finally, in other analysis of ECG signals, an ECG-derived Respiration Rate (EDR) was computed from raw-ECG throughout the procedure via a built-in algorithm of Kubios HRV Premium® 3.0.2 software: the algorithm examined the alterations of the amplitude of the R-peak caused by chest movements during each respiratory cycle.

### *Arithmetic calculation test*

Standard procedure of arithmetic calculation test was performed.[25-27] It is an efficient stimulus to induce cardiovascular reactivity by causing mental stress.[9,10,28-30] Subjects subtracted 17 starting from 1000. They were instructed to subtract as accurate as possible. For a single subtraction time allowed was 5 second (s) and it was signaled by a sound. They told the result and after each answer subjects received verbal confirmation ("right" or "wrong"). They continued successive subtracting even when the result was wrong. The subjects did not talk during calculation between verbalization of answers.

### *New indexes proposed*

The Gini coefficient is a measure of how incomes are unequally distributed between social groups. A coefficient of zero means that all subgroups have exactly the same income per capita, meaning that there is no dispersion of income; a very large coefficient would result if all the incomes accumulate only in one subgroup and all the remaining groups have zero incomes. When the total population size approaches to infinity but all the incomes belong only to one individual, Gini coefficient approaches to 1.[31]

*Sequential Gini*

Absolute delta histogram of pairs of adjacent normal R-R intervals |NN-(NN+1)| were employed measuring on discrete scale with bins of 1/128 s. (7,8125).

*Non-sequential Gini*

Histogram of normal R-R intervals (NN) were employed measuring on discrete scale with bins of 1/128 s. (7,8125)

The sequential and non-sequential histograms can be constructed using bins of 7,8125 milliseconds length. Let *n* be the number of classes. Let *A* be a sequence $a_1$, $a_2$,…,$a_n$ such that $a_i$ is the number of elements in the i-th class, and let *B* be the sequence $b_1$,$b_2$,…,$b_n$ such that $b_i$ is the mean of the limits of the i-th class. Now then, we can say that *A* is a sequence of population sizes of some subgroups, and B represents the income per capita in these different subgroups.

The Brown Formula is used to calculate an approximate value of the Gini coefficient and is defined as follows:

$$i \ni \frac{\sum(p_i - q_i)}{\sum p_i} \text{, such that: } p_i = \frac{a_1 + a_2 + \cdots + a_i}{a_1 + a_2 + \cdots + a_n} \quad q_i = \frac{b_1 a_1 + b_2 a_2 + \cdots + b_i a_i}{b_1 a_1 + b_2 a_2 + \cdots + b_n a_n}$$

And i = 1,2,…,n-1. Therefore, $p_i$ is the cumulated proportion of the population variable and  is the cumulated proportion of the variable. In *Non-Sequential Gini,* pi is cumulated proportion of the NN intervals in the different bins of RR Histogram and $q_i$ is the cumulated proportion of values of the different bins of RR Histogram. In *Sequential Gini,* $p_i$ is cumulated proportion of the absolute delta NN intervals in the different bins of absolute delta RR Histogram and $q_i$ is the cumulated proportion of values of the different bins of absolute delta RR Histogram.

***Data analysis and statics***

The Wilcoxon Signed-Rank Test for related samples was employed with p<0,05. All values were expressed as Mean, Standard Deviation (SD) and Coefficient of Variation (CV %). The p values < 0,05 were considered statistically significant. Effect size with Cohen's d was calculated and effect sizes of 0,25, 0,5, and 0,9 should be interpreted as small, medium, and large effects [32]. Pearson Correlation was applied to the data with normal distribution, or Spearman’s correlation, for the ones that did not accept this distribution. The normality of the data was initially determined using the Shapiro-Wilk test. The distributions of bins scaling histograms are showed. All the statistical calculations were carried out in SPSS 22 statistic software and mathematical calculations as well as the processing of the signals were carried out in Matlab 2012b.

***The Ethics Committee Approval Statement***

This study was approved by the Medical University of Santiago de Cuba Ethics Committee under protocol number 22/2017. All procedures involved in this study are in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

## RESULTS

In Table 1 can be observed that mental stress caused changes in the values of heart rate and RR intervals. The traditional indexes of HRV that exhibited statistically significant variations by the cardiovascular hyperactivity are pNN50, RMSSD and SD1. Mental stress also produced a significant increment of Sequential Gini and Non-Sequential Gini. Sequential Gini reported the lowest values of inter-individual variation both in rest and during mental stress.

Figure 1 shows the Lorenz curves of temporal distribution inequality of both Gini indexes during rest and mental stress, in a random subject. The Lorenz coefficient result from the space between the colored zone and the diagonal line, which is highest during mental stress in both Sequential Gini and Non-Sequential Gini indexes.

Table 2 shows the Pearson’s correlations between Gini temporal HRV indexes and traditional HRV indexes, which illustrates that there was no correlation between Non-Sequential Gini and the other indexes in any of the status. Nevertheless, Sequential Gini at rest showed a significant negative correlation with respiratory rate, which means that lowest values of Sequential Gini are related to highest values of respiratory rate; a positive correlation was also founded between Sequential Gini and pNN50. Otherwise, Sequential Gini during mental stress correlated with TINN, and in the total sample it correlated with SDNN, HRVTi, TINN and SD2.

| **Table 1.** Traditional and Gini Temporal HRV indexes during psychophysiological status | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **HRV Indexes** | **Rest** | | | **Mental Stress** | | | **Effect Size** | |
| | **Mean** | **SD** | **CV (%)** | **Mean** | **SD** | **CV (%)** | **Cohen's d** | **p** |
| HR | 80,32 | 10,52 | 13,09 | 96,41 | 11,78 | 12,21 | 1,44 Large | 0,004 [a] |
| RR | 763,3 | 93,0 | 12,18 | 636,9 | 75,1 | 11,79 | 1,49 Large | 0,004 [a] |
| Respiratory Rate | 14,9 | 3,1 | 20,8 | 12,63 | 2,99 | 23,67 | 0,74 Medium | 0,101 |
| SDNN | 60,59 | 24,37 | 40,22 | 62,03 | 22,66 | 36,53 | 0,06 Small | 0,650 |
| pNN50 | 28,32 | 21,20 | 74,85 | 14,91 | 15,03 | 100,8 | 0,72 Medium | 0,007 [a] |
| RMSSD | 47,36 | 22,95 | 48,45 | 33,52 | 17,98 | 53,63 | 0,67 Medium | 0,009 [a] |
| HRVTi | 11,72 | 2,32 | 19,79 | 12,83 | 3,07 | 23,96 | 0,40 Small | 0,263 |
| TINN | 229,0 | 94,7 | 41,35 | 232,7 | 100,6 | 43,23 | 0,03 Small | 0,753 |
| SD1 | 33,53 | 16,24 | 48,43 | 23,73 | 12,72 | 53,60 | 0,67 Medium | 0,009 [a] |
| SD2 | 78,49 | 31,35 | 39,94 | 84,26 | 30,01 | 35,61 | 0,18 Small | 0,311 |
| Sequential Gini | 0,420 | 0,043 | 10,23 | 0,491 | 0,057 | 11,60 | 1,41 Large | 0,004 [a] |
| Non-Sequential Gini | 0,044 | 0,015 | 34,09 | 0,055 | 0,018 | 32,72 | 0,66 Medium | 0,046 [a] |

CV: Coefficient of variation. SD: Standard deviation. [a] Statistically significant.

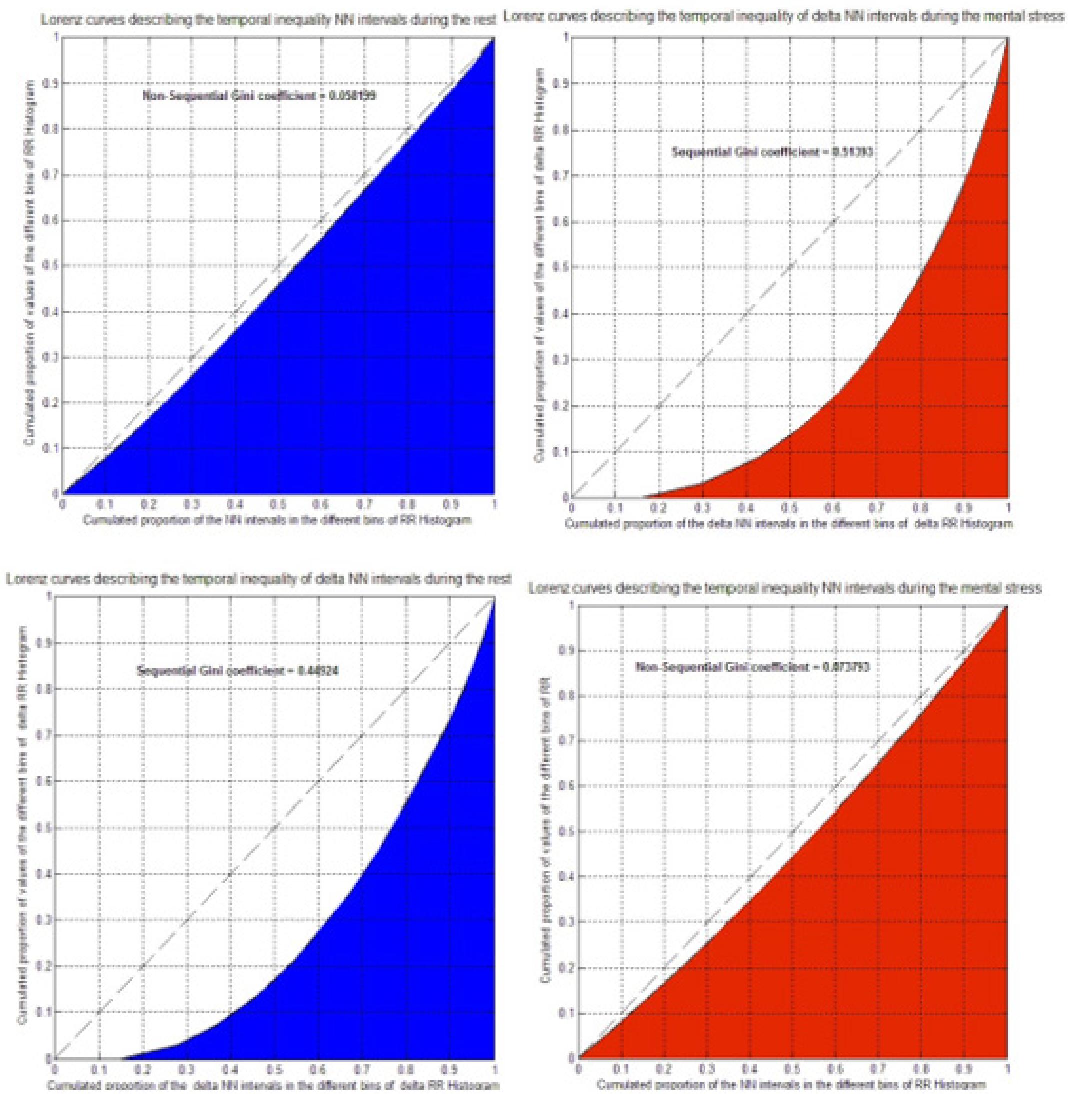


**Figure 1.** Lorenz curves of temporal distribution inequality of Sequential Gini and Non-Sequential Gini during psychophysiological status.

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**Table 2.** Correlation between Gini temporal HRV indexes and traditional HRV indexes during psychophysiological status

| HRV Indexes | Rest | | Mental Stress | | Global | |
|---|---|---|---|---|---|---|
| | Non-Sequential Gini | Sequential Gini | Non-Sequential Gini | Sequential Gini | Non-Sequential Gini | Sequential Gini |
| Heart Rate | 0,027 | -0,152 | 0,102 | -0,327 | 0,241 | -0,188 |
| RR intervals | -0,026 | 0,227 | -0,112 | 0,350 | -0,242 | 0,186 |
| Respiratory Rate | 0,304 | -0,698** | 0,089 | 0,353 | 0,054 | -0,294 |
| SDNN | -0,137 | 0,434 | -0,334 | 0,490 | -0,216 | 0,389* |
| pNN50 | -0,039 | 0,559* | -0,374[a] | 0,360[a] | -0,239[a] | 0,104[a] |
| RMSSD | -0,043 | 0,495 | -0,346 | 0,514 | -0,269 | 0,178 |
| HRVTi | -0,184 | -0,159 | 0,062 | 0,521 | 0,062[a] | 0,413[a]* |
| TINN | -0,138 | 0,351 | -0,189 | 0,595* | -0,152 | 0,408* |
| SD1 | -0,043 | 0,495 | -0,344 | 0,514 | -0,268 | 0,178 |
| SD2 | -0,148 | 0,410 | -0,329 | 0,481 | -0,199 | 0,414* |
| Sequential Gini | -0,022 | - | -0,012 | - | 0,269[a] | - |

*Statistically significant. ** p < 0,01. [a]Spearman's correlation, for the HRV indices that did not accept normal distribution in Shapiro-Wilk test.

Table 3 shows the evaluating parameters of the discriminatory capacity of traditional and Gini temporal HRV indexes to be related to stress status, provided by the Receiver Operating Characteristic (ROC) curve. Sequential Gini shows a poor discriminatory capacity to relate with stress, from which it is inferred that its highest values are associated with rest. Non-Sequential Gini by the other hand showed a good discriminatory capacity to be related with stress. Its cut point could be proposed to be evaluated as an indicator of mental stress.

**Table 3.** Efficacy of traditional and Gini temporal HRV indexes in the discrimination of psychophysiological status

| Variables | Cut point | Sensitivity | Specificity | Youden Index | Area Under Curve | p |
|---|---|---|---|---|---|---|
| RR intervals | 723,700 | 0,000 | 0,231 | -0,769 | 0,136 | 0,002 |
| SDNN | 41,950 | 0,692 | 0,077 | -0,230 | 0,503 | 0,980 |
| pNNx | 4,60 | 0,538 | 0,077 | -0,384 | 0,302 | 0,086 |
| RMSSD | 24,850 | 0,538 | 0,077 | -0,3846 | 0,325 | 0,130 |
| Heart Rate | 83,350 | 1,000 | 0,769 | 0,769 | 0,870 | 0,001 |
| HRVTi | 9,750 | 0,923 | 0,308 | 0,230 | 0,621 | 0,293 |
| TINN | 284,400 | 0,385 | 0,846 | 0,230 | 0,467 | 0,778 |
| SD1 | 17,600 | 0,538 | 0,077 | -0,384 | 0,325 | 0,130 |
| SD2 | 91,250 | 0,462 | 0,769 | 0,230 | 0,538 | 0,739 |
| Gini non-secuencial | 0,034 | 1,000 | 0,308 | 0,307 | 0,669 | 0,144 |
| Gini secuencial | 0,447 | 0,923 | 0,769 | 0,692 | 0,876 | 0,001 |
| Respiratory Rate | 15,121 | 0,154 | 0,462 | -0,384 | 0,308 | 0,096 |

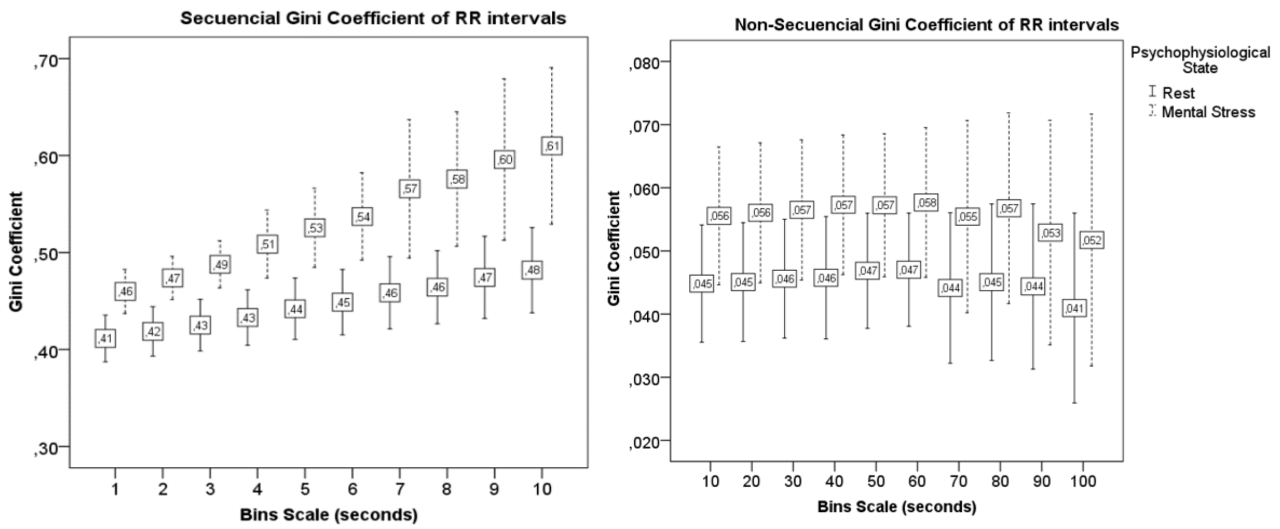


**Figure 2.** Distribution of class intervals (bins) of Gini temporal HRV indexes in both psychophysiological states.

In Figure 2 can be observed the class intervals (bins) of Gini temporal HRV indexes in both psychophysiological statuses to evaluate the unequal distribution of elements. It was founded that during stress both Gini temporal HRV indexes are more concentrated, while during rest they are more dispersed. In the analysis of each index separately, Sequential Gini increases when bins sizes increases, while Non- Sequential Gini keeps stable no matter the sizes of bins.

## DISCUSSION

The response of the cardiovascular system to acute stress causes activation of the autonomic nervous system, matching energy supply to increased metabolic demands (increase in blood pressure, heart rate, vascular peripheral resistance, etc.), and it can clarify the pathophysiological mechanism in which various cardiovascular conditions develop.(33) The effect of stress on HRV has been extensively studied, and the changes it produces in traditional parameters have been demonstrated, such as increased heart rate, SD2/SD1 ratio, short-term fluctuations α, low frequencies, multiscale entropy and the sympathetic index; as well as significant decrease in the RR interval, SDNN, RMSSD, pNN50, sample entropy and the parasympathetic index. (9,34) These variations respond to the adjustment of the parasympathetic branch that make these variables look like within the allotted time, in the sense that they adapt to the changes and demands of the organism to face different life situations and they adjust to signals that travel down the hypothalamic-pituitary-adrenal axis causing acute catecholamine release and subsequent cortisol release. These explain the increase in other variables such as blood pressure, heart rate or peripheral vascular resistance.(4,8)

An increasing number of indicators have been developed and applied to quantify HRV in the time and frequency domain since the beginning of the new century.(16) Sassi et al.(15) provided a critical review of newer methods highlighting their contribution to the technical understanding of HRV and their ability to quantify the complex regulation mechanism of the heart rate not covered by traditional methods. But Gini index as a statistical measure that has only been investigated in a previous study for its application to the study of HRV during an acute stimulus induced by a mental stressor.(18)

In the present study, a significant increase in the inequality of heartbeat variations (Sequential Gini) during mental stress was found, which in the bivariate analysis exceeded the rest of the traditional HRV indicators in statistical significance. This result agrees with the previous obtained by Sánchez-Hechavarría et al. (19) where he determined the efficacy of the Spectral Gini coefficient (SpG) of each traditional HRV indicator to compare between baseline and mental stress. In that study, a significant increase in SpG (LF) (p = 0,009) and SpG (LF2) (p = 0,033) was found that also surpassed the traditional indicators. However, the significance value of the Sequential Gini in the present study is higher than that of SpG, which means that there were more and better differences with the index proposed here. Also, the coefficient of variation of the Sequential Gini showed that its appearances to be more homogeneous than the rest of the indexes studied, which improves the strength of this result. Regarding the result obtained in the Non-Sequential Gini analysis (distribution of beats), it is worth highlighting its statistical significance that is higher than most traditional HRV indexes.

The obtained results show that with the use of the new proposed indicators a greater homogeneity of the data is achieved in the study of HRV against various stimuli, taking into account the value of its coefficient of variation, which results are higher. This increases the validity and feasibility of using this indicator to assess mental stress against the other indicators.

The Lorenz curve is used together with the Gini coefficient as a graphical representation to measure the inequality in the distribution of numerical data and to express the relative distribution of a variable in a domain; the perfect data distribution would result in a slope of one and a Gini coefficient of zero, whereas severe data inequity results in higher Gini values closer to one.(35) In this study it was found that there is a more unequal distribution in the Sequential Gini than in the Non-Sequential Gini, since the coefficient was higher. It was also found that the distribution of the data resulted to be more irregular during mental stress than during rest. This result is completely novel, since the application of the Lorenz curve to measure the inequality in spectral values of HRV, and its comparison with mental stress has been applied in this study for the first time.

The study of HRV has shown that it is a measurement coupled with the fact it is relatively affordable, non-invasive and pain free, which makes it very accessible to many researchers.(16) But it is true that most HRV indicators currently studied are highly correlated with each other, which constitutes a difficulty in their interpretation and a possible bias when correlating them with external stimuli, since the values of some of them are toughly influenced by the other values such as heart rate and respiratory rate.(36) Another difficulty in the study and application of HRV is the large number of indicators and the high correlation between them that in many cases is possible to estimate one indicator from another, not providing valuable information, but making its interpretation difficult.

In this study, little correlation was found between the proposed indicators and the traditional variables, which provides new information not previously described. The Non-Sequential Gini did not correlate with any of the traditional indicators; However, Sequential Gini did show a correlation with some geometric HRV indicators, which opens the possibility of classifying this new indicator as geometric, by its possibility of being calculated and geometrically represented, and in the fact of using the concept of Data binning, which a characteristic of many geometric indicators of HRV.[37] The benefit of having greater homogeneity and the possibility of visualizing the most obvious changes produced by mental stress in the heart is reported in this study.

Regarding results obtained in the analysis of the discriminatory capacity of the traditional indexes and the Sequential and Non-Sequential Gini, it was found that the area under the Sequential Gini curve resulted as poor discriminatory capacity to discriminate mental stress. However, the Non-Sequential Gini did show a good discriminatory capacity to be associated with stress, with high sensitivity and specific values.

The analysis of discriminatory capacity has been used in several previous investigations of HRV, and cut-off points have been established for various variables, which aim to predict a better or worse cardiovascular function or a better adaptation of them to stressful physical or mental stimuli. [38,39] In this study a cut-off point is proposed to be investigated in future.

It was observed that the new indicators have different characteristics when the sizes of the Bins vary. In the Sequential Gini (which indicate the inequality of the clustered variations of the heartbeats) it was obtained that as the size of each bin increases, there are fewer class intervals, therefore fewer groups within the distribution and it consequently increases the possibility that these beat variations are concentrated in these class intervals. These differences are principally observed during induced mental stress, since it has been shown that slow oscillations tend to concentrate.[18] This result demonstrates the need to use a standard Bins size when calculating the Sequential Gini. In this study, 8-second Bins were used, which is very similar to the value recommended for geometric methods.[40] It was also observed that the Non-Sequential Gini (inequality of the distribution of the beats) performs in a constant manner regardless of the size of the Bins used, so the limitations that the Sequential Gini presents is not applicable to Non- Sequential Gini. These results allow describing the mathematical characteristics of the indicators proposed in the studied sample.

Results of this investigation show that Gini index in its Sequential and Non-Sequential categories was found to be associated with mental stress in the individuals studied, more effectively than the traditional HRV indexes. Temporal inequality measured by the Gini index was bigger during mental stress, and the proposed indexes seems not to be affected by other indicators, which they could be very valuable in the study of the cardiovascular autonomic response to various stimuli, with great potentialities in the objective to assess the consequences of psychosomatic affections and the anxiety disorders.

The main limitation of this study is related to the small sample size, which could affect the performance of methods for qualitative sample comparison and distribution of studied parameters values in the population. Although the sample size accomplishes sufficiently the minimal recommended sample size for biomedical experiments,[41] the effect size was assessed in every single analysis, by calculating Cohen's kappa coefficient.

## CONCLUSIONS

Temporary inequality of the RR intervals analyzed from the Gini coefficient could be an adequate indicator of sympathetic-adrenal activity present during mental stress.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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