Chapter

Heart Rate Variability as Biomarker for Prognostic of Metabolic Disease

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Abstract

Lifestyle emerging diseases like obesity, metabolic syndrome (MeS), and diabetes mellitus are considered high-risk factors for lethal arrhythmias and side effects. A Poincaré plot is constructed with the time series of RR and PP electrocardiogram (ECG) intervals, using two stages: the new phase and the old phase. We proposed this diagram of two dimensions, a way to quantify and observe the regularity of events in space and time. Therefore, the heart rate variability (HRV) can be used as a biomarker for early prognostic and diagnostic of several metabolic diseases; additionally, this biomarker is obtained by a noninvasive tool like the electrocardiogram.

Keywords: Poincaré plot, heart rate variability, metabolism, biomarker

1. Introduction

The biological phenomena could be explained by classical physics, and most of these phenomena are characterized by cycles. Usually, the time period required to "complete a cycle" is not constant. The study of time period fluctuations represents a way to assess interactions between other systems and the intrinsic properties of the same system.

The light/dark cycle (circadian rhythm) and the cyclic seasons that divide the year by changes on weather, ecology, and hour of daylight allowed the evolution of life on earth [1]. These series of events have influenced the organisms inducing cycles that are essential for life (hormonal, organ function, behavior, production of neurotransmitters, reproduction, and others); all cycles are fluctuations related to several biological phenomena. The study and knowledge of the fluctuations of biological phenomena are valuable to analyze the intrinsic properties of a system and the interaction with other cyclic systems [2].

The quantification of biological variability has been used to study several physiological phenomena, among them, fluctuations on the heart rate using the RR interval period of the electrocardiogram (ECG). The heart rate variability (HRV) is a useful health indicator [3], and in this chapter we detail how this tool is used for the prognosis and diagnosis of metabolic diseases.

2. Biological variability

All organisms present dynamic and complex oscillations in their function. The time between every oscillation is called period, and it represents biological rhythms. These rhythms regulate all physiological processes with periods of milliseconds as neuronal activity, seconds as the heart rate, hours as hormone release, monthly as the ovarian cycle, and annually as the growth and migration. The biological rhythms are present in all levels of biological organization at the molecular, organelle, cell, and tissue levels; these organizations are present in vertebrates, invertebrates, and plants (**Figure 1**).

The study of period variations is essential because these fluctuations represent the interaction of the cycles with other systems or alterations on the intrinsic properties of the same system, individual and interspecific variability [4].

The periods can oscillate only under the influence of an external periodic signal originating exogenous rhythms; these allow changes in the variability of biological rhythm associated with external environmental synchronizer [5]. However, when the light/darkness external synchronizer is removed, a self-sustaining oscillation is shown, so it is said that the system has an autonomous endogenous rhythm.

The biological rhythms with a periodicity of 24 h are denominated circadian rhythms (*circa* = about, *diem* = a day). These circadian rhythms develop an endogen rhythm with one period close to 24 h under constant darkness, the free running, but can be synchronized again with the light and darkness; this phenomenon is called circadian entrainment. The circadian rhythms of longer period are infradians, such as the menstrual cycle, while shorter periods are ultradians, such as cardiac frequency, the autonomic system regulation, electrical activity of neurons, and secretion of hormones, among others (see **Figure 1**).

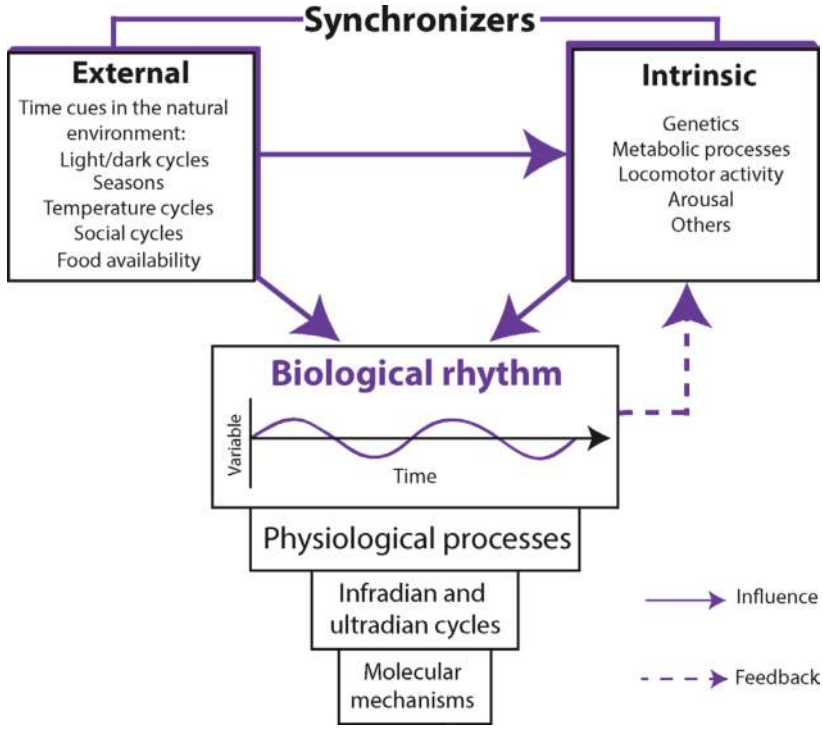

Figure 1.
Variability biologic system. All organisms develop the variability of biological systems for environmental adaptation. Physiological and metabolic processes depend on the interaction between the central and peripheral rhythms.

The autonomous nervous system (sympathetic and parasympathetic) regulates the cardiovascular system that involves the heart rate variability. The relevance that the intermittent oscillations of peripheral clocks modulate the variability of the central clock is fundamental for health process. The coordination and communication among peripheral and central clock are essential for metabolic, enzymatic, molecular, and physiological process.

3. Heart rate

The heart rate (HR) is determined by the activity of the sinoatrial node. The electrical activity propagates to the atria and then to the atrioventricular node, and, finally, the electrical activity reaches the ventricles triggering its contraction from apex to base. Any change in the origin and propagation of this electrical activity is denominated arrhythmia. The contraction and relaxation of cardiac tissue is a process named heartbeat. It is a cyclical event, the beats per minute produce the heart rate. Heart rate is a parameter that serves to diagnose some health problems in patients. When HR is increased, it is called tachycardia and the decrease of HR is called bradycardia. Tachycardia is related to exercise, emotions, the fight or flight phenomenon, among other activities. Bradycardia is related to sleep and rest. To measure HR there are several methods that are used in the clinic, like pulse taking, auscultation, and electrocardiography.

4. Heart rate variability

The interaction of the organisms with its environment causes changes in the metabolic requirements of the multiple tissues that are depending on the circulating blood to supply oxygen and nutrients and remove metabolic waste, i.e., age, physical conditioning or exercise, behavior (emotions, pathologies, spice that is being studied, activity that takes place when the HR is taken, hemorrhages, heart attacks, addictions). In response to these demands, the heart adapts its interbeat intervals.

These intervals vary thanks to the intrinsic properties of the heart (spontaneous activity of the sinoatrial node [6] and atrial and ventricular electrical properties along with extracellular matrix composition) and especially the influence of the autonomic nervous system (ANS), a communication pathway between the heart and the whole body. This system modulates the spontaneous activity of the sinoatrial node and conduction system of the heart (**Figure 2**).

The ANS regulates heart rate, visceral activities, and glandular functions to keep homeostasis. The ANS innervation on the heart can be divided in sympathetic (SNS) and parasympathetic (PNS) nervous system. They both have opposing effects on the heart activity. The sympathetic nervous system is responsible for the "fight or run" response, increasing the myocardium contractile properties and the rate of spontaneous activity of the sinoatrial node (SAN), the natural pacemaker of the heart, augmenting the heart rate. On the other hand, the parasympathetic nervous system has an inhibitory effect on the peacemaker and atrioventricular node (NAV) activity (see **Figure 2**), adjusting to rest states by means of a decrease in the heart rate [2].

Sympathetic innervation secretes norepinephrine, a neurotransmitter that links to β1 receptors on the cardiac sarcolemma activating G proteins. This union induces a conformational change that dissociates the α_s subunit activating adenylyl cyclase. The activated adenylyl cyclase catalyzes the conversion of ATP to AMP_c, which joins directly to ionic channels responsible for the hyperpolarization activated pacemaker

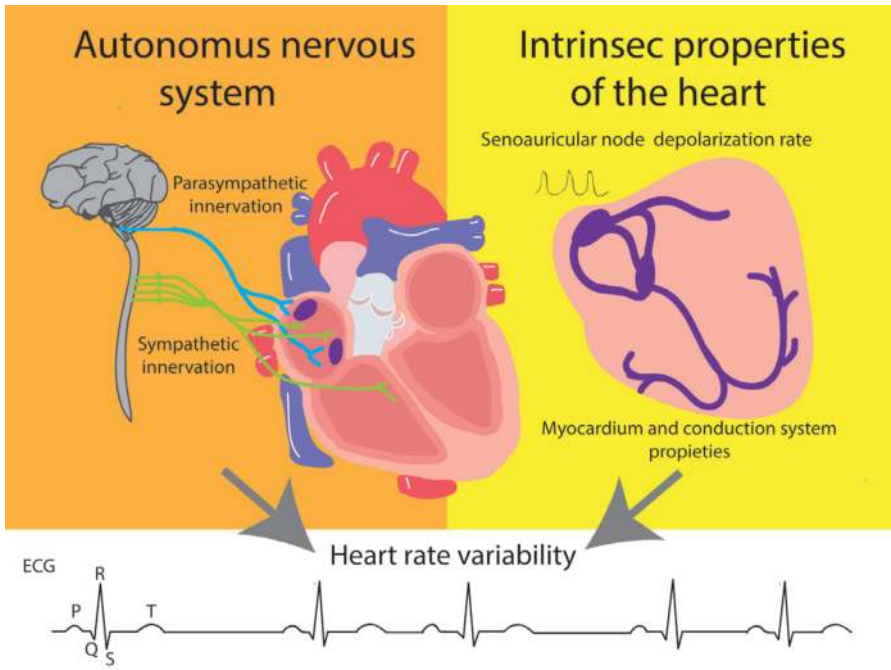

Figure 2.
Heart rate variability sources. The time interval variations between consecutive heartbeats are result of the interaction between the autonomous nervous system modulation and intrinsic properties of the heart regulating its function.

current (I_f) increasing the SAN depolarization rate. This stimulus also increases the opening probability and the inward calcium current enhancing the strength of cardiac contraction. Parasympathetic innervation releases acetylcholine, a neurotransmitter that binds to M_2 receptors on the cardiac sarcolemma activating inhibitory G proteins, inducing a conformational change in G_i protein that dissociates the α_i subunit inhibiting adenylyl cyclase leading to a decrease in the formation of AMP_c, thus decreasing the SAN depolarization rate. The dynamical interaction between SNS and PNS enables the heart to fulfill the organism requirements in the short and long term.

Since the heartbeat is a cyclic phenomenon that repeats continually as a result of the interaction between spontaneous SAN activity [7], passive and active properties of the myocardium, conduction system, and ANS influence, it can be regarded as a result of the interaction of multiple coupled systems that oscillate. This complex nonlinear interaction reflects on interbeat interval variability; such phenomenon is called heart rate variability.

The interbeat intervals are usually assessed as the time between the R-wave peaks of the ECG signal (RR time series). This registry is consequence of the spatial and temporal sum of the electrical activity of the whole heart, and each wave is characteristic of specific electrical events. The R wave is representative of the QRS complex, which is the result of the ventricular transmural depolarization heterogeneity [8]. In view on the fact that the time from the start of the depolarizing wave at the sinoatrial node to the ventricle depolarization can account as other oscillation sources, inter-beat interval indicated by the PP interval (depolarization of the atria) can provide some insights that can be concealed by the RR interval (**Figure 2**).

HRV analysis is a valuable noninvasive method to quantify modifications caused by aging, disease progression, and other physiologic or pathologic changes. These alterations influence the oscillating systems or the way they couple, as sympathetic and parasympathetic heartbeat modulation besides intrinsic properties of the heart

that rely on extracellular matrix, sarcolemma composition (ionic channel density and kinetics, gap junction density, lipid composition), myocyte size, adipocyte, and fibroblast distribution. The etiology of these alterations is often related to metabolic diseases [9].

5. Poincaré plots

5.1 Time series

To analyze HRV using the ECG signal, we used the R waves of the QRS complex. The evaluation of the time between an R-wave peak (R_1) and the next immediate R-wave peak (R_2), the time interval between the appearance of an R wave and the next (t_{1-2}) will be called heart period. The RR intervals are organized in chronological order, with an organized set of numbers. This set will be called the "heart activity time series."

The heart rate (number of beats per unit of time) can be estimated as the inverse of the time period. When the frequency is stable, it is always the same, so are the period and the time series. When the time series is plotted against its order appearance, a time series graph is obtained [10]. The times series values determine the shape of the graph. When the frequency is constant, the graph is a parallel line to the time axis. And in the case that the frequency has variations (HRV), the graph is like in **Figure 3**.

The time series has all the information of the variability of system; then, to determine that two time series are similar, numerical values were allocated to this variability. The first tool used was RR time series spectral analysis, this technique is based on the use of all periodic signals consisting of sums of sine and cosine functions with different frequencies and amplitudes, with the purpose to determine which frequencies are involved in the formation of the time series [10]. The frequencies obtained by this mathematical tool have been associated to the nervous system, breathing, and other physiological functions. The frequencies and power spectrum of the different components of the time series are the parameters used to quantify the variability with this method [11].

The disadvantage (if it can be considered as one) of using this method is that not everybody is expert in Fourier series; therefore it's difficult to analyze, interpret, and perform. The second tool we use is the Poincaré plots. They require graphing

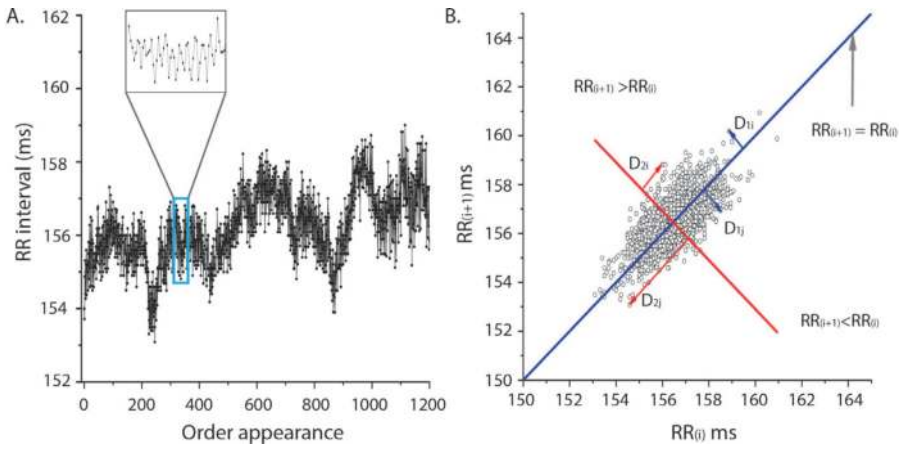

Figure 3.
Heart rate variability analysis. (A) Time series with chronological order and (B) Poincaré plot of time series.

the time series as follows: the first RR_1 interval is assigned as x value, and then the value of the RR_2 interval is assigned as y; this ordered pair is plotted on a Cartesian coordinate axis. Now we consider RR_2 as x, and RR_3 as y to plot it. After that the rest of the RR intervals are graphed in the same way, where the x is the $RR_{(i)}$ interval and the y is the interval $RR_{(i+1)}$; we plot each RR interval against the next immediate one. The resulting graphs are converted into spot stains; this chart is known as Poincaré plot (**Figure 3**). Now the question is how to quantify the spots. Before we give an answer to the problem, we will first describe the advantages of Poincaré plot.

5.2 Advantages of the Poincaré plots

First of all, it is important to mention that the heart rate will be $1/RR_i$; consequently the analysis of the interval variations gives us information of the heart rate variation. That is, the Poincaré plots give us information about changes in heart rate even if this parameter is not explicitly represented in this graph. As above mentioned, the frequency (F) is the inverse of the time period (T), hence F*T = 1.

In the plot we trace the identity straight line $RR_{(i+1)} = RR_{(i)}$, this line divides the plane into three parts: one where the $RR_{(i+1)}$ is equal to $RR_{(i)}$ (blue line in **Figure 3**), another where $RR_{(i+1)} > RR_{(i)}$ which is the top of the identity line. And the third where $RR_{(i+1)} < RR_{(i)}$ which is the part that is under the identity line (**Figure 3**). Therefore, just by looking at the point localization, we can say that the next interval has a higher value, i.e., the frequency is less. In other words, when the points fall above the identity straight line, the period i + 1 is greater and the further the point is from the identity line, the value of the period i + 1 will be greater; otherwise when the points are under the identity graph, the period i + 1 will be smaller, and the frequency will be higher [3].

5.3 SDD_1 and SDD_2 calculation

The distance between the points and the identity straight line tells us what the instantaneous (or sequential) changes of the RR interval will look like, as mentioned in the short term [12]. As an example, we will mention that when the distance from the points to the identity straight line is zero $RR_{(i+1)} = RR_{(i)}$, there are no changes in the interval, but if this distance becomes greater, the variation between RR is greater. These distances are called D_{1i}; the D_{1i} distances that are above the identity straight line will be positive and those below will be negative in such a way that the average of these distances are zero, but the standard deviation of these distances (SDD_1) will be different from zero, and this parameter will be used to characterize the width of the Poincaré plot. The width or SDD_1 will be used to determine the variability of D1; this parameter is related to the short-term variability of the RR, and this relates to the interaction of the sympathetic system and the heart. To calculate SDD_1 all distances from points to the identity line are calculated the average and standard deviation [3, 13]. Thus it is found that

$$D_{1i} = \sqrt{\left(\frac{RR_i - RR_{i+1}}{2}\right)^2} \quad (1)$$

All D_{1i} distances are added and divided by the number of distances to get the average; the standard deviation to the latter is called SDD_1. SDD_1 is a parameter that characterizes short-term variability.

Secondly, calculate the distance from all points to the perpendicular line that crosses the identity line at the coordinate point of the mean value (RR_m, RR_m). This

distance is called $D2_j$ (Eq. (2) and **Figure 3**). All D_{2j} distances are added and divided by the number of distances to get the average; the standard deviation to the latter is called SDD_2.

The RR variability changes can be obtained based on the RR time series without using explicitly the time:

$$D2j = \sqrt{2\left(\frac{2\overline{RR_j} - RR_j - RR_{j+1}}{2}\right)^2} \quad (2)$$

where $\overline{RR_j}$ is the average value of the sum of all RR intervals.

By obtaining the values of SDD_1 and SDD_2, we quantify the variability of the heart rate in the short and long term. This data defines the coefficient of variability as SDD_1/SDD_2.

Using the Poincaré plots, the quantification of the variability in the heart rate is determined by calculating SDD_1, SDD_2, and the SDD_1/SDD_2 ratio. The advantage of this method is that the calculation of these parameters is clearly arithmetic, and just by looking at the Poincaré, plot you have an idea of how the variability is given.

6. Biomarkers

A biological marker or biomarker is any substance, structure, or process that is objectively measured and evaluated as an indicator of normal biological processes. The biomarkers in the medical science field play essential role for disease detection, pathogenic responses, and therapeutic intervention. These markers are observational side products with potential utility in clinical and research studies [14]. Additionally they are used in new treatment strategies for clinical management. The biomarker field opens the opportunity to originate new knowledge in the complex health scheme.

7. Metabolic disease

Metabolic alterations cause metabolic diseases as result of changes in chemical reactions in the organism by several enzyme deficit, developing alterations like lipid metabolism disorders. These diseases are associated with synthesis and degradation of fatty acids. The principal and general symptoms of metabolism injury are lethargy, weight alteration, inflammatory process, seizures, and jaundice.

8. Metabolic syndrome

For the last decade, the cardiovascular diseases have been the first cause of death worldwide, and the deadly arrhythmias have increased in the industrialized countries; this fact is related to lifestyle and metabolic alterations such as sedentarism and diet [15, 16]. Obesity and metabolic syndrome are disorders associated with metabolic modifications.

The metabolic syndrome has been described as a cluster of several signs like abdominal obesity, hyperglycemia, dyslipidemia, and high blood pressure (**Figure 4**). These factors predispose to develop cardiovascular diseases, and each component is strongly correlated with CVD. An opportune diagnosis is necessary to know the progression of MeS and predisposition to develop lethal risks. HRV analysis is a tool to assess cardiac function in patients with several pathologic conditions.

However, relationships between HRV and cardiac rhythm with changes in MeS have not been found, improving considerably the prognostic and diagnostic of MeS, as well as the side effects.

The ECG is a biomarker for early diagnosis of metabolic diseases [3], and to asses HRV, a five random minute interval must be measured and analyzed. When more time is analyzed, the characteristic SDD_1 and SDD_2 will be lost [3]. In humans, the MeS showed changes in RR intervals; SDD_1 or short-term variability was modified in young adults, while in woman and elderly human, the alterations were vagal as sympathovagal balance (SDD_1 and SDD_2 [17]).

Also, spectral analysis with Fourier transform was used for the 24-h ECG record; this analysis showed that in human, the high frequencies (HF 0.15–0.40 Hz), which represents sympathetic modulation, were lower only in women with metabolic syndrome [18] and at low frequencies (LF 0.04–0.15 Hz), which represent parasympathetic modulation, heart rate was not altered by MeS. Furthermore, individual components of the MeS were highly correlated with imbalance cardiac autonomic system; the obesity modifies sympathetic nervous system [19]; hyperglycemia alters parasympathetic system [20]; and microalbuminuria, dyslipidemia, and hypertension do not alter neither of them but decrease LF/HF index (see **Figure 4**) [21, 22].

Rats with obesity and hypertension presented similar cardiovascular changes as humans: a decrease in parasympathetic system without any increase in sympathetic modulation [23], and only temporary alterations in sympathetic nervous system were reported in rats with high sucrose diet, insulin resistance, and visceral fat (epididymal fat) [24]. However, the rats with high sucrose diet showed higher LF than control [25], and also the heart rate was decreased showing sinus bradycardia and a threefold increase of heart rate variability, SDD_1 15 ± 0.4, and SDD_2 69 ± 1, compared with control animals 5.5 ± 0.1 and 26 ± 0.1, respectively. In addition, sinoatrial node doubled its variability as shown in the SDD_1/SDD_2 index = 0.25 for control condition and MeS: SDD_1/SDD_2 = 0.55 [26]. In genetically modified rats, cardiac alterations were observed independently on individual characteristic of MeS (see **Figure 4**).

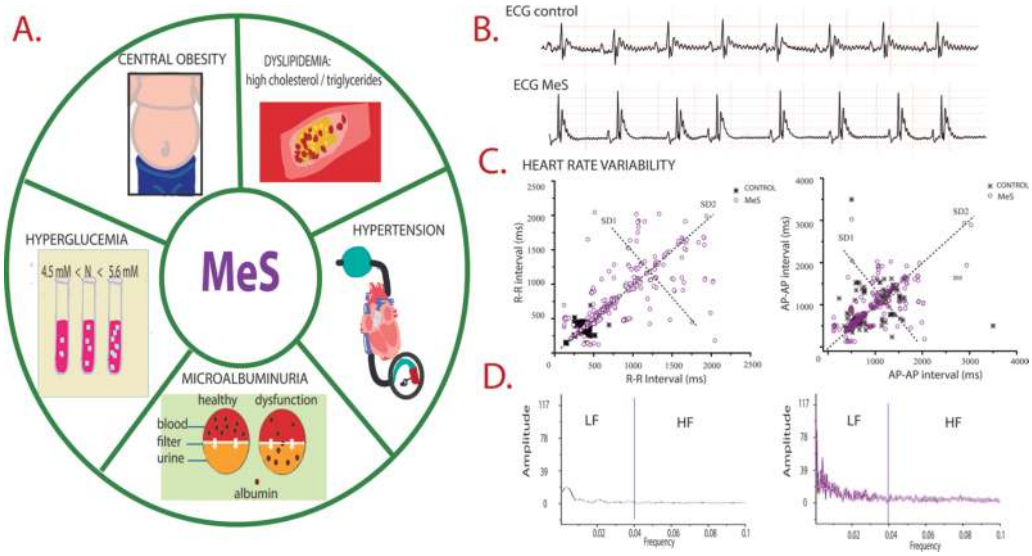

Figure 4.
The metabolic syndrome increases the heart rate variability. (A) The cluster signs of MeS increase the risk to develop cardiovascular diseases (CVD) and diabetes mellitus. (B) ECG of control and MeS rats, showing lower heart rate in MeS rats. (C) Poincaré plots exhibiting lower balance between parasympathetic and sympathetic systems. (D) Fourier analysis indicating that lower frequencies predominate in MeS rat RR time series.

9. Diabetes mellitus

The cardiac arrhythmias in diabetes mellitus are due to structural and functional remodeling, which are alterations in the architecture of the heart that include fibrosis, fat deposition, hypertrophy, modification in the utilization, and production of energy. In addition, electrical activity remodeling includes failure in electrical conduction, dysregulation in ion channels and gap junctions [27], and all these changes are added to the autonomic imbalance between the sympathetic and parasympathetic nervous systems until it becomes cardiac autonomic neuropathy (CAN), which is recognized as a risk for development of atrial fibrillation and sudden cardiac death (see **Figure 5**) [28].

In order to realize clinical diagnosis of CAN, the performing cardiac autonomic reflex test or neuropathy Ewing's battery is recommended. It consists of the assessment of the HRV in rest condition, while standing, during paced deep breathing, during sustained muscle contraction with the use of a handgrip dynamometer (handgrip exercise), and during and after a provoked increase in intrathoracic/abdominal pressure (maneuver of Valsalva) (see **Figure 5**) [29, 30].

Unfortunately, these tests have limitations: patients must be aware so they can perform each of the tests, and it is necessary to suspend medications that could alter the results of the test (e.g., the avoidance of medications that cause hypotension, such as diuretics, tricyclic antidepressants, and vasodilators) [31].

Due to these disadvantages, the measurement of HRV has been used as an alternative for CAN diagnosis in recent years because it is a noninvasive test, it does not provoke pain in the patient, the analysis is performed in a short time, it is reliable, and it is a low-cost technique. In addition, this methodology allows the HRV analysis to be performed in less time because it is not necessary to have specialized knowledge in statistics or mathematics since the values of SDD_1, SDD_2, and SDD_1/SDD_2 are obtained by means of relatively simple arithmetic calculations and it does not need specialized software to perform them [3]. Another improvement is that the Poincaré plot analysis can be done with only 100 RR intervals, which excludes the use of a Holter registry without reducing the reliability and sensitivity of the test [32].

Several authors have reported a decrease in HRV in patients with DM types 1 and 2 regardless of the method used to measure it (frequency-domain HRV or time domain). The decrease in HRV in diabetic patients is associated with an early phase of the evolution of CAN. There is a loss of parasympathetic function with a relative increase of sympathetic function causing an imbalance of the sympathetic/parasympathetic tone (without parasympathetic denervation). The patient experiments an increase in resting heart rate. In the next stage, sympathetic denervation takes place increasing the risk of arrhythmias [33]. Despite the existence of a large number of studies on HRV in diabetic patients, we still do not have a relationship that allows us to know the stage of damage in which the autonomic nervous system is found.

On the other hand, we have validated the use of HRV and the measurement of SDD_1, SDD_2, and the Poincaré SDD_1/SDD_2 index (Eqs. (1) and (2)) as a biomarker for diagnosis and prognosis of cardiac autonomic neuropathy. For this purpose, a model of type 1 diabetes pharmacologically induced by STZ was used. This model was developed in CD1 mice in which the progress of disease is allowed for 10 weeks without insulin administration (the time compared with human 8 years of disease progression), which produces a decrease in the values of SDD_1 (1 vs. 0.9), SDD_2 (1.3 vs. 0.8), and SDD_1/SDD_2 (0.8 vs. 1.1) compared to the control (**Figure 6**) [32].

In this stage of the disease, no decrease in heart rate was reported, which suggests that CAN was in the early stages. However, after a time period equivalent to

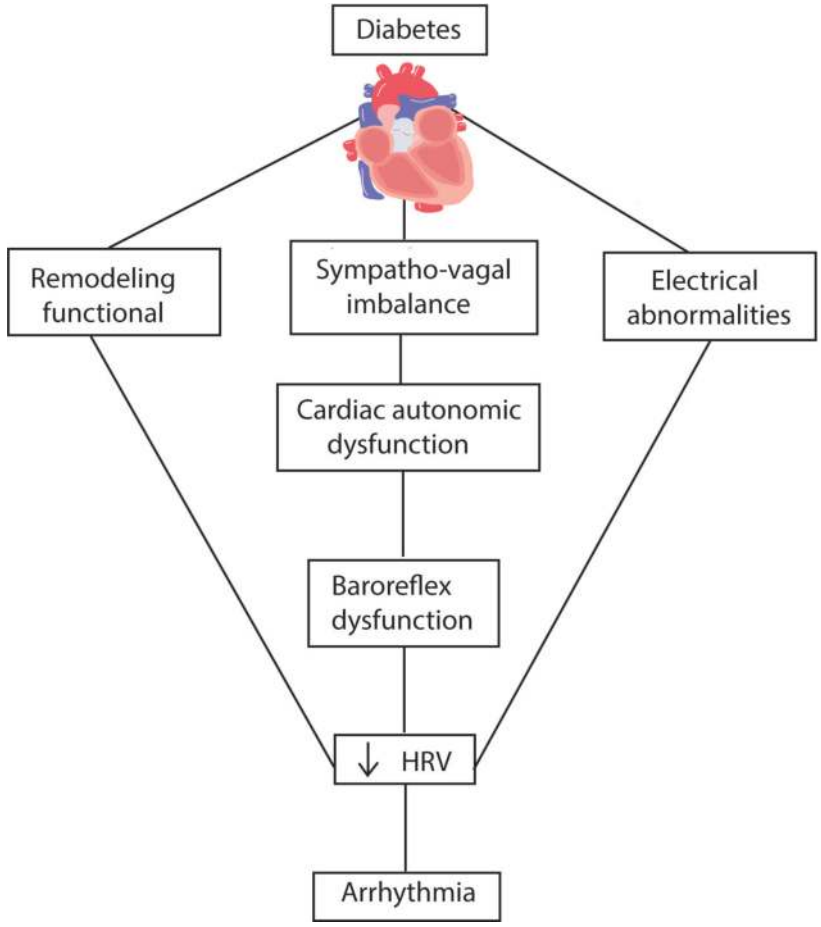

Figure 5.
Relations between alterations in DM and cardiac arrhythmias. The alterations in the architecture of heart tissue and functions produce a decrease in HRV during diabetes, which increment the risk of arrhythmias.

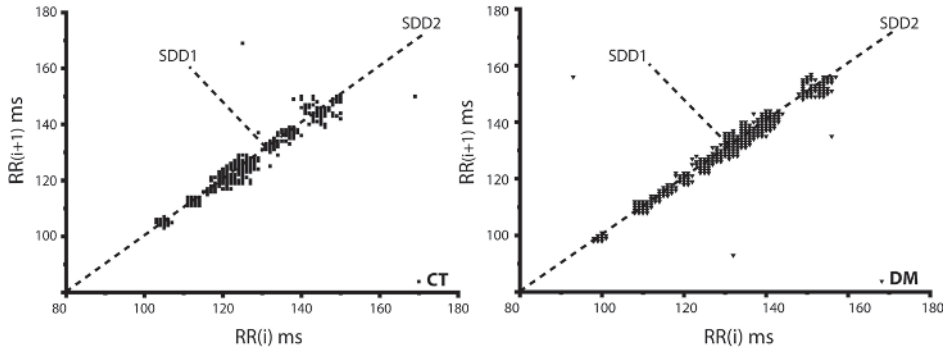

Figure 6.
Poincaré plots of interval RR of ECG. The HRV in conditions of control vs. 8 years of DM development. The influence of the ANS allowed maintaining the balance of an elliptical shape.

15 human years of DM induction without hypoglycemic treatment CAN, a decrease in HRV is developed (**Figure 6**). Additionally, in this second stage, mice showed denervation in the pacemaker tissue [13]. We conclude that the use of HRV and Poincaré plots could detect CAN even in early stages of the disease, and therefore it will allow introducing therapeutic maneuvers to control the symptoms and delay the damage to the ANS due to DM.

10. Conclusions

The periodic oscillations in biological phenomena are quantified with the purpose to use them as a health indicator (biomarker) in mammalian. By means of the ECG interval analysis, HRV is quantified using RR and PP time series. Poincaré plots were constructed, and three indicators were obtained: SDD_1, SDD_2, and SDD_1/SDD_2 index. The behavior of these indicators is related with health or metabolic disease. In MeS, a sympathovagal imbalance was reported, and the parasympathetic system showed alterations with a twofold increase in SDD_2 indicator. Furthermore, the three indicators were decreased by DM. These biomarkers have the advantages of being based on a noninvasive tool, being objective, and being obtained by easy arithmetic calculus. In addition, the shape of the Poincaré plots offers qualitative information by only looking at it.

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