HEART RATE VARIABILITY: CLINICAL APPLICATIONS AND INTERACTION BETWEEN HRV AND HEART RATE

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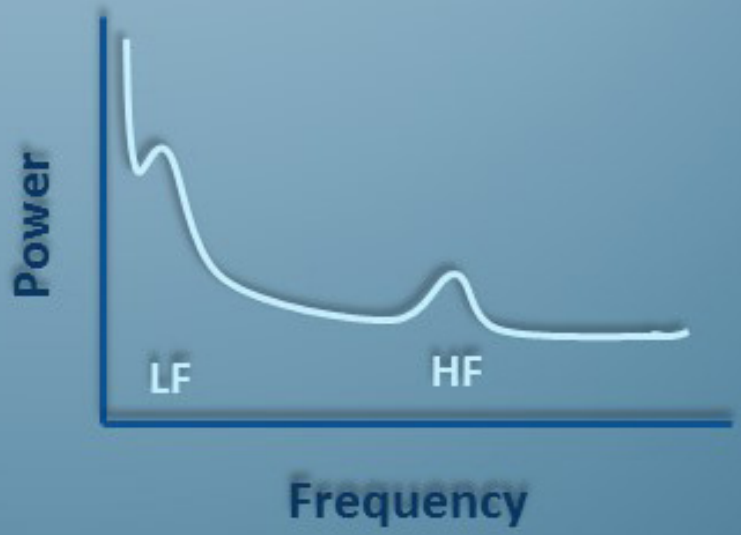

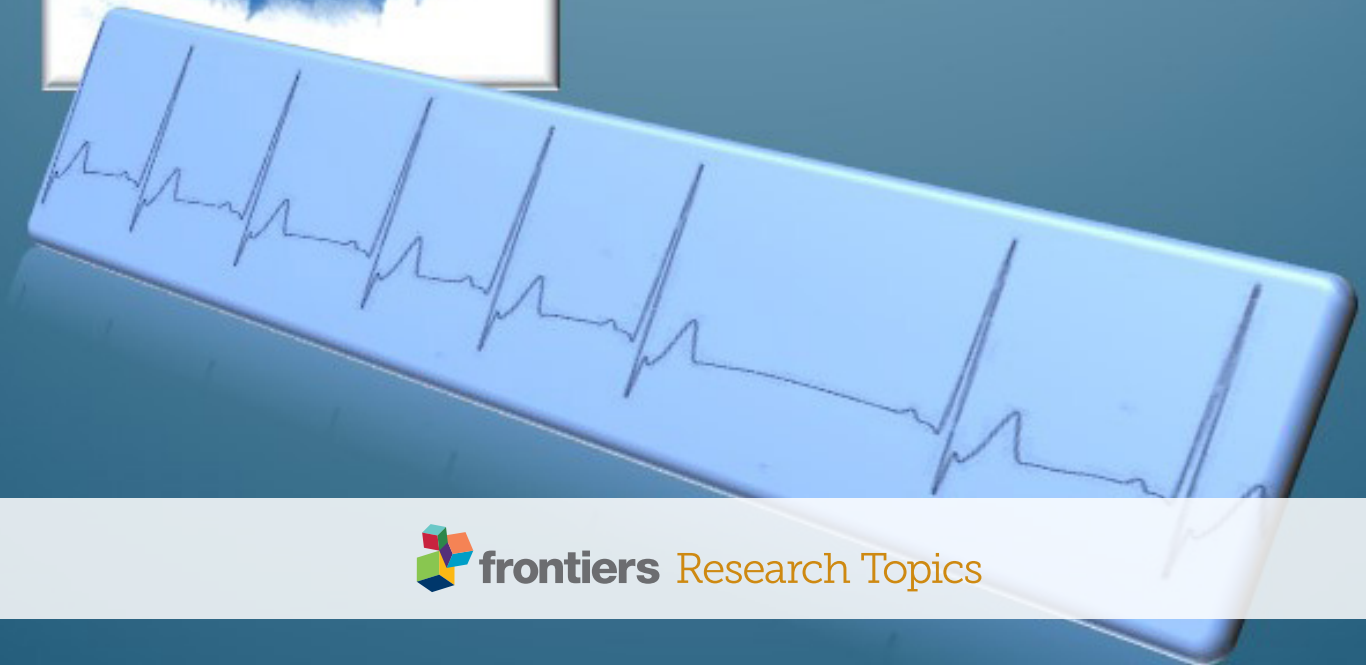

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HEART RATE VARIABILITY: CLINICAL APPLICATIONS AND INTERACTION BETWEEN HRV AND HEART RATE

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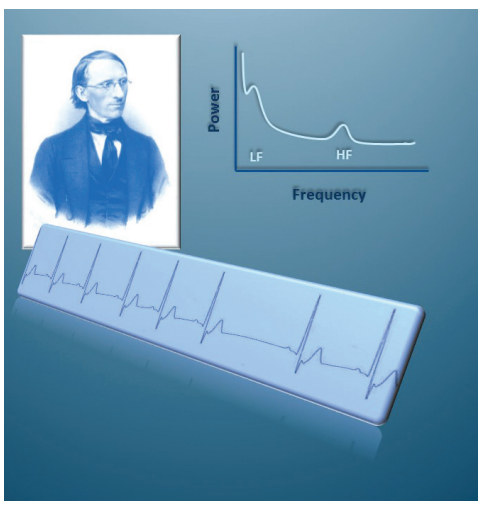

This figure contains a photograph of Dr. Carl Ludwig, the first individual to record HRV, together with an ECG strip illustrating the beat-to-beat variations in the R-R interval and a frequency domain analysis of HRV. Illustration by George E. Billman.

Over the last decades, assessment of heart rate variability (HRV) has increased in various fields of research. HRV describes changes in heartbeat intervals, which are caused by autonomic neural regulation, i.e. by the interplay of the sympathetic and the parasympathetic nervous systems. The most frequent application of HRV is connected to cardiological issues, most importantly to the monitoring of post-myocardial infarction patients and the prediction of sudden cardiac death. Analysis of HRV is also frequently applied in relation to diabetes, renal failure, neurological and psychiatric conditions, sleep disorders, psychological phenomena such as stress, as well as drug and addiction research including alcohol and smoking. The widespread application of HRV measurements is based on the fact that they are noninvasive, easy to perform, and in general reproducible – if carried out under standardized conditions. However, the amount of parameters to be analysed is still rising. Well-established time domain and frequency domain parameters are discussed controversially when it comes to their physiological interpretation and their psychometric properties like reliability and validity, and the sensitivity to cardiovascular properties of the variety of parameters seems to be a topic for further research. Recently introduced parameters like pNNxx and new dynamic methods such as approximate entropy and detrended fluctuation analysis offer new potentials and warrant standardization.

However, HRV is significantly associated with average heart rate (HR) and one can conclude that HRV actually provides information on two quantities, i.e. on HR and its variability. It is hard to determine which of these two plays a principal role in the clinical value of HRV. The association between HRV and HR is not only a physiological phenomenon but also a

mathematical one which is due to non-linear (mathematical) relationship between RR interval and HR. If one normalizes HRV to its average RR interval, one may get 'pure' variability free from the mathematical bias. Recently, a new modification method of the association between HRV and HR has been developed which enables us to completely remove the HRV dependence on HR (even the physiological one), or conversely enhance this dependence. Such an approach allows us to explore the HR contribution to the clinical significance of HRV, i.e. whether HR or its variability plays a main role in the HRV clinical value.

This Research Topic covers recent advances in the application of HRV, methodological issues, basic underlying mechanisms as well as all aspects of the interaction between HRV and HR.

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EDITORIAL
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An introduction to heart rate variability: methodological considerations and clinical applications

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Keywords: heart rate variability, heart rate, heart rate dynamics, autonomic nervous system, risk assessment, cardiovascular disease

Heart rate variability (HRV), the beat-to-beat variation in either heart rate or the duration of the R-R interval, has become a popular clinical and investigational tool (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Billman, 2011). Indeed, the term "heart rate variability" yields nearly 18,000 "hits" when placed in the pubmed search engine. These temporal fluctuations in heart rate exhibit a marked synchrony with respiration (increasing during inspiration and decreasing during expiration—the so called respiratory sinus arrhythmia) and are widely believed to reflect changes in cardiac autonomic regulation (Billman, 2011). Although the exact contributions of the parasympathetic and the sympathetic divisions of the autonomic nervous system to this variability are controversial and remain the subject of active investigation and debate, a number of time and frequency domain techniques have been developed to provide insight into cardiac autonomic regulation in both health and disease (Billman, 2011). It is the purpose of this book to provide a comprehensive assessment of the strengths and limitations of HRV techniques. Particular emphasis will be placed on the application of HRV techniques in the clinic and on the interaction between prevailing heart rate and HRV. This book contains both state-of-the art review and original research articles that have been grouped into two main sections: Methodological Considerations and Clinical Application. A brief summary of the chapters contained in each section follows below.

METHODOLOGICAL CONSIDERATIONS

The opening section provides a historical overview of the evolution in the concept of heart rate variability (Billman, 2011) and then describes time domain, frequency domain, and non-linear dynamic analysis techniques (and their limitations) that are commonly used to measure heart rate variability. Heathers (2014) and Billman (2013a) describe methodological issues in the analysis of short-term frequency-domain HRV such as the LF band, normalized units, or the LF/HF ratio as well as the influence of external factors on HRV data. These reviews provide substantial information on mathematical concerns in HRV analysis and on the interpretation of the underlying physiological background of HRV power and highlight the necessity of methodological improvement in HRV measurement. Peltola (2012) evaluates the methods used to edit R-R interval time series and how this editing can influence the results obtained by the HRV analysis. The effects of prevailing HR on HRV are further evaluated in series of review and original research articles.

It is not widely appreciated that HRV is significantly associated with average heart rate (HR) and that, as a consequence, HRV actually provides information on two quantities; i.e., HR and its variability (Sacha, 2014a,c). Sacha (2013, 2014b) demonstrate that interpretation of HRV data is further complicated by the inverse non-linear relationship between HR and R–R interval. Owing to this inverse (mathematical) relationship, the same fluctuations of HR yield higher R-R interval changes for the slow than for the fast average HR, and therefore the standard analysis of heart rate variability may be mathematically biased (Sacha and Pluta, 2008). Thus, one must calculate HRV normalized to HR in order to differentiate between physiologically and mathematically mediated changes in HRV (Sacha, 2013). This normalization is particularly important if one compares HRV between the patients with different average HRs or during interventions that change HR. The effect of these normalization procedures are explored further in a series of original research articles.

For example, the effects of HR on the HRV response to different autonomic interventions were examined using a canine model (Billman, 2013b). Maneuvers that accelerated HR (e.g., submaximal exercise) caused a decrease in HRV even after normalization for the HR changes while interventions that slowed down HR yielded mixed results (e.g., baroreceptor reflex activation provoked an increase in HRV even after normalization for reflexively mediated reductions in HR, while beta-adrenergic receptor antagonists reduced rather than increased HRV after normalization for the drug-induced HR reductions) (Billman, 2013b). In a review article, Billman (2013a) further demonstrated that, among other factors, both heart rate and mathematical considerations profoundly influence the LF/HF ratio such that it is not possible to determine the physiological basis for this widely use index (Billman, 2013a). He concluded that the preponderance of evidence confirms that the LF/HF ratio cannot accurately quantify cardiac "sympatho-vagal balance" either in health or disease (Billman, 2013a).

In another article, Grant et al. demonstrate (by employment of the normalization method) that HR is a better indicator of higher

fitness than HRV; i.e., an association between HRV indices and maximal oxygen intake (VO_2max) exists mainly due to the relationship between HR and VO_2max (Grant et al., 2013). On the other hand, the same normalization method enabled Carter et al. to show that an increase in HRV following dengue viral infection does not result from the accompanying reduction in HR, but reflects a real improvement in cardiac autonomic nervous control (Carter et al., 2014). Finally, Pradhapan et al. (2014) examined the impact of HR on HRV on the results of exercise stress testing and found that HR immediately before exercise was not a risk factor of death, and the removal of its influence improved the HRV predictive power. Conversely, HR during the recovery phase was a significant mortality predictor, and the enhancement of its impact (by using the method of Sacha et al., 2013) increased the HRV predictive ability (Pradhapan et al., 2014). These examples clearly show that it is very important to establish to what extent HRV changes associated with simultaneous HR alterations are physiologically and mathematically determined. Unraveling this remarkable interplay between HRV and HR may yield valuable prognostic information (Sacha, 2014b). Further studies are needed to determine which of the two, i.e. HR or HRV, provides better predictive performance for a given population and outcome as well as to what modifications of the HRV/HR relationship increase the prognostic power of HRV (Sacha, 2014b).

CLINICAL APPLICATIONS

HRV analysis has become an increasing important diagnostic tool in cardiology. For example, Lombardi and Stein (2011) review the relationship between HRV and heart rate turbulence (HRT, baroreceptor reflex mediated short-term oscillations in the heart period that occur after spontaneous ventricular arrhythmias) and "sympatho-vagal" balance while Zuern et al. (2011) and Huikuri and Stein (2012) evaluate HRV and HRT as tools for risk assessment in patients recovering from myocardial infarction. Non-linear indices of HRV are evaluated by Perkiömäki (2011) and Glass et al. (2011). Perkiömäki (2011) reports that novel HRV indices that quantify the non-linear dynamics of HR may have a greater prognostic value to identify patients with the greatest risk for adverse cardiovascular events than do conventional HRV indices, while Glass et al. (2011) analyzed the dynamic properties of premature ventricular complexes to reveal the underlying mechanisms responsible for these arrhythmias.

In a similar fashion, Papaioannou et al. (2013) investigated the association between changes in HRV and the inflammatory response in patients with cardiovascular diseases by assessing the relationship of inflammatory biomarkers such as CRP, TNF-a, IL6, or white blood cell count with different parameters of HRV. Bravi et al. (2013) further explored the different changes in HRV produced by physiological and pathological stress. Datasets of healthy subjects performing physiological exercise (physiological stress) were compared to those of patients who developed sepsis after a bone marrow transplant (pathological stress), showing similar responses during both conditions, however, with subtle differences. In another chapter, Jelinek et al. (2013) evaluated cardiac rehabilitation (CR) outcomes following a 6-week program of percutaneous coronary revascularization (PCI) and coronary artery bypass graft (CABG) patients by the analysis of HRV variables and comparing changes in the 6-min-walk-test and peak VO_2. It was shown that CR significantly improved exercise capacity and positively affected HRV changes especially in the CABG group. Hinojosa-Laborde et al. (2011) investigated whether any HRV index could accurately distinguish between individuals with high and low tolerances to simulated hemorrhage (i.e., lower body negative pressure). They report that, although a few HRV indices could accurately differentiate between low and high tolerance subjects when considered as group (i.e., difference in group means), a given individual's HRV value provided a poor indicator of tolerance to hypovolemia. Finally, Tobaldini et al. (2013) reviewed linear and non-linear analyses of HRV to assess autonomic changes during sleep under physiological as well as pathological conditions such as sleep-related breathing disorders, insomnia, or epilepsy/sudden unexplained death in epilepsy (SUDEP).

Thus, by understanding both the strengths and limitations of the various techniques used to quantify heart rate variability, the authors hope that this brief monograph will provide sufficient knowledge so that these indices can be used appropriately in the clinic not only to identify high risk patients but also to aid in the development of more effective therapies to treat the diseases that elicited the HRV changes.

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Heart rate variability – a historical perspective

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Heart rate variability (HRV), the beat-to-beat variation in either heart rate or the duration of the R–R interval – the heart period, has become a popular clinical and investigational tool. The temporal fluctuations in heart rate exhibit a marked synchrony with respiration (increasing during inspiration and decreasing during expiration – the so called respiratory sinus arrhythmia, RSA) and are widely believed to reflect changes in cardiac autonomic regulation. Although the exact contributions of the parasympathetic and the sympathetic divisions of the autonomic nervous system to this variability are controversial and remain the subject of active investigation and debate, a number of time and frequency domain techniques have been developed to provide insight into cardiac autonomic regulation in both health and disease. It is the purpose of this essay to provide an historical overview of the evolution in the concept of HRV. Briefly, pulse rate was first measured by ancient Greek physicians and scientists. However, it was not until the invention of the "Physician's Pulse Watch" (a watch with a second hand that could be stopped) in 1707 that changes in pulse rate could be accurately assessed. The Rev. Stephen Hales (1733) was the first to note that pulse varied with respiration and in 1847 Carl Ludwig was the first to record RSA. With the measurement of the ECG (1895) and advent of digital signal processing techniques in the 1960s, investigation of HRV and its relationship to health and disease has exploded. This essay will conclude with a brief description of time domain, frequency domain, and non-linear dynamic analysis techniques (and their limitations) that are commonly used to measure HRV.

Keywords: heart rate variability, respiratory sinus arrhythmia, time domain, frequency domain, autonomic nervous system

Variability is the law of life. . .

(William Osler, physician and educator, 1849–1919; Olser, 1903, p. 327)

INTRODUCTION

Heart rate variability (HRV), beat-to-beat variation in either heart rate or the duration of the R–R interval – the heart period (for an example see **Figure 1**), has become an important risk assessment tool. A reduced HRV is associated with a poorer prognosis for a wide range of clinical conditions while, conversely, robust periodic changes in R–R interval are often a hallmark of health (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Bigger, 1997; De Jong and Randall, 2005; Thayler et al., 2010). A major portion of these temporal changes in heart rate occur synchronous with respiration [heart rate increases (R–R interval shortens) during inspiration and decreases (R–R interval prolongs) during expiration] and, therefore, are referred to as the respiratory sinus arrhythmia (RSA). Although HRV and RSA are not quite the same, these terms are often used interchangeably and both are widely believed to reflect changes in cardiac autonomic regulation. The exact contributions of the parasympathetic and the sympathetic divisions of the autonomic nervous system to this variability are controversial and remain the subject of active investigation and debate (Parati et al., 2006). It is the purpose of this essay to provide a historical overview of the evolution of the concept of HRV and its application in the laboratory and in the clinic. Time and frequency domain techniques used to quantify HRV and their limitations will also be briefly discussed.

HISTORICAL OVERVIEW

A summary of some of the major events in the evolution of the HRV concept is displayed as a timeline (not drawn to scale) in **Figure 2**. Undoubtedly early humans were the first to notice that the heart beat varied, increasing, for example, during physical exertion or sexual arousal. However, the first written descriptions of heart rate (measured by the pulse) are found in the fragmentary writings of the ancient Greek physician and scientist Herophilos (Ήρόφιλος, Latinized as Herophilus, ca. 335- ca. 280 BC; **Figure 3**; Bedford, 1951; Bay and Bay, 2010). He was born in Chalcedon but spent the majority of his adult life in Alexandria. He was perhaps the first anatomist and published at least nine volumes of his findings, all of which have been lost (Bedford, 1951; Bay and Bay, 2010). Fortunately, his original text was extensively quoted in the works of other authors, particularly by the Greco-Roman physician Galen (Bedford, 1951; Boylan, 2007). Among his most notable findings was the demonstration that the veins carried blood, that veins and arteries were distinctly different, and that the arteries pulsed rhythmically (Bedford, 1951; Bay and Bay, 2010). These fragmentary quotations also suggest that Herophilos was the first person to measure heart rate (by timing the pulse

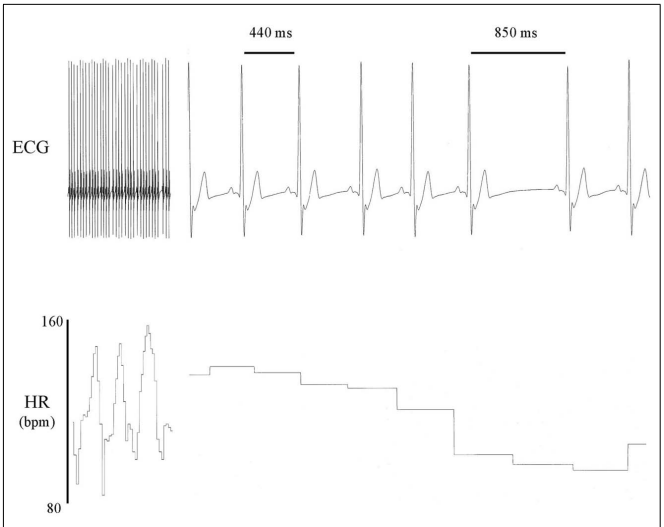

FIGURE 1 | Heart rate variability: representative electrocardiogram (ECG) recordings from a conscious dog that illustrate beat-to-beat variations in both R–R interval and heart rate.

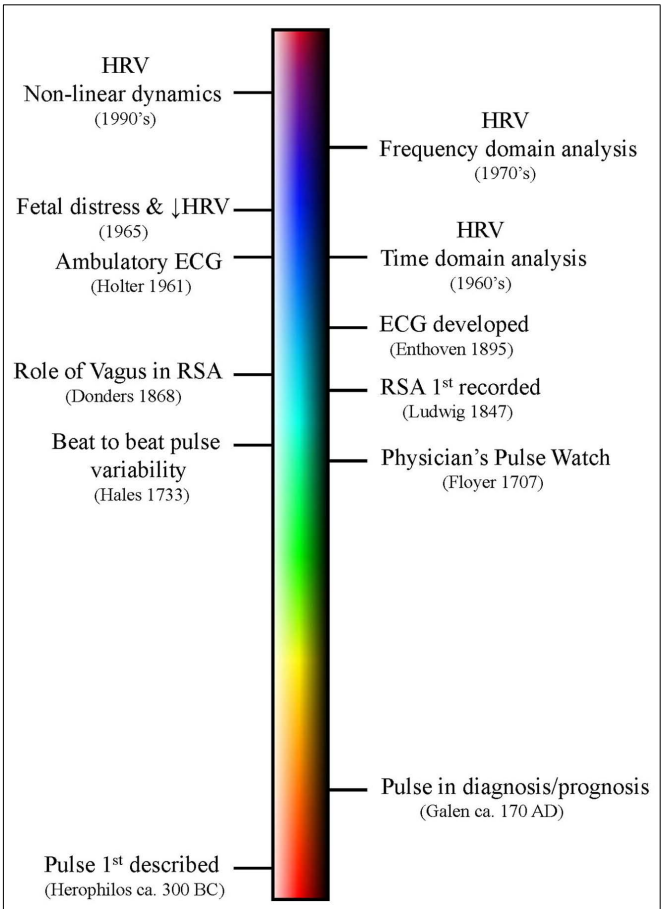

FIGURE 2 | A timeline of some of the major events in the discovery of heart rate variability (HRV). Please note that the timeline is not drawn to scale.

FIGURE 3 | Portrait of Herophilos (ca. 335–280 BC). He was the first to measure the heart beat using a water clock to time the pulse. Source: Reproduced with permission from the John P. McGovern Historical Collections and Research Center; Houston Academy of Medicine-Texas Medical Center Library; Houston, TX, USA. P-254, color photo; Artist: Joseph F. Doeve, painted in 1953.

using a water clock or clepsydra; Bedford, 1951; Bay and Bay, 2010). Galen also extensively cites and criticizes the description of the pulse made by Archigenes (Ἀρχιένης, fl. first century AD, born in Syria but practiced medicine in Rome; Bedford, 1951). Archigenes apparently described eight characteristics of the pulse, including observations on its regularity and irregularity (Bedford, 1951). The first individual by whom the original texts on the pulse have survived is Rufus of Ephesus (fl second century; Bedford, 1951). He was the first to recognize that the pulse was caused by the contraction and relaxation of the heart (Bedford, 1951).

Irrefutably, the most influential ancient physician/scientist was Galen of Pergamon (Γαληνός, Latinized as Claudius Galenus, 131–200 AD; **Figure 4**). He wrote at least 18 books on the pulse including at least 8 treatises that described using pulse for the diagnosis and predicting the prognosis of disease (Bedford, 1951; Boylan, 2007). His teaching on pulse dominated medical practice for almost sixteen centuries through the Middles Ages and the Renaissance to dawn of modern era. Among his many findings, he was the first to report on the effects of exercise on pulse. For example, in "The Pulse for Beginners" he states:

> "Exercise to begin with – and so long as it is practiced in moderation – renders the pulse vigorous large, quick, and frequent. Large amounts of exercise, which exceed the capacity of the individual, make it small, faint, quick and extremely frequent."
>
> (Galen, 1997, p. 332)

FIGURE 4 | Portrait of Galen of Pergamon (131–200 AD). He wrote extensively about the pulse and used it for both the diagnosis and predicting the prognosis of disease. Source: National Library of Medicine (the history of medicine public domain image files). Lithograph by Pierre Roche Vigneron (Paris: Lith de Gregoire et Deneux, ca. 1865).

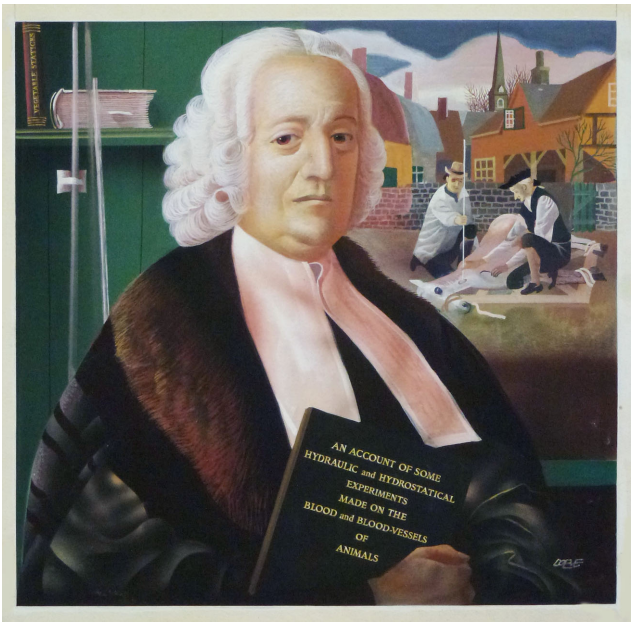

FIGURE 5 | Portrait of Rev. Stephen Hales (1677–1761). He was the first to report periodic fluctuations in arterial pressure and the beat-to-beat interval that varied with respiration. These pioneering studies were performed on conscious horse. Source: Reproduced with permission from the John P. McGovern Historical Collections and Research Center; Houston Academy of Medicine-Texas Medical Center Library; Houston, TX, USA. P-261, color photo; Artist: Joseph F. Doeve, painted in 1953.

It was not until the early eighteenth century that the more accurate measurement of time allowed for more quantitative evaluations of heart rate. John Floyer (1649–1734), an English physician, is credited with inventing what he called the "The Physician Pulse Watch," a portable clock that added a second hand and push-piece that could stop the watch (Floyer, 1707, 1710). Using this device, he tabulated both pulse and respiration under a variety of conditions. He published his findings in two volumes (Floyer, 1707, 1710) and became a strong advocate of using the timing of the pulse so that "*we may know the natural pulse and the excesses and defects from this in disease*" (Floyer, 1707, p.13).

With the increased availability of accurate time-pieces, periodic fluctuations in the arterial pulse were soon described. In 1733, the Rev. Stephen Hales (1677–1761; **Figure 5**) was the first to report that the beat-to-beat interval and arterial pressure level varied during the respiratory cycle (Hales, 1733). In 1847, Carl Ludwig (1816–1895; **Figure 6**) using his invention, the smoked drum kymograph (a device that allowed for the measurement of mechanical activity), was the first to record periodic oscillations in the amplitude and timing of the arterial pressure waves that varied during the respiration (Ludwig, 1847). Using the dog, he noted that pulse regularly increased during inspiration and slowed during expiration, thereby providing the first documented report of what subsequently became know as the RSA (Ludwig, 1847). In the late nineteenth and early twentieth century Willem Einthoven (1860–1927), using galvanometers to measure accurately changes in electrical currents, produced the first continuous recordings of the electrical activity of the heart (Einthoven, 1895; Katz and Hellerstein, 1982; Hurst, 1998). With the development and standardization of the electrocardiogram, it became possible to evaluate beat-to-beat changes in the cardiac rhythm. In the early 1960s, ambulatory ECGs could be obtained over long periods of time (e.g., 24 h) using a small portable recorder developed by Norman "Jeff" Holter (1914–1983; Holter, 1961) which further sparked the interest in understanding the relationship between beat-to-beat variation in the heart interval and disease. With the advent of modern digital signal processing techniques (Cooley and Tukey, 1965), it became possible to quantify and to analyze subtle beat-to-beat variations in cardiovascular parameters. Beginning in the early 1970s several groups applied power spectral analysis to investigate the physiological basis for the individual frequency components that compose the periodic variations in heart rate (Hyndman et al., 1971; Sayers, 1973; Chess et al., 1975; Hyndman and Gregory, 1975; Peňáz et al., 1978; Akselrod et al., 1981; Kay and Marple, 1981; Pagani et al., 1984, 1986; Pomeranz et al., 1985; Myers et al., 1986; Malliani et al., 1991). Since these pioneering studies the field has rapidly expanded. Both time and frequency and time domain techniques have been used to quantify HRV. Recently, techniques derived from the new science of deterministic "chaos" have been used to evaluate the non-linear dynamic characteristics of HRV (Goldberger and West, 1987; Denton et al., 1990; Bigger et al., 1996; Lombardi et al., 1996; Mäkikallio et al., 1997, 1999a,b; Huikuri et al., 1998, 2000, 2003; Pikkujämsä et al., 1999). Some of these methodologies will be briefly discussed in a subsequent section of this essay.

FIGURE 6 | Photograph of Carl Lugwig (1816–1895). He is credited with inventing the smoked drum kymograph and used it to record periodic oscillations in the amplitude and timing of arterial pressure that varied during respiration. Using the dog, he reported that the pulse rate increased during inspiration and decreased during expiration, thereby providing the first documented recordings of the respiratory sinus arrhythmia. Source: National Library of Medicine (the history of medicine public domain image files). Picture made in 1856.

The physiological basis that underlies HRV has been the subject of intensive investigation and still remains an unresolved question. In later half of nineteenth century, several investigators proposed that changes in neural activity were responsible for the periodic changes in the arterial pressure interval (Traube, 1865; Donders, 1868; Hering, 1869, 1871; Cyon, 1874; Mayer, 1876; Frédéricq, 1882). Ludwig Traube (1818–1876) proposed that "irradiation" from central neural (medullary) respiratory neurons unto the cardiovascular centers was responsible for arterial waves (Traube, 1865) while in 1871 Karl Ewald Hering (1834–1918) concluded that these periodic changes originated from the reflex activation of afferent fibers located in the lungs (Hering, 1871). Frédéricq (1851–1935) demonstrated that arterial pressure variability continued when the lung motion ceased (by opening the chest cavity) and conversely, the RSA was eliminated by the inhibition of respiratory motor activity following hyperventilation (Frédéricq, 1882). Later, Francis A. Bainbridge (1874–1921) proposed that the RSA did not involve the nervous system but rather results from mechanical distortion of the atria due to changes in thoracic pressure during the respiratory cycle (Bainbridge, 1930). The first systematic evaluation of these competing hypotheses was reported by Gleb von Anrep (1891–1955) and associates (Anrep et al., 1936a,b). They performed studies in dogs that clearly demonstrated that either central respiratory neural activity or the activation of pulmonary stretch receptors could maintain RSA when the other factor was controlled (Anrep et al., 1936b). They concluded that both central and peripheral mechanisms can contribute to these beat-to-beat changes in heart rate. It has also been subsequently suggested that cyclic activation of the arterial baroreceptor, thermoregulatory control, and the renin–angiotensin system may also contribute to oscillations in heart rate (Sayers, 1973; Hyndman, 1974; Akselrod et al., 1981; Madwell et al., 1989). Despite nearly 90 years of subsequent investigation, the relative contribution of the central and peripheral mechanisms responsible for RSA (Eckberg, 2003) and its functional significance (Hayano et al., 1996; Sin et al., 2010) remain the subject of considerable controversy and active investigation.

With regards to efferent neural contribution to periodic changes in heart rate, Franciscus C. Donders (1818–1889) suggested that the changes in heart period associated with respiration resulted from activation of the cardiac vagus nerves (Donders, 1868). This view soon gained wide-spread acceptance. By 1910, Heinrich E. Hering (1866–1948) could write that "*it is known with breathing that a demonstrable lowering of heart rate . . . is indicative of the function of the vagi*" (Hering, 1910). Hamlin et al. (1966) convincingly demonstrated that RSA in the dog resulted from activation of the vagal nerves, an observation that has been confirmed in other mammalian species (cats: Chess et al., 1975; Yongue et al., 1982; rats: McCabe et al., 1985; Cerutti et al., 1991; horse and ponies: Hamlin et al., 1972; Rugh et al., 1992), including human (Davies and Neilson, 1967; Melcher, 1976; Hirsch and Bishop, 1981; Selman et al., 1982; Eckberg, 1983). Sympathetic neural activation was also found to contribute significantly periodic arterial pressure changes (Guyton and Harris, 1951; Preiss et al., 1975). Arthur C. Guyton (1919–2003) and co-workers reported that vasomotor waves occur synchronous with increases in sympathetic nerve activity (Guyton and Harris, 1951). Similarly, a strong correlation between respiration, sympathetic nerve outflow, and changes in arterial pressure have been reported (Preiss et al., 1975). By the early 1970s several investigators began to apply modern digital processing techniques to evaluate the relationship between the autonomic neural regulation and in subtle changes in both arterial pressure waves and heart rate (Katona et al., 1970; Hyndman et al., 1971; Sayers, 1973; Chess et al., 1975; Hyndman and Gregory, 1975; Peñáz et al., 1978). For example, Katona and Jih (1975) proposed that periodic changes in heart rate that corresponded to the respiration could be used as non-invasive maker of cardiac parasympathetic regulation. A multitude of studies have been performed since these pioneering studies were competed nearly 40 years ago (for reviews see: Appel et al., 1989; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Bigger, 1997; Cohen and Taylor, 2002; Grossman and Taylor, 2007; Thayler et al., 2010). Today, it is now clear that the rhythmic changes in the heart rate at any given moment reflect the complex interactions between parasympathetic nerve fibers (activation decreases heart rate), sympathetic nerve fibers

(activation increases heart rate), mechanical, and other factors on the pacemaker cells usually located in the sinoatrial node.

HEART RATE VARIABILITY TECHNIQUES

A number of techniques have now been developed to quantify this beat-to-beat variability in order to provide indices of cardiac autonomic regulation in both health and disease (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Bigger, 1997; Denver et al., 2007; Grossman and Taylor, 2007; Thayler et al., 2010). There are two primary approaches for the analysis of HRV: time domain and frequency domain methods (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Denver et al., 2007). The time domain measures of this variability are easier to calculate but tend to provide less detailed information than the frequency domain approaches. The time domain methods employ either statistical or geometric approaches (**Table 1**). Each approach shares the common feature that either heart rate at any point in time (instantaneous heart rate) or the intervals between successive normal beats are determined from a continuous ECG record. Only the normal QRS complexes are used for the calculation; that is, only beats that result from the normal electrical activation pattern (i.e., depolarization originating from the sinoatrial node) are included, any abnormal beats (atrial or ventricular arrhythmias) are excluded. Thus, the normal-to-normal (NN) interval (the interval between adjacent normal QRS complexes) or the instantaneous heart rate (heart rate calculated on a beat by beat basis) is determined and simple descriptive time domain variables such as the mean NN interval, mean heart rate, and the range (longest NN minus the shortest NN) for a given time interval can be calculated (Kleiger et al., 1987). More detailed information is provided by the statistical analysis of a continuous sequence of normal beats (NN interval) for the time period of interest. Due to the ease of calculation, the SD (i.e., the square root of the variance) of the NN interval (SDNN) is one of the most widely used time domain indices of HRV (Kleiger et al., 1987). This calculation measures the total variability that arises from both periodic and random sources (equivalent to total power as determined by frequency domain spectral analysis). Artifact recognition also can influence time domain measurements of HRV (Malik et al., 1993). As such, these approaches cannot differentiate between the various factors that contribute to the total variance. Other approaches to quantify RSA involves obtaining the difference between the peak and the valley (or trough) of heart rate that occurs during a respiratory cycle (for each inspiration and expiration; Hirsch and Bishop, 1981; Eckberg, 1983; Fouad et al., 1984) or determining the number of adjacent pairs of normal beats that differ by more than 50 ms, NN50 (Ewing et al., 1984). The peak-to-valley techniques attempt to extract periodic variability from a baseline heart rate. If the amplitude of the RSA is large relative to the baseline variance of heart rate or at slower respiratory frequencies, this technique

Table 1 | Conventional heart rate variability measurements.

Variable	Units	Definition
TIME DOMAIN MEASURES		
a. Statistical		
SDNN	ms	SD of all normal R–R intervals
SDANN	ms	SD of the average normal R–R intervals calculated over short time periods (usually 5 min) for the entire recording period (usually 24 h)
RMSSD	ms	The square root of the mean squared differences between adjacent normal R–R intervals
SDNN index	ms	Mean of the SD of the normal R–R intervals calculated over short periods time (usually 5 min) for the entire recording period (usually 24 h)
NN50		The number of pairs of adjacent normal R–R intervals that differ by more than 50 ms
pNN50	%	NN50 divided by the total number of normal R–R intervals × 100
b. Geometrical		
HRV triangular index		Number of normal R–R intervals divided by the height of the histogram of all the normal R–R intervals measured on discrete scale with bins of 1/128 s (7.8125 ms)
TINN	ms	Baseline width of the minimum square difference of triangular interpolation of the highest peak of the histogram of all normal R–R intervals
FREQUENCY DOMAIN MEASURES		
Total	ms^2	Area under the entire power spectral curve (usually ≤0.40), variance of all normal R–R intervals
ULF	ms^2	Ultra low frequency power (≤0.003 Hz)
VLF	ms^2	Very low frequency power (0.003–0.0.04 Hz)
LF	ms^2	Low frequency power (0.04–0.15 Hz)
HF	ms^2	High Frequency power (usually 0.15–0.40 Hz*)
LFnu	nu	Normalized low frequency power (LF/LF + HF)
HFnu	nu	Normalized high frequency power (HF/LF + HF)
LF/HF		Ratio of the low-to high frequency power

*Nu, normalized units; * HF is shifted to higher ranges (0.24–1.04 Hz) in infants and exercising adults.*

provides a reasonable estimate of RSA that correlates well with other time domain indices (Grossman et al., 1990). However it is less accurate at higher respiratory frequencies and cannot quantify dynamic changes in the HRV on a beat by beat basis (Grossman et al., 1990). Other widely used statistical time domain calculations are listed in **Table 1**.

A series of NN intervals can also be plotted to provide a geometric pattern of the variability (Mayer-Kress et al., 1988; Malik et al., 1989; Farrell et al., 1991). Measurement of the geometric pattern (the width of the distribution) or the interpolation of a mathematically defined shape such as a triangle is used to provide a measure of the HRV (**Table 1**). One common non-linear technique graphs the sequence of normal R–R intervals using Poincaré (return or recurrence mapping) plots, where the beat (n) is plotted against the next beat ($n + 1$; Woo et al., 1994; Huikuri et al., 1996; Tulppo et al., 1996). The resulting shape provides graphical display of the variability such that the greater the scatter the greater the variability.

Although time series approaches provide information about changes in the total variability, with one notable exception (see below) these techniques are less useful in identifying specific components of this variability. Beginning in the late 1960s investigators applied techniques to partition the total variability into frequency components (Hyndman et al., 1971; Sayers, 1973; Chess et al., 1975; Hyndman and Gregory, 1975; Peñáz et al., 1978; Akselrod et al., 1981; Kay and Marple, 1981; Pagani et al., 1984, 1986; Pomeranz et al., 1985; Myers et al., 1986; Malliani et al., 1991; Laude et al., 1995). Power spectral density analysis produces a decomposition of the total variance (the "power") of a continuous series of beats into its frequency components (i.e., how the power distributes as a function of frequency; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Denver et al., 2007). The spectral power for a given frequency can then be quantified by determining the area under the curve within a specified frequency range. The two most common spectral analysis approaches are fast Fourier transform analysis (FFT) and autoregressive (AR) modeling (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Denver et al., 2007). FFT is based upon the assumption that a time series is composed of only deterministic components while with AR the data are viewed as being composed of both deterministic and random components. For shorter duration recordings (2–5 min) three main peaks are often identified: very low frequency (VLF) <0.04 Hz, low frequency (LF), 0.04–0.15 Hz, and high frequency (HF) 0.15–0.4 Hz. It should be noted that in infants and in response to exercise HF is shifted to a higher frequency ranges (0.24–1.04 Hz; Berntson et al., 1997). A fourth peak, ultra low frequency (ULF) 0.003–0.04 Hz), is obtained during longer recording periods (24 h). The absolute power at a given frequency is reported as ms^2, but LF and HF power are often measured in normalized units (nu) obtained by dividing the frequency band of interest by total power minus VLF (in practice, since total power largely reflects the combination of VLF, LF, and HF; LF + HF is used as the divisor). Finally, the ratio of LF to HF (LF/HF, no units) has been used as an index of the sympathetic/parasympathetic balance (Pagani et al., 1984, 1986; Malliani et al., 1991). However, this concept has been challenged (Kingwell et al., 1994; Koh et al., 1994; Hopf et al., 1995; Eckberg, 1997; Houle and Billman, 1999; Billman, 2009) as there is considerable controversy concerning the relationship between these frequency components and a particular division of the autonomic nervous system (Kollai and Mizse, 1990; Randall et al., 1991; Kingwell et al., 1994; Koh et al., 1994; Hedman et al., 1995; Hopf et al., 1995; Eckberg, 1997; Houle and Billman, 1999; Taylor et al., 2001; Parati et al., 2006; Denver et al., 2007; Grossman and Taylor, 2007; Billman, 2009).

One time domain approach can also be used to partition HRV within specific frequency bands, similar to those obtained by frequency domain techniques (Porges et al., 1980; McCabe et al., 1985; Billman and Dujardin, 1990; Denver et al., 2007). This method applies a moving polynomial to the heart period (R–R interval) time series to remove slow trends from the data (Porges et al., 1980; McCabe et al., 1985; Billman and Dujardin, 1990; Denver et al., 2007). A specified bandpass filter is then applied to the detrended data to remove all variance outside of the target frequency band. The variance of the residual data set then provides an estimate of the HRV within the target frequency band (Porges et al., 1980; McCabe et al., 1985; Billman and Dujardin, 1990; Denver et al., 2007). This procedure provides a time domain equivalent of spectral analysis with two important advantages; relatively short sequences of beats are required for calculation of the variance within the bandwidth of interest and the "moving filter" allows for the extraction of the RSA from non-stationary baselines Porges et al., 1980; McCabe et al., 1985; Billman and Dujardin, 1990; Denver et al., 2007). Thus, this technique can investigate the dynamic regulation of HRV in response to physiological challenges such as exercise, its onset, and its termination (Billman and Hoskins, 1989; Halliwill et al., 1998; Houle and Billman, 1999; Smith et al., 2005; Billman, 2006a,b; Billman and Kukielka, 2007), or myocardial ischemia (Collins and Billman, 1989; Halliwill et al., 1998; Houle and Billman, 1999; Billman and Kukielka, 2006).

As was previously noted, non-linear dynamic analysis approaches as derived from Chaos Theory have also been used to evaluate HRV. It is beyond the scope of the present essay to provide a detailed history of the development of this exciting new branch of science (for an outstanding non-technical account see Gleick, 1987). However, a brief discussion of some of the central tenets of Chaos Theory is merited.

Chaos is perhaps a less than ideal word choice, as in common usage this word conveys a sense of total disorder, unpredictability, and instability. Chaos Theory describes something entirely different: an underlying order in a seemingly randomly varying sequence of events. Truly random behavior never repeats itself, it is unpredictable and disorganized while, in contrast, periodic behavior always repeats in a predictable way over some finite time interval. One might say that chaos falls somewhere between total randomness and monotonically repeating periodic behavior and has characteristics of both: an apparent randomness that emerges as a consequence of a deterministic process. There is method found in the madness. A chaotic system exhibits aperiodic behavior with a subtle but regular pattern. The behavior never quite repeats itself exactly and is constrained within a range of values. Thus, the system is stable; it does not wander off into infinity as would a random

system. Deterministic chaos is non-linear, such that small changes in initial conditions lead to large changes with reiteration and also in that a single value y can be associated with more than one value of x (known mathematically as folded non-linearity; Denton et al., 1990). It is this ambiguity that gives rise to the "chaos." In biological systems, deterministic chaos promotes the stability (variation within limits) and flexibility (more than one value of x for each y) that allows an organism to maintain an optimal internal environment as it adapts to changing external demands, a new "wisdom of the body" that updates our concept of homeostasis (West, 2010). Lorenz (1963) was the first to describe deterministic aperiodic behavior in a weather simulation model (**Figure 7**). He recognized that exceedingly small changes in initial conditions eventually resulted in totally different weather patterns, an observation that has become know as the butterfly effect (i.e., a butterfly flapping its wings in China produces tornados in Kansas).

Beginning in the 1980s, evidence began to accumulate that strongly indicated that heart rate was the not the product of a regular periodic oscillator (a sine wave generator) but rather displayed complex non-linear dynamic behavior (Guevara et al., 1981; Goldberger and West, 1987). As a consequence, simple statistical approaches to analyze heart rate time series may lack the sensitivity necessary to detect subtle non-linear changes in HRV. Therefore, analytical approaches based upon Chaos Theory and fractal mathematics have been employed to evaluate HRV (Goldberger and West, 1987; Denton et al., 1990; Bigger et al., 1996; Lombardi et al., 1996; Mäkikallio et al., 1997, 1999a,b; Huikuri et al., 1998, 2000, 2003; Pikkujämsä et al., 1999). These techniques do not measure the HRV magnitude but provide an estimate of its complexity. The most common methods evaluate fractal-like properties of the heart rate time series. For example, the heart rate frequency (f) spectrum exhibits an inverse power law relationship ($1/f$; Saul et al., 1987; Bigger et al., 1996; Huikuri et al., 1998, 2000; Mäkikallio et al., 1999a,b; Pikkujämsä et al., 1999) that is a defining characteristic of fractal regulatory networks (Bassingthwaighte et al., 1994). The slope of the relationship between (log) frequency and (log) spectral density (power) from 10^{-4} to 10^{-2} Hz (an analysis of $1/f$ characteristics) was steeper in post-myocardial infarction and cardiac transplant patients than in healthy subjects and provided an excellent predictor of mortality following infarction (Bigger et al., 1996). These non-linear HRV measures were often better at predicting adverse cardiovascular events then were traditional markers of HRV (Bigger et al., 1996; Huikuri et al., 1998).

There are several other non-linear methods that have been used to evaluate HRV (Denton et al., 1990; Goldberger, 1990; Skinner et al., 1993; Pincus and Goldberger, 1994; Iyengar et al., 1996; Ho et al., 1997; Voss et al., 1998; Mäkikallio et al., 2001a,b; Perkiömäki et al., 2001a,b,c; Jokinen et al., 2003; Tulppo et al., 2005; Laito et al., 2006; Tuzcu et al., 2006; Perkiömäki, 2011). For example, detrended fluctuation analysis is a technique that detects the presence or the absence of fractal properties in R–R interval time series (Peng et al., 1995; Iyengar et al., 1996; Mäkikallio et al., 1999b, 2001b; Pikkujämsä et al., 1999; Perkiömäki et al., 2001a,c; Tulppo et al., 2005). Altered fractal properties have been shown to precede the onset of lethal cardiac arrhythmias; changes that traditional markers of HRV failed to detect (Mäkikallio et al., 1999b). In a similar manner, multiscale (Norris et al., 2008a,b) or approximate entropy (Pincus and Viscarello, 1992; Fleisher et al., 1993; Richman and Moorman, 2000 have been used to measure of the complexity of the system, that unlike other techniques (e.g., Lyapunov exponent, Wolf et al., 1985; Denton et al., 1990) can be applied to short data sets (Pincus and Viscarello, 1992; Richman and Moorman, 2000).

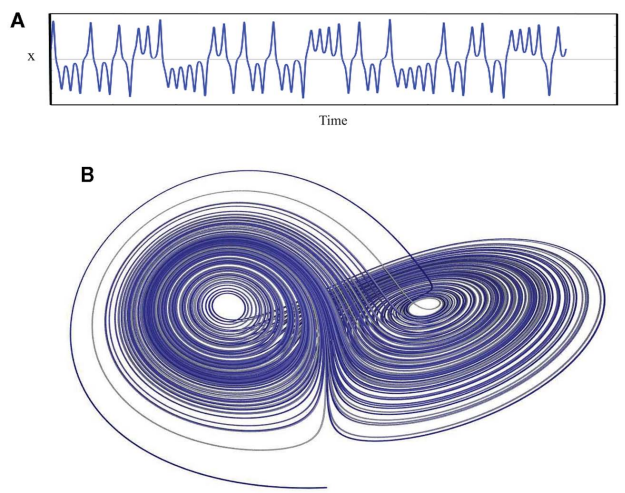

FIGURE 7 | A plot of the Lorenz attractor. (A) represents a the time series for single variable (x) while **(B)** illustrates the changing relationship between all three variables. The weather model that produced this plot consists of three differential equations: (1) $dx/dt = \sigma(y - x)$, (2) $dy/dt = x(\rho - z) - y$, (3) $dz/dt = xy - \beta z$. σ = Prandtl number, ratio of fluid viscosity to its thermal conductivity, ρ = Rayleigh number, heat transfer- the temperature difference between the top and the bottom of the gaseous system, and β = a geometric expression, the ratio of width to height of the containing holding the gaseous system. Lorenz used 10 for σ, 28 for ρ and 8/3 for β. $x(t)$ amplitude of the convection current, $y(t)$ temperature diffusion behavior (temperature difference between rising and falling air currents), and $z(t)$ normal temperature deviations. Some applets that can be used to create the Lorenz attractor are found at the following websites: www.cmp.caltech.edu/~mcc/Chaos_Course/Lesson1/Demo8.html, www.geom.uiuc.edu/~worfolk/apps/Lorenz/, http://www.exploratorium.edu/complexity/java/lorenz.html.

SOME LIMITATIONS AND CAVEATS

Although it is beyond the scope of the present review to analyze extensively the strengths and weaknesses of the various indices used to measure HRV, a brief discussion of some of the limitations with these techniques is merited. For a more detailed presentation the reader is encouraged to read one or more of the review articles that eloquently address the technical issues concerning the HRV and its relationship to cardiac autonomic regulation (Appel et al., 1989; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Bigger, 1997; Eckberg, 1997; Parati et al., 2006; Denver et al., 2007).

Respiratory parameters can profoundly alter heart rate and R–R interval variability independent of changes in cardiac autonomic regulation (i.e., against a constant background level of automatic regulation; Peñáz, 1957; Koepchen and Thurau, 1959; Angelone

and Coulter, 1964; Davies and Neilson, 1967; Hainsworth, 1974; Melcher, 1976; Hirsch and Bishop, 1981; Brown et al., 1993). It is now well established that increases in respiratory frequency reduce the amplitude of heart rate oscillations (Peñáz, 1957; Angelone and Coulter, 1964; Melcher, 1976; Hirsch and Bishop, 1981; Brown et al., 1993) while either increases in tidal (Koepchen and Thurau, 1959; Davies and Neilson, 1967; Melcher, 1976; Hirsch and Bishop, 1981; Eckberg, 1983; Kollai and Mizse, 1990; Brown et al., 1993) or static lung volume (Hainsworth, 1974) provoke increases in the R–R interval variability. Conversely, reductions in respiratory frequency increase HRV (Peñáz, 1957; Angelone and Coulter, 1964; Melcher, 1976; Hirsch and Bishop, 1981; Brown et al., 1993) while decreases in tidal volume lead to reductions in the R–R interval variability (Koepchen and Thurau, 1959; Davies and Neilson, 1967; Melcher, 1976; Hirsch and Bishop, 1981; Eckberg, 1983; Kollai and Mizse, 1990; Brown et al., 1993). Thus, it is critical to control breathing (paced or timed breathing) in order to interpret HRV data accurately. For obvious reasons, it is much more difficult to control respiratory parameters in conscious animal than in human studies. However, these respiratory parameters frequently are not controlled even in human studies (Brown et al., 1993). Brown et al. (1993), reviewed the human literature and found that only about 51% controlled respiratory rate, and even fewer studies controlled for tidal volume (11%). They further reported that respiratory parameters not only altered HF power but also strongly influenced the LF components of the R–R interval power spectrum, a component that previously was viewed to vary independently of changes in respiration (Brown et al., 1993).

It also must be emphasized that HRV only provides an indirect assessment of cardiac autonomic activity and does not provide a direct measurement of either cardiac parasympathetic or sympathetic nerve activity. Thus, any relationship between HRV and cardiac autonomic regulation is qualitative rather than quantitative in nature. In other words, a low or high amount of HRV may reflect a decreased or increased cardiac autonomic regulation but does not provide a quantification of the actual cardiac nerve firing rate. Furthermore and as previously noted, there is considerable debate as to the exact relationship between changes in cardiac autonomic activity and a particular branch of the autonomic nervous system (Kollai and Mizse, 1990; Randall et al., 1991; Kingwell et al., 1994; Hedman et al., 1995; Hopf et al., 1995; Eckberg, 1997; Houle and Billman, 1999; Taylor et al., 2001; Parati et al., 2006; Denver et al., 2007; Grossman and Taylor, 2007; Billman, 2009). For example, frequency domain analysis of HRV usually reveals two or more peaks, a LF (<015 Hz), and a higher frequency peak (>0.15 Hz) that are often assumed to correspond to cardiac sympathetic and cardiac parasympathetic neural activity, respectively (Pagani et al., 1984, 1986; Malliani et al., 1991). However, accumulating evidence clearly demonstrates this assumption is naïve and greatly oversimplifies the complex non-linear interactions between the sympathetic and the parasympathetic divisions of the autonomic nervous system. This is particularly true with regards to the relationship between LF power and cardiac sympathetic regulation (Randall et al., 1991; Kingwell et al., 1994; Hopf et al., 1995; Eckberg, 1997; Houle and Billman, 1999; Parati et al., 2006; Billman, 2009).

Low frequency power was found to be reduced by selective parasympathectomy and also was not totally eliminated when the denervation was combined with beta-adrenoceptor blockade (Randall et al., 1991). Furthermore, interventions that would be expected to increase cardiac sympathetic activity, such as acute exercise or myocardial ischemia, not only failed to increase LF power but actually provoked significant reductions this variable (Houle and Billman, 1999). Thus, LF component of HRV reflects both sympathetic, parasympathetic and other as yet unidentified factors. Accordingly, LF power should not be used as an index of cardiac sympathetic regulation.

Although the vast majority of the clinical and the experimental studies demonstrate a strong association between HF power and cardiac parasympathetic activity (Appel et al., 1989; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Bigger, 1997; Billman, 2009; Thayler et al., 2010), this concept has also been challenged (Kollai and Mizse, 1990; Hedman et al., 1995; Taylor et al., 2001; Parati et al., 2006). Just as parasympathetic activation exerts profound influences on the LF component of HRV, sympathetic neural activity may modulate the HF component of the R–R interval variability (Taylor et al., 2001; Cohen and Taylor, 2002). Taylor et al. (2001) found that cardioselective beta-adrenergic receptor blockade (drugs that should not indirectly alter vagal outflow via action within the central nervous system) increased RSA amplitude over a wide range of respiratory frequencies (i.e., the increases were not restricted to lower frequencies, <0.15 Hz). They concluded that "*cardiac sympathetic outflow can oppose vagally mediated R-R interval oscillations and sympathetic blockade removes this effect*" (Cohen and Taylor, 2002). Thus, differences in cardiac sympathetic activation during a physiological challenge (e.g., exercise or postural changes) in healthy subjects or that occur as consequence of cardiovascular disease (following myocardial infarction) could restrain vagally mediated changes HRV. These data further demonstrate that HRV is a complex phenomenon that should not be solely attributed to changes in cardiac vagal efferent nerve traffic.

In addition to autonomic influences, a portion of the HRV occurs as a consequence of the mechanical events (due to stretch of the atria that results from both changes in cardiac filling and the changing thoracic pressure that occur during respiration) as was first proposed by Bainbridge (1930). This conclusion is supported by the observation that heart transplant patients, despite the absence of cardiac nerves, still exhibit small (∼2–8% of normal) change in R–R interval associated with the respiratory cycle (Bernardi et al., 1989). Taylor et al. (2001) further demonstrated that atrial stretch can exert significant influences on R–R interval in subjects with complete autonomic blockade. They found that after combined cholinergic and adrenergic receptor blockade slow deep breathing could still provoke oscillations of ∼120 ms in healthy human subjects (Taylor et al., 2001). Thus, given the complex interactions between cardiac sympathetic and cardiac parasympathetic nerves that are confounded by the mechanical effects of respiration, HRV data should be interpreted with appropriate caution.

CLINICAL APPLICATIONS

Heart rate variability has gained wide-spread acceptance as a clinical tool for the evaluation of cardiac autonomic changes in patients (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Bigger, 1997; Hohnloser et al., 1997; Billman, 2009; Thayler et al., 2010). The term "HRV" yields nearly 14,000 "hits" when placed in the pubmed search engine. A variety of cardiovascular risk factors and disease states have all been shown to reduce HRV, including diabetes (Murray et al., 1975; Ewing et al., 1985; Vinik et al., 2003; Rosengard-Barlund et al., 2009), smoking (Mancia et al., 1997; Karakaya et al., 2007), obesity (Skrapari et al., 2007), Work stress (Thayler et al., 2010), hypertension (Pagani et al., 1984; Pagani and Lucini, 2001; Maule et al., 2008), and heart failure (Saul et al., 1988; Binkley et al., 1991; Woo et al., 1994; Adamopoulos et al., 1995; Kiilavuori et al., 1995; De Jong and Randall, 2005).

Eppinger and Hess (1915) provide the first suggestion that HRV could be used to provide some insight in abnormalities in autonomic regulation in disease. They wrote "*clinical facts, such as respiratory arrhythmia, habitual bradycardia, etc. have furnished the means of drawing our attention to variation in the tonus of the vagal system*" (Eppinger and Hess, 1915, p. 12). They further emphasized that pharmacological manipulation of the cholinergic system might provide an avenue for treatment (Eppinger and Hess, 1915). However, the first reports of the applications of HRV in the clinic only began to appear in the mid 1960s. Hon and Lee (1965) noted that fetal stress was preceded by reduction in the inter-beat interval even before any appreciable change in average heart rate could be detected. Fetal heart rate monitoring has now become the standard of care and has contributed to reductions in morbidity associated with fetal distress. In the 1970s, Ewing and co-workers used short-term changes in R–R interval in response to simple autonomic challenges to detect autonomic neuropathy in diabetic patients (Murray et al., 1975; Ewing et al., 1985).

About the same time, Wolf was the first to demonstrate a relationship between HRV and mortality following myocardial infarction (Wolf et al., 1978). This observation has subsequently been confirmed (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Bigger, 1997; Hohnloser et al., 1997; Thayler et al., 2010). Specifically, HRV is reduced in patients recovering from a myocardial infarction and, further, those patients with the greatest reduction in this variable also have the greatest risk for sudden death (Myers et al., 1986; Kleiger et al., 1987; Farrell et al., 1991; Bigger et al., 1992). Kleiger and co-workers (Myers et al., 1986; Kleiger et al., 1987; Bigger et al., 1992) found that in patients recovering from myocardial infarctions, those with the smallest HRV (SD of R–R interval) had the greatest risk of dying suddenly. The relative risk of mortality was 5.3 times greater in patients with R–R interval variability less than 50 ms compared to patients with variability greater than 100 ms (Kleiger et al., 1987). This finding has been subsequently confirmed by numerous more recent clinical studies; reductions in HRV following myocardial infarction now represent one of the strongest independent predictors of mortality following infraction. (La Rovere et al., 1988, 1998; Malik et al., 1989; Mazzuero et al., 1992; Huikuri et al., 1996; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Bigger, 1997; Hohnloser et al., 1997; Lanza et al., 1998). To cite just one example, La Rovere et al. (1988, 1998), reporting for the ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) group, found that post-myocardial infarction patients with either low HRV or a small heart rate response to an increase in blood pressure (i.e., baroreceptor reflex sensitivity) had a much greater risk of sudden death than those with well preserved cardiac vagal tone. The greatest risk for mortality was observed in patients with a large reduction in both markers of cardiac vagal regulation (La Rovere et al., 1998).

Similar results have also been obtained using animal model of human disease (Billman, 2006a). For example, HRV was reduced to a greater extent in animals susceptible to ventricular fibrillation as compared to animals resistant to these malignant arrhythmias (Billman and Hoskins, 1989; Collins and Billman, 1989; Halliwill et al., 1998; Houle and Billman, 1999; Smith et al., 2005; Billman, 2006a,b; Billman and Kukielka, 2006). In particular, the susceptible animals exhibited a much greater reduction (withdrawal) of cardiac vagal regulation in response to either submaximal exercise (Billman and Hoskins, 1989; Halliwill et al., 1998; Houle and Billman, 1999; Billman, 2006a,b; Billman and Kukielka, 2006) or acute myocardial ischemia (Collins and Billman, 1989; Halliwill et al., 1998; Houle and Billman, 1999; Billman, 2006a; Billman and Kukielka, 2006). Heart rate recovery and the reactivation of cardiac parasympathetic regulation following the termination of exercise were also impaired in the animals subsequently shown to prone to ventricular fibrillation (Smith et al., 2005; Billman and Kukielka, 2007); an observation that has been noted in patients. For example, post-infarction patient with the slowest heart rate recovery following an exercise stress test also exhibited the highest mortality rate during the observation period (up to 12 years; Cole et al., 1999, 2000; Nishime et al., 2000; Morshedi-Meibodi et al., 2002; Nissinen et al., 2003; Jouven et al., 2005). Thus, despite the limitations noted in a previous section, HRV has proven to be an important tool for the identification of patients at risk for adverse cardiovascular events.

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Origin of heart rate variability and turbulence: an appraisal of autonomic modulation of cardiovascular function

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Heart period constantly changes on a beat to beat basis, due to autonomic influences on the sinoatrial node, and changes can be quantified as heart rate variability (HRV). In addition, after a premature ventricular beat, there are reproducible variations in RR interval, also due to baroreflex mediated autonomic influences on the sinoatrial node, that can be measured as heart rate turbulence (HRT). Impaired autonomic function as measured by HRV and HRT has proven to predict adverse outcomes in clinical settings. The ability of reduced HRV and HRT to predict adverse outcomes has been explained by their dependency on vagal mechanisms that could reflect an increased sympathetic and a reduced vagal modulation of sinus node, thus favoring cardiac electrical instability. Analysis of non-linear dynamics of HRV has also been utilized to describe the fractal like characteristic of the variability signal and proven effective in identify patients at risk for sudden cardiac death. Despite the clinical validity of these measures, it has also been evident that the relationship between neural input and sinus node responsiveness is extremely complex and variable in different clinical conditions. Thus, abnormal HRV or HRT on a clinical Holter recordings may reflect non-neural as well as autonomic mechanisms, and this also needs to be taken into account when interpreting any findings. However, under controlled conditions, the computation of the low and high frequency components of HRV and of their normalized powers or ratio seems capable of providing valid information on sympatho-vagal balance in normal subjects, as well as in most patients with a preserved left ventricular function. Thus, analysis of HRV does provide a unique tool to specifically assess autonomic control mechanisms in association with various perturbations. In conclusion, HRV measures are of substantial utility to identify patients with an increased cardiac mortality and to evaluate autonomic control mechanisms, but their ability to capture specific levels of autonomic control may be limited to controlled laboratory studies in relatively healthy subjects.

Keywords: autonomic modulation, sympathetic and vagal control, baroreflex mechanisms, spectral analysis, non-invasive evaluation of cardiac function

HEART RATE VARIABILITY

Heart rate variability (HRV) was the first non-invasive methodology extensively used to evaluate autonomic modulation of the sinus node in normal subjects and in patients with different cardiac and non-cardiac diseases and to identify patients at risk for an increased cardiac mortality. Since the initial report of Wolf et al. (1978) of the relationship of decreased RR variability on ECG and morality in post-MI patients, different methodologies have been developed and are now available to measure HRV in the experimental laboratory and in the clinical setting (Malik and Camm, 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). These range from simple statistical descriptors to complex non-linear mathematical parameters. Indeed, the possibility of obtaining a non-invasive evaluation of autonomic control mechanisms has resulted in a relative broad utilization of this methodology, often in a "black box" manner, by people who have bought HRV software. However, a precise understanding of and a consensus on the autonomic underpinnings of most HRV parameters has not been achieved.

TIME DOMAIN HRV PARAMETERS

In almost all normal subjects and patients, the heart period changes on a beat to beat basis even during resting, controlled conditions. These variations, largely due to respiration and sympathetic activity are almost abolished by simultaneous parasympathetic and sympathetic blockade (**Figure 1**). When the recording period is prolonged to several hours and conditions are no longer tightly controlled, additional factors may influence HRV. Among important influences are: environmental factors, level and extent of physical activity, emotional stress, meal times, talking vs. silence, sleep and quality of sleep, and recording duration.

Overall variability is largely dependent on the strength of the circadian rhythm with a predominant sympathetic activity during day-time and a predominant vagal activity during night-time. These autonomic changes can be easily inferred by considering the

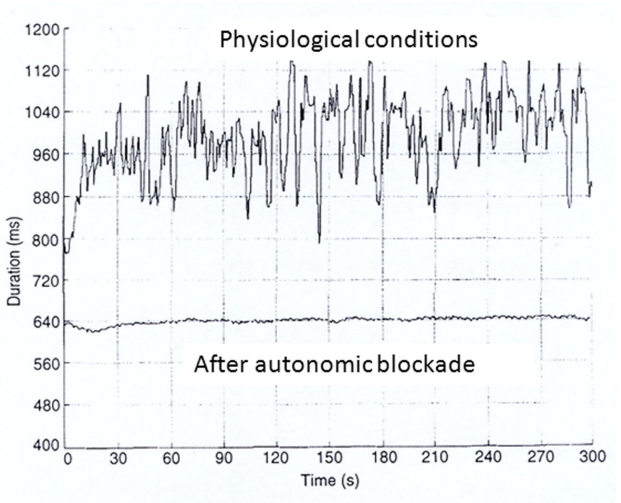

FIGURE 1 | RR interval time series during resting controlled conditions before and after autonomic blockade (atropine 0.04 mg/kg and propranolol 0.2 mg/kg).

variation in 24 h heart rate patterns. A reduced SDNN or triangular index is generally due to failure of heart rate to decrease at night and reflects an impaired vagal activity. Moreover, an increased resting heart rate is often present in patients with sign of sympathetic activation. These autonomic alterations are made worse by low day-time activity levels. Thus, both a diminished vagal and an increased sympathetic modulation of the sinus node may be reflected by a reduction in HRV (Kleiger et al., 1987; Malik and Camm, 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Fauchier et al., 1997; Nolan et al., 1999; Rashba et al., 2006). This interpretation is in agreement with experimental evidence indicating a pro-arrhythmic effect of sympatho-excitation (Lown and Verrier, 1976) and also with the findings that a reduction of these parameters is associated with an increased cardiac mortality in almost all clinical conditions characterized by an autonomic imbalance, e.g., after myocardial infarction, in patients with heart failure, hypertension, or diabetes (Kleiger et al., 1987; Malik and Camm, 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Fauchier et al., 1997; Nolan et al., 1999; Rashba et al., 2006).

The finding that time domain parameters were inversely correlated with aging and influenced by the extent of left ventricular dysfunction made clear that the causes of reduction in HRV and the interpretation of their predictive value were more complex than originally assumed and also involved neural and non-neural mechanisms (Malik and Camm, 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Indeed, the relationship between neural discharge, heart rate, and HRV is complex and cannot be explained by a linear model. Experimental findings, indicate (Zaza and Lombardi, 2001) that neural modulation of cycle length is rather dependent on where on the curve they are, much as cardiac contractility is not uniform over different amounts of stretch of the cardiac muscle. Thus, although the finding of an intrinsic rate dependency of autonomic indices does not limit the prognostic value of HRV, it markedly complicates its physiological interpretation. Moreover, it has also to be considered that at the sinus node transduction level acetylcholine-mediated effects occur with a faster kinetics than those of norepinephrine, thus having a larger potential for inducing near-term variability. Although this forms the basis of measuring acetylcholine-mediated vagal control of heart rate by examining more rapid changes in cycle length, it also complicates the interpretation of slower changes. In addition two other factors need to be considered: first, an increase in resting heart period duration may amplify the effects of any source of variability affecting the sinus node and second, that many cardiovascular drugs may directly affect the sinus pacemaker. All these factors have to be taken into account when interpreting HRV findings (Malik and Camm, 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Time domain variables can be divided into indexes of total variability such as SDNN, SDANN, or triangular index, preferably computed on long term recordings, and beat to beat indexes such as rMSSD or pNN50 that are more specific markers of vagal/respiratory effects but are also exaggerated by subtle arrhythmias that are not of respiratory origin, especially in older populations or in subjects with underlying clinical disease. These latter parameters can also be assessed on short-term recordings and have been frequently used to evaluate alterations in vagal modulation, although unless the subjects being studied are young and healthy, verification, by using a graphical analysis, that the measures truly reflect respiratory sinus arrhythmia, is suggested (Stein et al., 2005b).

FREQUENCY DOMAIN PARAMETERS

Spectral analysis allows the identification and quantification of the principal oscillations that characterize HRV especially during resting controlled conditions. Since the beginning, interest in this approach was driven by the possibility of correlating short-term spectral components to neural discharge (Akselrod et al., 1981; Pagani et al., 1986; Malliani et al., 1991; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) and of obtaining indirect information on neural modulation of the sinus node. In normal subjects under controlled conditions (**Figure 2**), two principal components are easily identified: a respiration-related high frequency (HF) component (0.15–0.4 Hz) and a lower frequency (LF) component (0.04–0.15 Hz) with a peak at about 0.1 Hz synchronous with the low frequency oscillations of arterial pressure (Akselrod et al., 1981; Pagani et al., 1986; Malliani et al., 1991; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The former was considered to reflect phasic vagal activity triggered by respiration. The origin of LF was more controversial, being the result of more complex neural mechanisms related to sympathetic and vagal outflows. The LF/HF ratio has been used as an index of sympatho-vagal interaction to explore autonomic control of

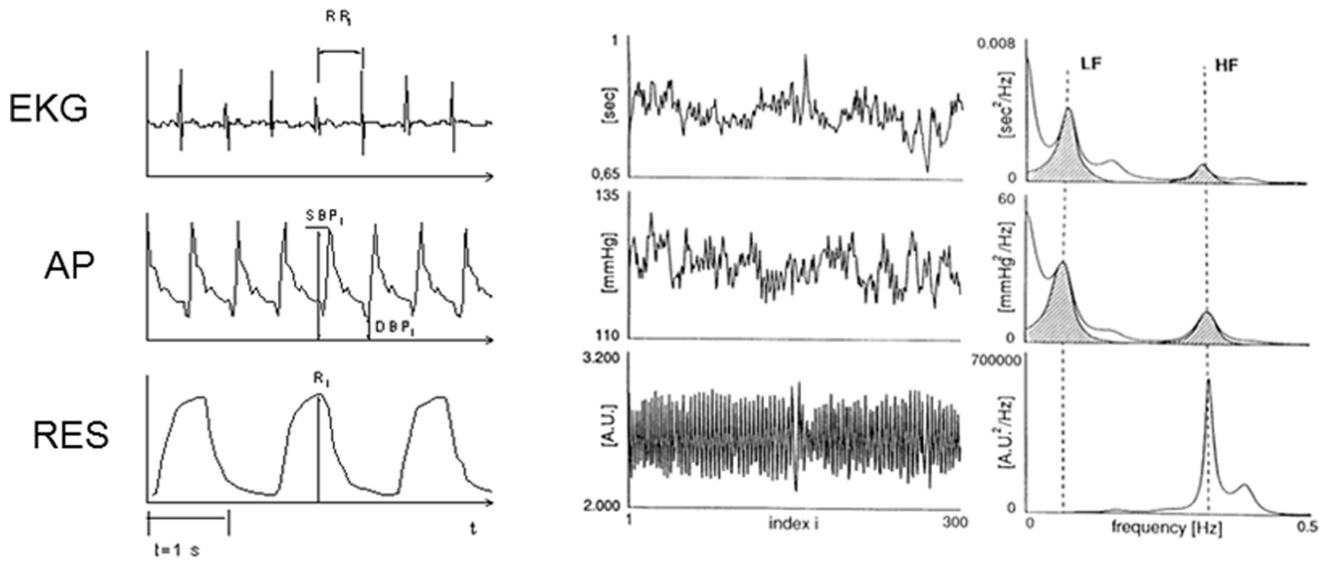

FIGURE 2 | Spectral analysis of short-term heart rate, arterial pressure and respiration variability. Signal recordings are presented in the left part of the figure. In the central panels, the time series of RR interval, systolic arterial pressure and respiratory movements are displayed. In the right panels the power spectrum of heart rate, systolic arterial pressure and respiration are presented. Two distinct components at low (LF: ∼0.01 Hz) and high (HF: ∼0.25 Hz) frequency are detectable (shadowed areas) in the autospectra of heart rate and systolic arterial pressure variability. A single HF component characterizes respiration. EKG, electrocardiogram; AP, arterial pressure; RES, respiratory movements.

sinus node during experimental intervention capable of producing reflex sympathetic or vagal activation (Akselrod et al., 1981; Pagani et al., 1986; Malliani et al., 1991; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). This was the case, for example, in patients during the acute phase of myocardial infarction or in the initial stages of heart failure (Akselrod et al., 1981; Pagani et al., 1986; Lombardi et al., 1987; Malliani et al., 1991; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In these patients, values of LF/HF ratio greater than 2 were considered to reflect a shift of sympatho-vagal balance toward a sympathetic predominance. More controversial was the interpretation of spectral component in patients with a marked reduction of ventricular function, where in spite of clinical signs of sympathetic activation, a reduction rather than an increase in LF component is commonly observed (van de Borne et al., 1997). Different factors including an altered central pattern of discharge at vasomotor centers, a loss of rhythmicity in sympathetic outflow as a consequence of a reduced baroreflex modulation, as well as a reduced responsiveness of the sinus node to neural inputs have been repeatedly advocated as explantation without, however, definitive experimental data (Malliani et al., 1991; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Burger et al., 1997; Grassi et al., 1999).

The issue of the meaning of LF, HF, or LF/HF is even more controversial when interpreting long term recordings (**Figure 3**) whereas environmental factors, extent of physical activity, and quality and duration of sleep are the major determinants of HRV and these components vary significantly over time (Malliani et al., 1991; Bigger et al., 1992; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

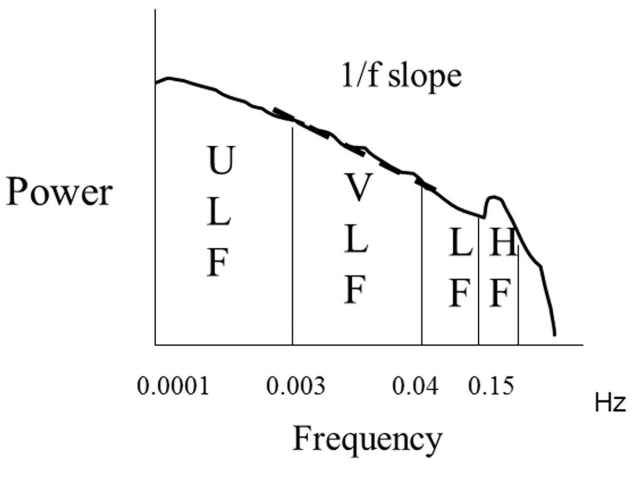

FIGURE 3 | Spectral analysis of ln transformed 24 h heart rate variability. Almost 90% of the power is distributed within the ultra low (ULF) and very low (VLF) frequency ranges. The slope of the relationship between ln power and frequency between 10^{-2} and 10^{-4} Hz is indicated and provides the value of $1/f$ slope.

In conclusion, spectral analysis of HRV must be restricted to short-term recordings under controlled conditions in order to definitely relate them to autonomic nervous system functioning. However, just as ST depression on a resting ECG is useful for finding clinical problems, a more definitive stress test

is needed to fully unmask ST depression. Similarly, short-term supine, controlled measures of HRV, which reflecting current autonomic status, may identify patients with extremely poor autonomic functioning but will miss autonomic abnormalities that are not present at rest. In addition, when associated with simultaneous continuous recording of arterial pressure and of respiratory activity (Pagani et al., 1986; Parati et al., 1988; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), spectral analysis offers the unique opportunity of evaluating the effects of arterial pressure changes on heart period and to compute baroreflex sensitivity, an important marker of cardiovascular integration.

NON-LINEAR DYNAMICS

The appraisal of the complexity of the different neural and non-neural feedback loops impacting on the sinus node and determining HRV has stimulated the search for novel indexes capable of reflecting the complexity and the correlation properties of the signal rather than the magnitude of variability (Goldberger, 1996; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Indices such as the exponent β of 1/*f* slope for long term analysis (Goldberger, 1996; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Lombardi, 2000) or the scaling exponent α^1 for short-term recordings (Peng et al., 1995; Mäkikallio et al., 2005) provide measures of fractal correlation properties of RR intervals at different time scales. These parameters do not reflect a specific component of autonomic modulation and therefore cannot be used to evaluate the presence of increased sympathetic activation or of a reduced vagal tone. They rather reflect the characteristics of heart rate behavior and, in particular, its complexity or randomness which are dependent upon the functional integrity of autonomic control mechanisms and sinus node responsiveness (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

In normal subjects the power-law slope has been reported to be equal to −1, although this assertion is yet not supported by epidemiologic data. It becomes more negative after myocardial infarction (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Lombardi, 2000) and with aging (Stein et al., 2008). In ICD recipient patients, analysis of the storage electrograms revealed a further reduction of power-law slope of slow fluctuations in the minutes preceding the onset of ventricular tachycardia in comparison to control (Lombardi et al., 2000).

The detrended fluctuation analysis technique quantifies the relations of heart rate fluctuations at different time scales. In younger populations, the short-term fractal scaling exponent α^1 (or DFA1; correlations on the scale of 3–11 beats) in the 1.20–1.35 range is commonly observed. In the Cardiovascular Health study (Stein et al., 2008), DFA1 of 1.05 was the cut-point value for risk of mortality. Lower values in post-MI studies and in CHF patients (<0.80 or 0.85, respectively) indentify patients at higher risk (Huikuri et al., 2000). The short-term fractal scaling exponent has been particularly effective in identifying patients at risk for sudden cardiac death (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Mäkikallio et al., 1999, 2005; Huikuri et al., 2000; Lombardi et al., 2001; Stein et al., 2005a, 2008). The analysis of short-term fractal properties of HRV has been demonstrated to be superior to conventional HRV measures in term of prognostic value in predicting both arrhythmic and non-arrhythmic cardiac death in post myocardial infarction patients (Mäkikallio et al., 2005). Longer term DFA without correction is not very useful for risk stratification and DFA2 (α^2, correlations on the scale of 12–20 beats) has not been shown to add to risk stratification in most situations.

Altered short-term fractal scaling exponent has also been observed to precede ventricular fibrillation onset among patients who experienced this arrhythmia during Holter recordings (Mäkikallio et al., 1999).

HEART RATE TURBULENCE

Whereas alterations in RR intervals induced by premature ventricular beats can affect computation of time domain parameters and the LF and HF components by increasing, respectively, overall variability as well as power in the HF range (Malik and Camm, 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), they are the basis for heart rate turbulence (HRT) analysis.

This methodology, initially described by Schmidt et al. (1999), is based on the fact that the changes in RR intervals following a premature ventricular beat in healthy people are well-defined and consist of an initial shortening followed by a progressive lengthening until there is a return to the pre-arrhythmia baseline. This pattern of changes, although not necessarily the exact magnitude, is consistent within the same subject and is the result of the interaction of complex neural control mechanisms (Bauer et al., 2008).

PHYSIOLOGICAL INTERPRETATIONS OF HRT

Since the original description of HRT methodology (Schmidt et al., 1999; Bauer et al., 2008), it was hypothesized that the neural reflexes responsible for HRT were due to two principal components: the initial acceleration was the result of a transient vagal inhibition and sympathetic activation in response to brief inhibition of baroreflex afferent input due to a hemodynamically inefficient ventricular contraction (Lombardi et al., 1989; Schmidt et al., 1999; Davies et al., 2001; Bauer et al., 2008). This explanation proved to be incomplete. The initial HR acceleration is a fast response phenomenon thus making unlikely that a surge in sympathetic outflow could play a major role. Then, after the compensatory pause, it was hypothesized that the overshoot of arterial pressure, due to increased ventricular filling, was responsible for the subsequent deceleration of heart rate through a combined sympathetic and vagal recruitment. Thus two distinct phenomena characterize HRT: the initial shortening, which is defined by the term turbulence onset (TO) and the subsequent lengthening that is described by the term turbulence slope (TS; **Figure 4**). A more complete appraisal of HRT mechanisms, took in account the fact

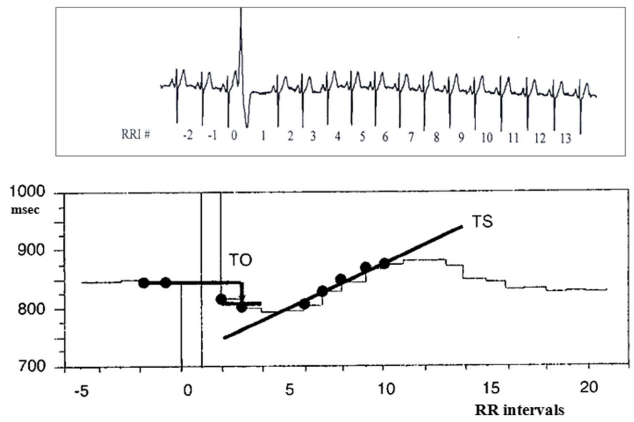

FIGURE 4 | Schematic representation of the RR interval changes induced by a premature ventricular contraction that are used to compute the two indexes of heart rate turbulence: turbulence onset (TO) and turbulence slope (TS).

that under physiologic conditions, systolic arterial pressure progressively increases after a premature ventricular beat and reaches its maximal value within eight cardiac cycles, thus activating a spontaneous baroreflex mechanism, with an initial sympathetic inhibition followed by a transient predominant vagal activation (Lombardi et al., 1989; Schmidt et al., 1999; Davies et al., 2001; Bauer et al., 2008). This explanation is supported by the finding that greatest variations in HRT indexes were observed in response to more premature spontaneous or induced ventricular contractions, which were associated with a greater fall in systolic pressure and longer compensatory pause (Davies et al., 2001; Roach et al., 2002; Watanabe et al., 2002; Savelieva et al., 2003; Wichterle et al., 2006; Bauer et al., 2008).

Heart rate turbulence is computed as TO and TS. TO is computed (Schmidt et al., 1999; Bauer et al., 2008) by determining the percentage differences between the two sinus RR intervals following the compensatory pause (RR_1 and RR_2) and the two RR intervals immediately preceding the premature beat coupling interval (RR_{-2} and RR_{-1}) divided by $RR_{-2} + RR_{-1}$. The initial acceleration is therefore computed on the first two sinus cycles following the premature ventricular beat and if there is no acceleration or an actual deceleration, both abnormal, TO is zero or positive. TS is defined as the maximum regression slope measured on any 5 consecutive sinus cycles within the first 15 sinus intervals after the compensatory pause and cannot be calculated when there are fewer than 15 sinus beats after the premature ventricular contraction. The slowing is due to a baroreflex mediated vagal activation and it is expressed in ms/RR interval. Computation of HRT requires at least five qualifying ventricular premature beats and TO and TS are computed from a "signal average" of the responses to all premature ventricular beats on the recording.

Both TO and TS were found to correlate with baroreflex sensitivity assessed by the phenylephrine method. The physiological changes in sinus cycle duration measure by HRT were abolished by vagal blockade with atropine and were almost unaffected after beta-blockade (Bauer et al., 2008).

The correlation between HRT and HRV is modest, suggesting that additional neural and non-neural factors are likely to play a major role in determining variations in the parameters that describes the two methodologies and that there is potential to use them in combination to leverage the risk stratification properties of both. In support of this, addition, the use of a combination of a decreased short-term fractal scaling exponent and abnormal HRT identified population-dwelling older adults at higher risk of cardiovascular death than the use of either parameter alone.

FACTORS INFLUENCING HRT

Left ventricular ejection fraction significantly affects HRT parameters. In patients with structural heart disease and depressed left ventricular function, HRT indexes are markedly depressed as a result of mechanical and autonomic factors: the enhanced post-extrasystolic potentiation and the altered autonomic pattern with signs of sympathetic activation and reduced vagal modulation account for such a finding (Bauer et al., 2008). However, a recent study showed that abnormal values for HRT identify older adults who are considered to be healthy by multiple measures of cardiovascular function including HRV, but who were, in fact, at almost eight times greater risk of cardiovascular death on follow up than healthy older adults with normal HRT (Stein and Barzilay, 2011).

Aging is also associated with a reduction of HRT parameters in a manner similar to most autonomic indexes. Finally HRT is reduced at higher heart rate, as a result of either a baseline sympathetic activation or of a heart rate dependency associated with the non-linear relationship between sinus node properties and vagal neural activity (Schwab et al., 2004; Bauer et al., 2008).

CONCLUSION

Heart rate variability and HRT are two non-invasive ECG-based techniques capable of providing relevant information on the autonomic modulation of the sinus node. They can be considered as complementary, taking into account that HRV mainly reflects the continuous interaction between neural modulatory mechanism and sinus node function while HRT can be considered a physiological stimulus–response test in which sinus node function and autonomic modulatory activity are perturbed by a transient stimulus either occurring spontaneously or induced by programmed electric stimulation.

The predictive value of these methodologies will be discussed in the next section. Of clinical relevance is the finding that depressed values for both HRV and HRT indexes are commonly observed in those clinical conditions associated with an increased cardiovascular mortality (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Bauer et al., 2008, 2009). Finally, the association between a reduced SDNN and markers of subclinical inflammation suggests that reduced vagal activity may mediate inflammation which opens new perspectives, not only for a better understanding on the physiopathological mechanisms responsible for cardiac mortality, but also for the identification of high-risk patients by combining autonomic with inflammatory markers (Lombardi, 2004; Stein and Barzilay, 2011).

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Role of editing of R–R intervals in the analysis of heart rate variability

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This paper reviews the methods used for editing of the R–R interval time series and how this editing can influence the results of heart rate (HR) variability analyses. Measurement of HR variability from short and long-term electrocardiographic (ECG) recordings is a non-invasive method for evaluating cardiac autonomic regulation. HR variability provides information about the sympathetic-parasympathetic autonomic balance. One important clinical application is the measurement of HR variability in patients suffering from acute myocardial infarction. However, HR variability signals extracted from R–R interval time series from ambulatory ECG recordings often contain different amounts of artifact. These false beats can be either of physiological or technical origin. For instance, technical artifact may result from poorly fastened electrodes or be due to motion of the subject. Ectopic beats and atrial fibrillation are examples of physiological artifact. Since ectopic and other false beats are common in the R–R interval time series, they complicate the reliable analysis of HR variability sometimes making it impossible. In conjunction with the increased usage of HR variability analyses, several studies have confirmed the need for different approaches for handling false beats present in the R–R interval time series. The editing process for the R–R interval time series has become an integral part of these analyses. However, the published literature does not contain detailed reviews of editing methods and their impact on HR variability analyses. Several different editing and HR variability signal pre-processing methods have been introduced and tested for the artifact correction. There are several approaches available, i.e., use of methods involving deletion, interpolation or filtering systems. However, these editing methods can have different effects on HR variability measures. The effects of editing are dependent on the study setting, editing method, parameters used to assess HR variability, type of study population, and the length of R–R interval time series. The purpose of this paper is to summarize these pre-processing methods for HR variability signal, focusing especially on the editing of the R–R interval time series.

Keywords: heart rate variability, artifact, editing, R–R intervals

INTRODUCTION

Heart rate (HR) variability quantifies the fluctuations in the time intervals between individual heart beats. HR variability can be easily obtained, e.g., from Holter recordings, which is a non-invasive technique and has a relatively good reproducibility. The analysis of HR variability can provide insights into autonomic nervous function and provide information about the sympathetic-parasympathetic autonomic balance and cardiovascular health (Malik and Camm, 1995; Task Force of ESC and NASPE, 1996).

Numerous studies have been published during the last three decades about HR variability in many pathological conditions or during exercise or different physiological conditions. Analyses of HR variability have been considered as a way of quantifying the risk of different arrhythmic events or even death in conjunction with cardiac and non-cardiac disorders (Kleiger et al., 1987, 2005; Saul et al., 1988; Bigger et al., 1992; Nolan et al., 1998; Yoshikawa et al., 1999; Aronson and Burger, 2000; Huikuri et al., 2000; Malik et al., 2000; La Rovere et al., 2003; Aronson et al., 2004; Camm et al., 2004; Kataoka et al., 2004; Mäkikallio et al., 2005; Stein et al., 2005).

However, in most cases HR variability signals are imperfect, since they contain disturbances of technical or physiological origins. For instance, technical artifact may result from poorly fastened electrodes or be due to movement of the subject resulting missing beats or beats whose onset cannot be clearly identified. Premature beats (ectopic beats) and atrial fibrillation are examples of physiological artifact. Ectopic beats introduce a bias into HR variability results and represent a significant problem in the interpretation of these results (Task Force of ESC and NASPE, 1996; Berntson et al., 1997). For example, ectopic beats in R–R interval time series impair the reliability of the HR variability power spectrum by introducing false frequency components into the power spectrum. As HR variability analyses have become more popular, the need for and importance of artifact correction have been emphasized (Laguna et al., 1996; Task Force of ESC and NASPE, 1996; Salo et al., 2001; Mateo and Laguna, 2003; Thuraisingham, 2006; Tarkiainen et al., 2007; Sassi and Mainardi, 2008; Colak,

2009; Kumaravel and Santhi, 2010). Several pre-processing methods for HR variability signal have been introduced. For example, pre-processing can involve editing of artifact by deletion, interpolations, or filtering. However, these different editing methods may have their own distinct effects on HR variability results and one could end up with different values if the R–R interval time series have been edited by deletion or interpolation. This paper reviews the pre-processing methods for R–R interval time series as part of HR variability analysis. The main focus is to describe the common editing methods for R–R interval time series and how they influence the results of the analysis. In addition, common false beats occurring in R–R interval time series are described.

BACKGROUND OF HR VARIABILITY

The normal heart rhythm is defined by the rate of sinus node depolarization. Sinus rhythm oscillates around the mean HR, which is dependent on continuous regulation by the autonomic nervous system (ANS). The main components of the ANS are the vagal (parasympathetic) and sympathetic centers in the central nervous system and thus the HR represents the balance of the inputs from the sympathetic and parasympathetic (vagus) nerves. Parasympathetic activity has decelerating effects on the HR and correspondingly sympathetic activity elevates the HR. The dynamic balance between parasympathetic and sympathetic activity causes a continuous oscillation of the HR – this is called HR variability (Levy and Martin, 1979). Under resting conditions, parasympathetic activity is dominant and the fluctuations in HR are mainly controlled by changes in parasympathetic activity (Levy, 1971; Chess et al., 1975). HR variability can be used as a window into the cardiorespiratory control system and as a tool for examining the fluctuations of the sympathetic and parasympathetic arms of the ANS but interpretation of the results depends on the conditions under which the recording was obtained and the length of the recording itself. HR variability measures have been postulated to describe the complex interaction between the ANS and HR and their modulation by many physiological factors.

Heart rate variability can be analyzed by different methods, which are usually categorized as time domain methods, frequency-domain methods, and methods based on the non-linear dynamics of HR. HR variability analyses are performed using R–R interval time series obtained from continuous electrocardiographic (ECG) recording by detecting each QRS complex. Normal-to-normal (N–N) interval time series contain only R–R intervals resulting from sinus node depolarizations.

Time domain analyses consists of various statistical parameters such as SDNN, the standard deviation of N–N beats, rMSSD, the square root of the mean squared differences of the successive N–N interval and pNN50, the percentage of differences between adjacent N–N intervals that are by more than 50 ms (Task Force of ESC and NASPE, 1996). In addition, different geometrical approaches that utilize the sample density distribution of N–N interval durations or the sample density distribution of differences between adjacent N–N intervals have been used to examine HR variability in the time domain (Malik et al., 1989b; Farrell et al., 1991).

Heart rate variability analysis in frequency-domain entails the estimation of the power spectrum of the R–R interval time series. Power spectrum computation can be performed with parametric or non-parametric methods. Parametric methods involve modeling the signal with an autoregressive (AR) model. Non-parametric spectrum estimation usually contains a computation of the fast Fourier transform (FFT) or periodogram. Information about spectral estimates can be obtained by decomposing the spectrum of R–R interval time series into quantified frequency components or by integrating the signals over a defined frequency band. Decomposition of the HR variability power spectrum provides information of how the variance is distributed as a function of frequency. Four main frequency bands are distinguished in the power spectrum of the HR variability signal, i.e.: high frequency (HF, 0.15–0.4 Hz), low frequency (LF, 0.04–0.15 Hz), very low frequency (VLF, 0.0033–0.04-Hz), and ultra low frequency (ULF, $<$0.0033 Hz) components (Task Force of ESC and NASPE, 1996; Stein et al., 2000). Various physiological phenomena have been associated with these frequency bands (Penaz et al., 1968; Sayers, 1973; Akselrod et al., 1981; Cohen and Taylor, 2002). Power spectrum analysis of HR variability is a popular method for studying autonomic neural fluctuations and it has been investigated in many studies in different patient populations and study settings. In addition, HR variability analysis in frequency domain can include computation of higher order spectrum (Chandran and Elgar, 1993) and wavelet transform (Vetterli, 1992).

In addition to conventional time and frequency-domain HR variability analysis, there are other methods based on the non-linear system theory and beat-to-beat dynamics. Non-linear analyses include return maps, such as Poincaré plots, fractal scaling analysis (DFA analysis), different complexity measures (e.g., Lyaponov exponent, correlation dimension), and approximate entropy, for instance. The clinical utility of these non-linear HR variability analyses has been tested in various sets of R–R interval data (Skinner et al., 1991; Bigger et al., 1992, 1996; Peng et al., 1995; Lombardi et al., 1996b; Mäkikallio et al., 1996, 1999; Ho et al., 1997; Huikuri et al., 1998, 2000; Brennan et al., 2001; Berkowitsch et al., 2004; Camm et al., 2004; Carpeggiani et al., 2004). In addition, beat-to-beat dynamics can be studied with novel methods such as HR turbulence after ventricular premature beats (VPB; Schmidt et al., 1999; Bauer and Schmidt, 2003) and deceleration capacity of HR (Bauer et al., 2006).

More detailed information about the various HR variability measures and their physiological background have been reviewed previously (Cohen and Taylor, 2002; Watanabe, 2003; Reed et al., 2005; Freeman, 2006; Bansal et al., 2009; Huikuri et al., 2009; Valentini and Parati, 2009; Wheat and Larkin, 2010; Karim et al., 2011).

ASSESSMENT OF THE HR VARIABILITY SIGNAL

The HR variability signal is usually obtained from an ECG recording that is captured over a certain period of time. Recording of the ECG is traditionally performed with a portable, ambulatory, electrocardiography device, a Holter monitor that enables recording of the heart rhythm continuously in outpatients although currently multiday recordings are beginning to be obtained via outpatient telemetry. Holter monitors are worn by the patients during his/her normal daily activities. Subsequently, the ECG data are uploaded to a computer for further processing and analysis. QRS complexes are detected from the ECG and the R-peak is usually used as the fiducial point due to its readily distinguishable amplitude. In fact,

the true marker for HR is the P wave onset, since the P wave is a more accurate marker of onset of the atrial depolarization than the R-peak (Clifford, 2006). However, in most cases the amplitude of the P wave is too low and difficult to detect accurately. Detection of the R-peaks is an important stage in the acquisition of the HR variability signal and requires a robust algorithm, i.e., the more accurate the R-peak detection, the less error in the R–R interval time series and in the subsequent HR variability analysis. Thus, the importance of accurate R-peak detection and high-quality software cannot be over-emphasized.

QRS complexes or R-peaks can be detected with different algorithms based for example on Hilbert transform (Benitez et al., 2001) or some other digital filtering methods (Pan and Tompkins, 1985; Hamilton and Tompkins, 1986; Israel et al., 2005; Arzeno et al., 2008), pattern recognition (Mehta et al., 1996), and wavelet transform (Mehta et al., 1996; Romero et al., 2003; Abdelliche and Charef, 2009), etc. Usually these methods include a feature extraction phase and a decision phase. There can be differences in the quality between different R-peak detection methods. Problems may appear especially with ECG signals containing noise, irregular rhythm, or frequently varying morphology of the QRS complexes. The more accurate the R-peak detection method the fewer missed and false R-peaks will need to be edited in R–R interval time series (Köhler et al., 2002; Manikandan and Soman, 2012). However, currently there are no detailed or standardized recommendations in the literature about the R-peak detection methods for inclusion in HR variability analyses. High-quality R–R interval software should contain a visual view of the actual point positions in the ECG signal of the R-peak detection process and the possibility to correct any false points. The correction of false points should be performed with accurate algorithm instead of manual correction to avoid errors. More information about the QRS complex detection can be found in the review of Köhler et al. (2002).

In addition, the better the sampling frequency in the ECG recording the better the resolution of the R–R interval time series. Sampling rate is recommended to be at least 500–1000 Hz (Task Force of ESC and NASPE, 1996). A low sampling rate may cause dispersion in the R-peak estimation and furthermore it will introduce bias into HR variability results, especially into spectrum, and some non-linear measures (Merri et al., 1990).

In order to form R–R interval time series, first, the difference in time of each of two consecutive R intervals is computed. Next, durations of the consecutive R–R intervals are defined and they form the discrete R–R interval time series, called the R–R interval tachogram or the HR variability signal. The R–R interval time series is not sampled at uniform intervals due to differences in the duration of adjacent heart beats. The fact that R–R intervals are represented as a function of time has to be taken into account, especially in the frequency-domain analysis. To avoid this issue, different approaches have been used prior to spectrum analysis. One approach is to compute the power spectrum directly from R–R interval time series available in function of the beat index. However in this approach, the spectrum is not a function of frequency but instead it is a function of cycles per beat (DeBoer et al., 1984; Baselli et al., 1987). Another approach is to resample R–R interval time series with different interpolation methods, such as spline interpolation in an attempt to distribute the non-equidistantly sampled R–R intervals so that they are equally spaced (Task Force of ESC and NASPE, 1996). The third approach is to use an integral pulse frequency modulation (IPFM) model. The IPFM method utilizes delta functions representing a series of impulses occurring at those times when heart beats (DeBoer et al., 1985). The generation of the R–R interval time series is illustrated in **Figure 1**.

ARTIFACT IN THE HR VARIABILITY SIGNAL

In the ideal situation, HR variability analysis is performed with R–R interval time series including only pure sinus beats (N–N intervals). However, R–R interval time series obtained from ambulatory ECG recordings are in most cases imperfect, since they can contain a different number of abnormal beats, artifact. Abnormal R–R intervals differ from sinus rhythm in their length and they represent disturbances of both technical and physiological origins and are present in almost all Holter ECG recordings. Physiological artifacts occur especially in patients suffering from different cardiovascular diseases. For example, cardiac dysrhythmias can appear at one time or another in 90–95% of patients with acute myocardial infarction (Kamath and Fallen, 1995). If steady state conditions are maintained in a laboratory study with pre-termined duration, then it may be possible to obtain recording without artifacts, at least in healthy subjects who are not suffering from any cardiovascular disease. However, in infants or uncooperative patients or during ECG recordings lasting for several hours, it is virtually impossible to obtain steady state conditions throughout the entire recording. Therefore, artifact represent a significant problem for the measurement of HR variability since they have impact on the reliability of the results. For example, if ectopic beats are left unedited, they may bias the HR variability power spectrum by increasing the power of higher frequency bands. Ectopic beats cause also erroneously higher values of the standard deviation of the R–R intervals (Thuraisingham, 2006). In **Figure 2**, an example of a short-term HR variability power spectrum including false frequency components due to ventricular ectopic is shown.

Physiological artifacts appear when disturbed electrical activity in the heart produces abnormal heart rhythms. The normal rhythm originates from the sinoatrial (SA) node. Abnormal impulse formation produces disturbances such as premature (ectopic) beats and atrial or ventricular fibrillation, for instance. A premature beat of ventricular origin is known as a VPB, or a premature ventricular contraction (PVC or VPC). Correspondingly, an ectopic beat of atrial origin is known as a premature atrial contraction (PAC or SVE). These kinds of disturbances are observed in the ECG as divergent waves and QRS complexes. Disturbances in the impulse conduction can produce errors in ECG such as pauses, for example due to AV block, SA block, or variable R–R intervals due to wandering atrial pacemaker.

Almost everyone experiences ectopic beats. Ectopic beats can be common events, especially in patients with cardiovascular disease, but they can be present also in healthy subjects (Kamath and Fallen, 1995). It is possible that as many as one in every three healthy men exhibit one or more VBPs during a 1-h ECG recording (Bikkina et al., 1992). Nonetheless, the prevalence of ectopic beats will introduce a major source of error into HR variability measurement. In particular, VPBs are usually followed by a

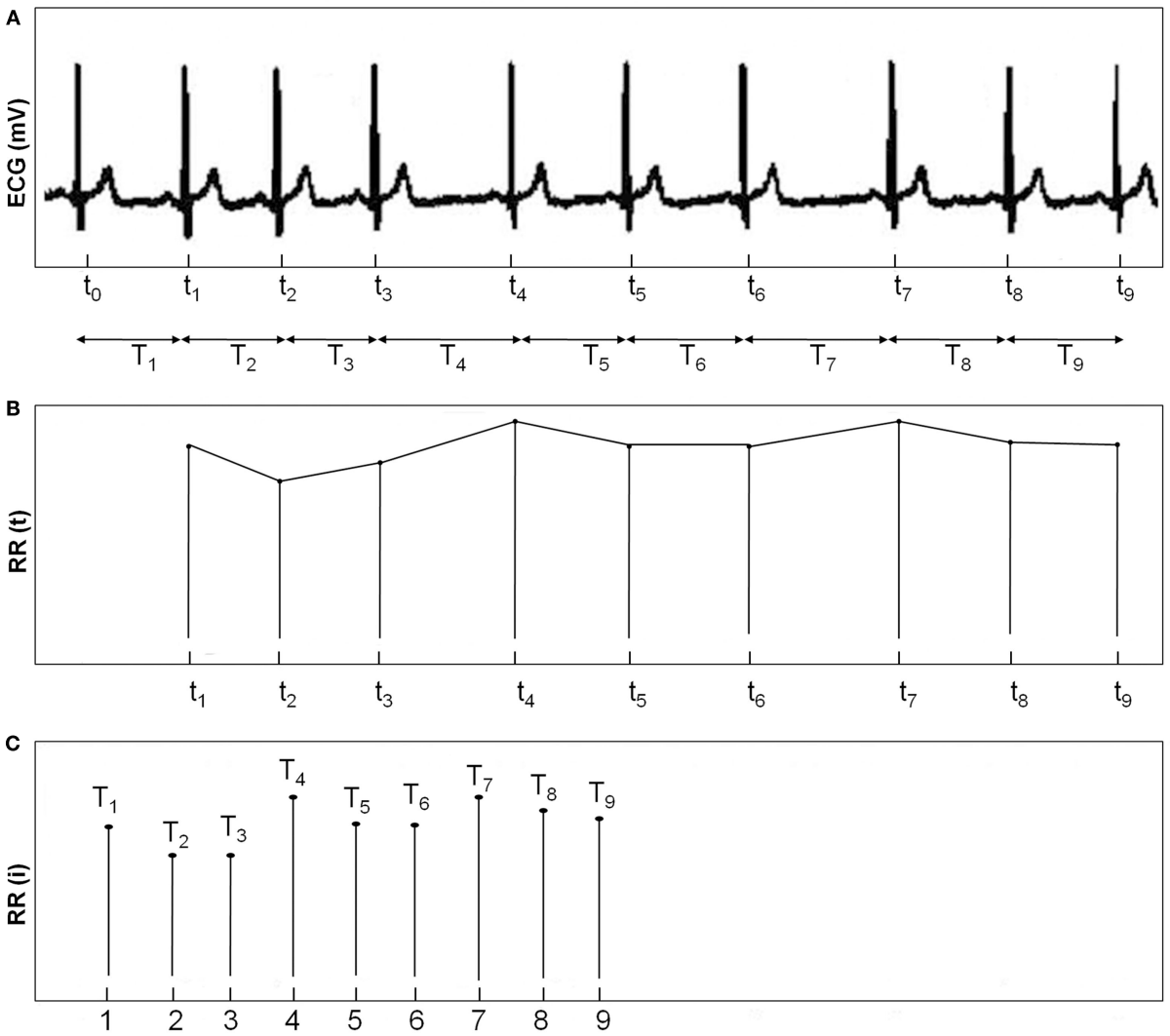

FIGURE 1 | (A) ECG with an event series of R-peaks. **(B)** Interpolated R–R interval time series **(C)** R–R interval time series.

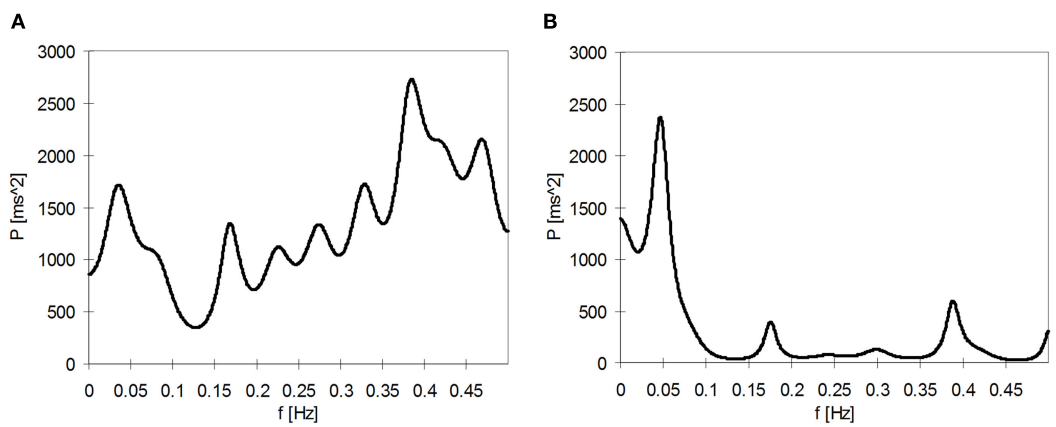

FIGURE 2 | (A) Power spectrum of a 3-min segment of the R–R interval time series containing a VPB. **(B)** Corresponding power spectrum, where the VPB is edited.

compensatory pause, before there is a return to the pre-ectopic baseline heart rhythm. In R–R interval time series, the appearance of a VPB with a compensatory pause is seen as an R–R interval of a short duration followed by a beat with a longer duration compared to normal sinus rhythm such as shown in **Figure 3**. Normally, both the ectopic beat and the following compensatory pause are edited.

In addition to physiological artifact, there may also be errors attributable to the technical aspects of the ECG recording. Problems may be traced to the software used to detect R–R intervals. For example, detection algorithm may fail if the threshold for R–R interval identification is set too low or too high. Technical artifact may also result from poorly fastened electrodes or from motion or sweating of the patient during the ECG recording. Technical artifact usually occur in larger epochs containing several consecutive false samples resembling random noise. Most of the Holter analysis software include some kind of automatic ECG recognition with the detection of QRS complexes and the classification of beats. Beat detection may include the possibility of labeling or annotating individual beats (Malik and Camm, 1995). However, the visual scanning of different morphologies of QRS complexes and labeling individual beats can be time consuming, especially in the case of long-term ECG recordings. For this reason, both high-quality HR data pre-processing software and the presence of an experienced Holter analysis specialist is very important if one wishes to obtain reliable HR data for analysis.

EDITING OF R–R INTERVALS

If the number of abnormal R–R intervals is relatively small and the artifact occurrence is infrequent, it is possible to reject or replace the artifacts by different correcting and editing methods before performing HR variability analysis. However, if the R–R interval time series contains many or recurrent artifacts, it is recommended that one should eliminate those entire segments with artifact prior to HR variability analysis or even to reject the entire recording (Malik and Camm, 1995). Several investigators have emphasized the importance of pre-processing of R–R interval time series to improve the quality of various HR variability analysis (Cheung, 1981; Malik et al., 1989a,b; Berntson et al., 1990, 1997; Cripps et al., 1991; Sapoznikov et al., 1992; Laguna et al., 1996; Task Force of ESC and NASPE, 1996; Salo et al., 2001; Mateo and Laguna, 2003; Thuraisingham, 2006; Tarkiainen et al., 2007; Peltola et al., 2008, 2011; Sassi and Mainardi, 2008; Colak, 2009; Kumaravel and Santhi, 2010). These studies confirm the view that the editing of R–R interval time series is an important aspect of HR variability measurement process.

Many methods and algorithms for editing or correcting the dubious R–R intervals have been developed and evaluated. Some of the common artifact correction and editing techniques involve deletion, interpolation of degree zero, interpolation of degree one (linear interpolation), and cubic spline interpolation. In addition, several other methods have been proposed for artifact correction such as comparing and merging method (Cheung, 1981), the predictive autocorrelation method (Albrecht and Cohen, 1988), non-linear predictive interpolation (Lippman et al., 1993), exclusion of R–R interval segments with divergent duration (Rottman et al., 1990; Lombardi et al., 1996a), impulse rejection (McNames et al., 2004), the integral pulse frequency model (IPFM; Mateo and Laguna, 2003; Solem et al., 2006), the sliding window average filter (Mietus, 2006), non-linear filtering combined with wavelet based

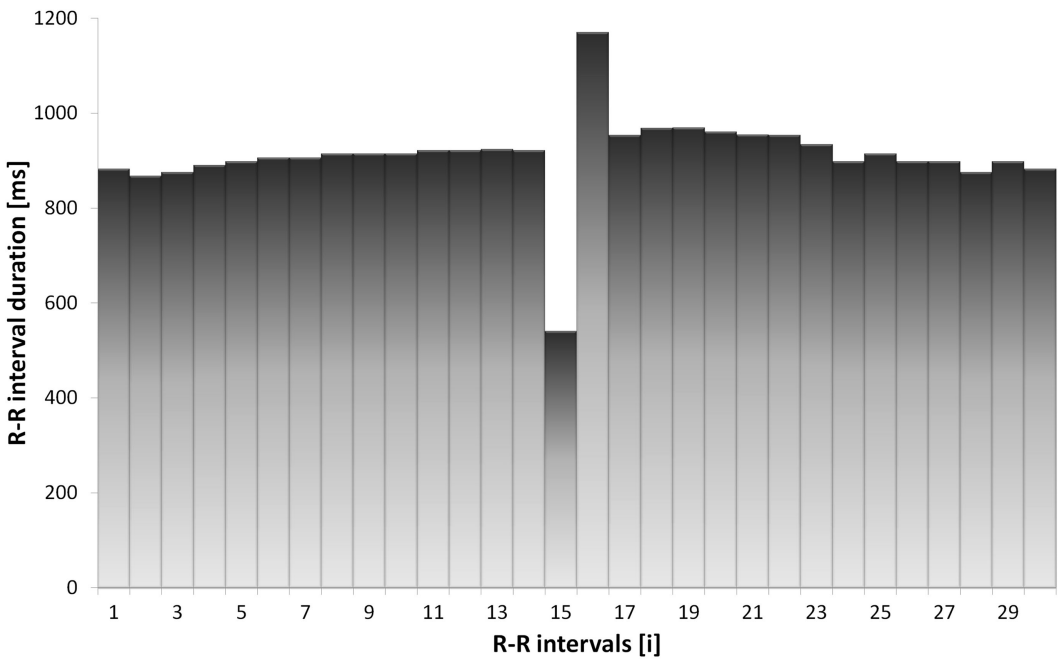

FIGURE 3 | An example of an ectopic beat in the R–R interval time series of a MI patient. The ectopic beat appears as a short R–R interval followed by a compensatory pause.

trend removal (Thuraisingham, 2006), and threshold filtering also with wavelet based trend removal (Lee and Yu, 2010).

Probably the simplest way of editing is to delete the false R–R intervals. In the deletion process, the abnormal R–R intervals are removed and the preceding normal R–R intervals are then shifted to replace the deleted ones. Deletion editing decreases the length of the HR variability signal, i.e., due to the loss of deleted R–R intervals. This may have significant influence on HR variability, especially when assessing the power spectrum or analyzing R–R interval segments of short duration. However, if all the segments containing ectopic beats or other artifacts are deleted in an attempt to eliminate out any possible interference in HR variability analysis, this can lead to an unacceptable and systematic loss of information (Salo et al., 2001).

Interpolation methods replace the non-normal R–R intervals with new interpolated R–R intervals. Unlike the deletion method, interpolation methods preserve the initial number of samples. There are various interpolation algorithms such as interpolation of degree zero, linear, spline, and non-linear predictive interpolation. Most interpolation methods can be considered as serving as low-pass filters with different filtering capacities. Interpolation of degree zero substitutes the abnormal R–R intervals with an average value that is computed from the neighboring normal R–R intervals. In interpolation of degree one, called the linear interpolation, a straight line is fitted over the abnormal R–R intervals to obtain new values. One popular spline interpolation method is a cubic spline interpolation, where smooth curves are estimated through a number of data points by fitting a third degree polynomial. It has been recommended to use interpolation methods when R–R interval time series contains occasional ectopic beats and artifacts. This concerns especially the power spectrum HR variability analysis (Kamath and Fallen, 1995).

Non-linear predictive interpolation for R–R interval artifact correction was introduced by Lippman et al. (1993). This algorithm is based on the fact that beat-to-beat variations in HR appear in a deterministic way. This algorithm utilizes methods of originating from chaos theory for locating ectopy-free portions of the R–R interval sequence. The purpose of the ectopy-free R–R interval sequence is to describe trajectories in the phase space that are locally similar to those of the segments containing ectopic beats. A trajectory is chosen such that it approximates most accurately to the particular segment with ectopic beats and this is then used to determine the replacement R–R intervals for the ectopic beats (Lippman et al., 1993).

R–R interval time series of longer duration can also contain slow linear trends and non-stationarities. Trend removal is a common way to preprocess R–R interval time series and diminish the effects of non-stationarities on HR variability analysis. Trend removal is usually performed with detrending methods that can be based on the first or higher order polynomial models (Litvack et al., 1995; Mitov, 1998). The so called smoothness priors approach (Tarvainen et al., 2002) is another filtering method to detrend signal. Non-stationarities have effects especially on the computation of FFT power spectrum. One additional method to avoid the effects of artifacts is to compute FFT spectrum estimates in finite length windows, which contains data of 2 or 5 min (Stein et al., 1995; Task Force of ESC and NASPE, 1996). Next, the window is moved along the signal producing consecutive estimates of the spectrum. Most studies reject segments that contain less than 80% of normal-to-normal beats. The aim of this method is to obtain correct data for FFT power spectrum analysis of 24-h ECG recording. This method is suitable for estimating the power of higher frequencies, HF and LF components. The power of lower frequency bands, VLF and ULF, cannot be reliably estimated using 5-min measure, because it does not contain fluctuations with longer cycle lengths (Kleiger et al., 2005).

EFFECTS OF DIFFERENT EDITING METHODS ON HR VARIABILITY ANALYSES

There are studies which have compared how the different editing methods impact on the results of HR variability analyses (Albrecht and Cohen, 1988; Birkett et al., 1991; Lippman et al., 1994; Salo et al., 2001; Peltola et al., 2004; Tarkiainen et al., 2007; Sassi and Mainardi, 2008). However, these studies can produce different results depending on the study setting. Despite the differences in the results, the message is the same, editing methods do have effects on HR variability analysis. Differences between results can be attributable to the type of study populations used, the length of R–R interval time series, editing methods, the type of HR variability analyses and the amount of edited samples, etc.

Spectrum analyses are sensitive to the signal length and any loss of samples and discontinuity of signal can affect the results. It has been recommended that one should avoid the deletion method in artifact correction and to use some other replacement methods in HR variability spectrum analyses (Task Force of ESC and NASPE, 1996; Salo et al., 2001; Mateo and Laguna, 2003). Deletion of R–R intervals may introduce step-like shapes into R–R interval time series, resulting in an increase in abrupt changes in the beat-to-beat variability and disruptions in the natural fluctuation. Deletion shortens the waveform of R–R interval time series and produces false frequency components in the HF and also in the LF area, resulting in a broadening of the spectra in the HF and LF bands. For example, in the study of Salo et al. (2001) with AMI patients, the errors in the HF and LF components of the short-term R–R interval time series were over 5% when less than 5% of the R–R intervals were deletion edited. These effects on the waveform make the deletion method unsuitable also for the analyses of the VLF and ULF components (Salo et al., 2001). Furthermore, it has been reported to prefer interpolation methods to deletion method in the DFA analysis of short- (α_1) and long-term (α_2) fractal scaling exponents (Peltola et al., 2004; Tarkiainen et al., 2007; Sassi and Mainardi, 2008). Deletion editing may produce a false increase in the values of α_1 in patients with acute myocardial infarction (Peltola et al., 2004) and in patients with coronary artery disease (Tarkiainen et al., 2007).

Although the deletion method may not be the most suitable method for editing, especially with respect to power spectrum HR variability analysis, it can be a feasible method of editing in the time domain for the analysis of SDNN and SDANN (Salo et al., 2001). However, in the same study of Salo et al. (2001), deletion editing was not recommended for the computation of pNN50 and RMSSD, again interpolation methods were considered superior. Nonetheless, deletion can be a suitable method for artifact removal in the case of the disturbances lasting for longer periods (Kamath

and Fallen, 1995). These include artifacts such as frequent ectopic beats and AF, the duration of which can last from several minutes to several hours (AF).

Different interpolation methods, artifact and trend removal methods and spectrum estimation methods have been proposed to especially improve the quality of the power spectrum HR variability analysis (Albrecht and Cohen, 1988; Birkett et al., 1991; Lippman et al., 1994; Salo et al., 2001; Mateo and Laguna, 2003; McNames et al., 2004; Clifford and Tarassenko, 2005; Colak, 2009; Kumaravel and Santhi, 2010; Lee and Yu, 2010). However, the results of these studies are dependent on the methods being used and on the type and length of R–R interval data. For example, Albrecht and Cohen (1988) claimed that the linear interpolation produced a more accurate power spectrum compared to the predictive autocorrelation method.

Lippman et al. (1994) compared the removal of ectopic beats with linear, cubic, and non-linear predictive interpolation in HR variability analysis with short-term R–R interval time series. They reported the necessity for editing and concluded that the removal of ectopic beats and non-linear predictive interpolation lead to better performance compared to linear and cubic spline interpolation, which overestimated the LF power and underestimated the HF power.

Salo et al. (2001) compared the performance of deletion editing with the interpolation of degree zero and one in HR variability of frequency-domain parameters. They examined short- and long-term R–R interval data of healthy subjects and patients with AMI. They found that the interpolation of degree zero and one perform at least equally well and in some cases was superior compared to the deletion method. However, the interpolation of artifacts can change the power of the frequency components by introducing false shapes and trends into R–R interval time series. For example, interpolation of degree zero leads to flat shapes in R–R interval time series since it uses the same average value over a whole segment of successive non-normal R–R intervals. Similarly, interpolation of degree one produces slope-like shapes, especially in R–R interval time series of high beat-to-beat variability. The longer the R–R interval segment is to edit the larger the flat or slope-like shape is. This generation of false trends due to interpolation may explain why linear and cubic spline interpolation have been reported to increase the power of the LF and VLF components in HR variability spectrum (Birkett et al., 1991; Salo et al., 2001). In addition, large segments cannot reasonably be edited with the interpolation methods due to the increase in false trends.

Interpolation methods have been examined and tested also for DFA analysis. Peltola et al. (2004) evaluated several interpolation methods including interpolation of degree zero and one and cubic spline interpolation and considered them to be more suitable for the analysis of fractal scaling exponents α_1 and α_2 than the deletion method. They found that the performance of the different interpolation methods depended on the scaling exponent (α_1 or α_2) and on the data type (healthy vs. AMI patient). Peltola et al. stated that the interpolation of degree zero was the most suitable method for the analysis of α_1. In their analysis of α_2, Peltola et al. observed that the deletion method produced much larger errors than the interpolation method. This was confirmed by Sassi and Mainardi (2008) who showed that the linear interpolation (interpolation of degree one) and substitution with local mean (interpolation of degree zero) performed better and introduced smaller errors in the long-term DFA analysis than the deletion method. The effects between different editing methods may not be very significant when the number of artifacts is small. Peltola et al. (2004) reported that the effects of editing in DFA analysis were minor, i.e., interpolation methods were as good as the deletion method when there was a small number of ectopic beats (<5%). This was confirmed by Tarkiainen et al. (2007) who did not detect significant differences between the performance of deletion and linear interpolation in the short-term DFA analysis and other non-linear HR dynamics when the number of ectopic beats was less than 10%.

Short-term HR variability analyses are more sensitive to artifacts and editing (Task Force of ESC and NASPE, 1996). The more R–R interval samples in the signal the better the capability of maintaining the original beat-to-beat variability despite the presence of edited R–R intervals. A 24-h R–R interval time series contains approximately 90000 R–R intervals. Correspondingly, a 5-min segment may contain about 300 R–R intervals. Even a small number of edited R–R intervals may impact on the results of HR variability analyses, especially with the short-term R–R interval data. Long-term time domain analysis including computation of SDNN and long-term spectrum analysis including ULF, VLF, and the slope of the lower frequencies (Bigger et al., 1996) mainly suffer from the deletion of the R–R intervals. For example, Salo et al. (2001) demonstrated that in long-term R–R interval time series (24 h) one could use the interpolation methods to edit up to 50% of all R–R intervals without causing any major changes in the results (error <5%). However, with short-term R–R interval time series, even a small number of edited intervals (<5%) can affect HR variability results not only with the spectrum analysis of HF and LF components but also with statistical time domain parameters such as pNN50 and RMSSD (Salo et al., 2001).

CONCLUSION AND FUTURE WORK

Ideally, the most reliable HR variability analysis is performed with R–R interval data with pure sinus beats. Technical developments in the ECG and R–R interval recording devices and improvement in the technology and materials of the electrodes have reduced the number of technical artifact. In addition, the importance of high-quality R-peak detection systems has also been noted. If one is able to perform an accurate R-peak detection, then it may be possible to decrease the number of artifact in the R–R interval time series. However, all the false R–R intervals, especially those of physiological origin, can never be eliminated. Since it is practically impossible to achieve data of 24-h free of artifact in ambulatory ECG recordings the focus must be directed to pre-processing and editing methods for the R–R intervals.

Accurate R–R interval artifact correction and editing methods are needed. It is possible to improve the quality of results of HR variability analyses with careful editing and by choosing appropriate editing methods. Various authors have proposed different approaches for handling ectopic beats and other artifacts. Nonetheless, currently there is no consensus about how to best edit artifact. The literature does not have any standard and detailed recommendations about suitable editing methods for different

HR variability analyses. Only one agreement exists concerning the power spectrum analysis of HR variability: in most studies artifact and ectopic beats should be interpolated instead of deleted. In addition, the maximum number for edited R–R intervals for HR variability analyses has not been standardized. Most investigators edit or reject the artifact and require at least 80% normal R–R intervals, especially in frequency-domain HR variability analysis. However, in different studies the amount of editing has ranged from 1 to 30% of the R–R intervals.

Various HR variability studies may use different R–R interval pre-processing and editing methods. This can lead to problems in any comparison of results obtained with HR variability analyses. This can be due to the different numbers of edited R–R intervals, analyzed HR variability parameter, type of the study population (high or low HR variability), length of the R–R interval time series or due to editing method selected. In the future, it is recommended that HR variability papers should contain an accurate description of the methods selected for pre-processing and editing.

The selection of editing methods or the absence of manual editing can be a problem also in current HR variability software. There can be a lack of information about how to deal with ectopic beats and other artifacts. Some HR variability software may include automatic systems for artifact correction. These can be based on some type of prematurity threshold extracting those R–R intervals that exceed the threshold value. Others may handle artifact by applying some trend removal processes. These kinds of automatic R–R interval editing are convenient to use and may perform adequately, especially in the cases of R–R intervals time series with low beat-to-beat variability and distinct ectopic beats or normal healthy adult with any or only small number of false beats. Nonetheless, many experts believe that manual editing with visual verification of the R–R intervals and a careful choice of the appropriate editing method is a more reliable method and can never be fully replaced by the current automatic correction systems. High-quality R-peak detection system together with accurate artifact editing are crucial for reliable HR variability analyses. Trustworthy R–R interval pre-processing software should contain (1) a possibility to view the ECG signal and the results of the R-peak detection process and a possibility to correct the false points and (2) manual or automatic editing of artifacts with different editing methods and a visual view of the edited R–R interval time series.

Despite the differences in HR variability study settings, many studies emphasize the importance of the artifact correction and appropriate editing if one wishes to conduct reliable HR variability analyses. In the future, more comparative studies will be needed to define standard recommendations for the suitable pre-processing and editing methods of R–R interval time series and the maximum number of edited R–R intervals which can be present in any short- and long-term HR variability analyses.

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Everything Hertz: methodological issues in short-term frequency-domain HRV

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Frequency analysis of the electrocardiographic RR interval is a common method of quantifying autonomic outflow by measuring the beat-to-beat modulation of the heart (heart rate variability; HRV). This review identifies a series of problems with the methods of doing so—the interpretation of low-frequency spectral power, the multiple use of equivalent normalized low frequency (LFnu), high frequency (HFnu) and ratio (LF/HF) terms, and the lack of control over extraneous variables, and reviews research in the calendar year 2012 to determine their prevalence and severity. Results support the mathematical equivalency of ratio units across studies, a reliance on those variables to explain autonomic outflow, and insufficient control of critical experimental variables. Research measurement of HRV has a substantial need for general methodological improvement.

Keywords: heart rate variability, autonomic nervous system, sympatho-vagal balance, sympathetic nervous system, parasympathetic nervous system

INTRODUCTION

Heart rate variability (HRV), the fluctuation of instantaneous heart period over time, is a correlate of cardiac autonomic regulation. HRV techniques have been applied in a broad range of contexts—they have been used to predict mortality after myocardial infarction (Buccelletti et al., 2009), as a correlate of stress (Berntson and Cacioppo, 2007) and psychopathology, to stratify attention (Mulder and Mulder, 1981), and have been incorporated into biobehavioral models of self-regulation (Porges, 1995; Thayer and Lane, 2000). The idea that reliable measurement of autonomic state may be obtained cheaply and non-invasively is obviously appealing. **Figure 1** illustrates a growing interest in HRV methods over time, a trend which seem likely to continue given the increasing access to heart rate data through recent technological advances—heart rate has recently been accurately calculated via smartphone (Heathers, 2013), microwave (Suzuki et al., 2008) and induction-powered indwelling device (Riistama et al., 2007).

Short recordings of HRV (i.e., less than 1 h) typically show two primary patterns of oscillation which are separated into frequency bands from ≈7 to 25 s (0.04–0.15 Hz; low frequency, or LF) and 2.5 to ≈7 s (0.15–0.4 Hz; high frequency, or HF)—lower frequencies than LF are generally not meaningful over the short term. LF and HF frequency bands are widely used to quantify parasympathetic and sympathetic regulation (Akselrod et al., 1981) and their interaction (Malliani et al., 1991).

As ease of access to HRV increases, establishing and maintaining correct methodology is important—redundant methodology may delay treatment, obscure valuable underlying effects, provoke Type I or II errors, and most importantly, potentially delegitimize both alternative useful results and the utility of HRV in general. However, the internal and external consistency of the methods used have received comparatively less research interest than the understanding of the autonomic, cardiac and circulatory which creates those methods (Billman, 2011).

Thus, this paper presents a focused review of HRV methodology in the frequency domain which serves two purposes. Firstly, to raise several inter-connected points concerning the collection, interpretation and interrelationship of frequency-domain HRV variables, and external factors which may influence their recording. While there are many unsettled questions concerning meaning and calculation frequency domain HRV, the points raised here are generally not in dispute—they are either derived from a strong base of evidence, or are defined mathematically.

Secondly, to formally outline the awareness of these methodological points with reference to a large convenience sample of work using HRV methods. This sample is drawn from the most recent complete calendar year (2012) at the time of writing.

THE INTERPRETATION OF LF POWER

All measures of HRV are necessarily complex as heart period over time is variously affected by multiple autonomic outflows, the modulation of those outflows at the sinoatrial node, their pacemaker response and competition, and the dynamic regulation of the vasculature, as well as endocrine, endothelial and mechanical factors. These interactions are further complicated by the realization that the individual mechanisms which may influence heart period are themselves incompletely understood. Examples include the source of cardiorespiratory coupling via either a central oscillator or the baroreflex (Eckberg, 2009; Karemaker, 2009), the mechanism behind periodic changes in blood pressure (Julien, 2006), the intrinsic meaning or function of respiratory

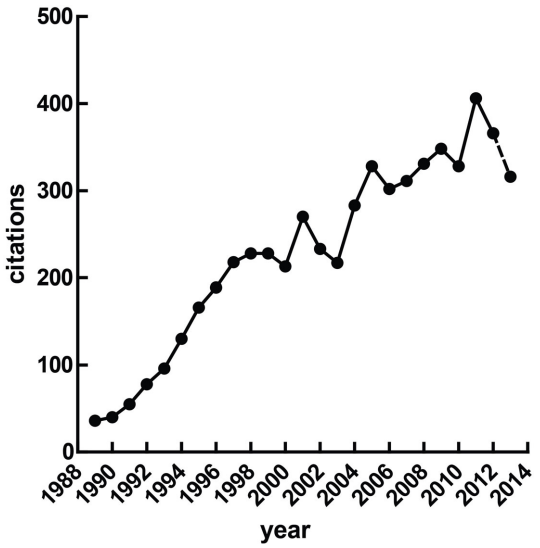


FIGURE 1 | Published research with "heart rate variability" in the title. At the time of writing, the value for 2013 was extrapolated from the publications Jan 1st through April 30th.

sinus arrhythmia (Hayano et al., 1996; Tzeng et al., 2009; Ben-Tal et al., 2012; Elstad, 2012).

Irrespective of this, the power spectral density of high frequency HRV is strongly associated with cardiovagal activity (Akselrod et al., 1981; Kamath and Fallen, 1993; Malik, 1996). Respiratory variation observed in heart period is linearly related to parasympathetic control of heart rate (Katona and Jih, 1975), and its modulation forms the theoretical center of most HRV analysis. However, it should be noted that HF HRV is not abolished by vagotomy (Tzeng et al., 2005, 2007), and shows a complex and only somewhat dose-dependent relationship with muscarinic blockade (Picard et al., 2009).

Alternatively, the debate over the characterization, meaning and utility of LF HRV is ongoing issue (Akselrod et al., 1981; Porges and Byrne, 1992; Hopf et al., 1995; Introna et al., 1995; Sleight et al., 1995; Eckberg, 1997; Grasso et al., 1997; Malliani et al., 1998; Sleight and Bernardi, 1998; Houle and Billman, 1999; Notarius et al., 1999; Notarius and Floras, 2001; Elghozi and Julien, 2007; Billman, 2011, 2013; Goldstein et al., 2011; Pagani et al., 2012; Reyes del Paso et al., 2013).

To fully describe the physiology involved above is beyond the scope of this review. Within the present context, we may confine ourselves to addressing one common claim about frequency analysis—the involvement of the SNS in vasomotor control (Julien, 2006), and the strong relationship between the baroreflex and LF power (Goldstein et al., 2011) has occasionally been extrapolated to the position that LF power is proportional to cardiac sympathetic nerve activity. The direct evidence against this claim is strong even if confined to just non-invasive or minimally invasive studies in humans.

For instance, beta-adrenergic antagonists have shown divergent effects on LF power. Jokkel et al. (1995), for instance, report an approximate doubling of LF power in response to total beta-adrenergic blockade with propanolol (a non-selective β-blocker). A modest increase in LF power (Chiladakis et al., 2004) or no difference to baseline (Taylor et al., 1998) have been reported subsequent to treatment with atenolol (a β_1-antagonist).

Likewise, cardiac 6-[^{18}F] fluorodopamine imaging in humans (Goldstein et al., 1990, 1993), which radiolabels catecholamine storage vesicles, has repeatedly shown no relationship between radioactivity subsequent to cardiac sympathetic activity and LF-HRV power (Alvarenga et al., 2006; Moak et al., 2007; Rahman et al., 2011). Likewise, there are dissociations between other measurements of SNS via impedance cardiograph (Goedhart et al., 2008), salivary alpha-amylase (Nater et al., 2007; Kobayashi et al., 2012), circulating epinephrine/norepinephrine (Sloan et al., 1996), and muscle sympathetic nerve activity (Grassi and Esler, 1999). This evidence has been recently covered at length (Goldstein et al., 2011; Reyes del Paso et al., 2013).

The connection between LF power and sympathetic activity, while frequently cited as representative of (Pagani et al., 1984, 1986), is a misrepresentation of the initial claim that *normalized* LF power is representative of relative sympathetic power as a measure of sympathovagal balance. This, and related theory, is dealt with below.

THE LF/HF RATIO

The ratio of low-frequency power to high-frequency power (LF/HF ratio), as popularized by (Pagani et al., 1984, 1986), is commonly used as a measure of sympathovagal balance—the putative balance between the mutually opposing branches of the autonomic nervous system. While widely used, this approach has been criticized on a number of grounds. The disconnection between this understanding of short-term spectral power within the heart series and the known physiology related to that power (Eckberg, 1997; Goldstein et al., 2011; Billman, 2013), and the response to those criticisms (Malliani et al., 1998; Pagani et al., 2012), have been covered in detail. As above, much of this is a natural extension of the argument that the numerator (i.e., LF power) reflects sympathetic outflow poorly, if at all.

From a methodological perspective, however, it is most concerning that there may be no mathematical basis on which to compare LF and HF power. Values of HRV are typically *internally consistent*, in that changes within a frequency band on individual sequential measurements may be directional or proportional. That is to say, it is meaningful that an individual under acute stress experiences a reduction in HF power from baseline, and that additive stress provokes additive change. However, those changes have less bearing on other measured quantities (i.e., a loss of HF power is compared to a loss of LF power; a loss of HF power between individuals, etc.). This is subsequent to considerations such that (a) fluctuations in HRV should more correctly be considered fluctuations in the *modulation* of autonomic tone, not a change in autonomic outflow (e.g., Katona et al., 1977), (b) the properties of interaction and competition between muscarinic and adrenergic outflow at the sinoatrial node are both non-linear (e.g., Levy, 1984) and mediated by neuropeptide co-transmitters (e.g., Revington and McCloskey, 1990), and c) changes in both low- and high- frequency power are mediated by both SNS and PNS (e.g., Taylor et al., 2001). This is often expressed simply

by characterizing HRV as a qualitative, not quantitative, variable (Notarius and Floras, 2001; Billman, 2011).

However, the possibility of measuring sympathovagal balance in the manner above has been repeatedly classed as heuristic (Malliani et al., 1998; Sleight and Bernardi, 1998; Malliani, 2005). This position has a great deal of merit, as it is inevitable that complex or poorly understood phenomena will be demonstrably related to other dependent or independent variables in advance of our ability to explain why this is so. In other words, a metric may be *useful* well before it appears *meaningful*. One argument related to the above is the clear interrelationship during graded orthostatic tilt between (1) tilt angle, (2) sympathetic outflow, and (3) LF/HF ratio, the conclusion being that as all of these positively covary, then LF/HF ratio well describes, and is capable of predicting tilt angle (Montano et al., 1994). This may be the case, but only indicates an association between these factors, rather than a reason for their association.

Orthostasis provides an interesting comparative example. **Figure 2** graphs mean pre-ejection period (PEP) against the angle of graded tilt with an overlaid quadratic regression (assuming that the relationship between cardiovascular response to tilt vs. angle is curvilinear). In this situation, the heuristic value of adjusted or unadjusted PEP is substantial, perhaps equivalent of some reports of spectral power (Bahjaoui-Bouhaddi et al., 2000), even without considering the individual regressions.

It seems very likely that predictions made in the manner of Malliani et al. (1997), where normalized units were successfully employed in a model to delineate posture, would be successful with PEP. Thus, as the following are clearly demonstrated:

(1) there is a predictable, positive relationship between PEP and positive tilt
(2) this relationship parallels an established positive relationship between SNS outflow and positive tilt (e.g., Chosy and Graham, 1965; Iwase et al., 1987)

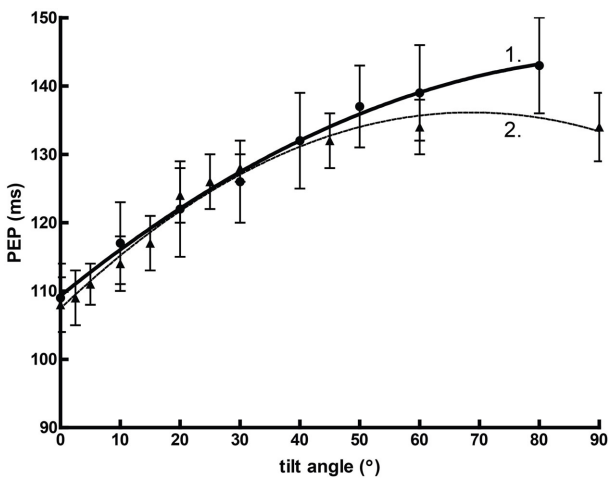


FIGURE 2 | The curvilinear relationship between pre-ejection period (PEP) and tilt angle during orthstatic stress. Data from Chan et al. (2007) (1) and Stafford et al. (1970) (2).

. . . we may draw a heuristic conclusion:
(3) PEP is positively related to sympathetic outflow.

However, the normal relationship between PEP and SNS outflow is precisely the opposite. Sympathetic activity, as measured by circulating catecholamines (Chosy and Graham, 1965) or by MSNA (Iwase et al., 1987), increases reliably during orthostatic tilt. In other contexts, this might well accompany a decrease in PEP (Newlin and Levenson, 1979). However, our model here fails to account for the effects of preload—the initial stretching of the myocardium due to passive factors prior to the cardiac cycle—which increases proportionally with tilt angle independently of sympathetic drive (Stafford et al., 1970). Thus, a heuristic variable formed between two robust associations may be precisely predictive but ultimately misleading. This is precisely the criticism leveled by Grassi and Esler (1999); that LF/HF ratio fails to describe SNS outflow outside of the demonstration provided by changes in orthostasis.

Finally, the source of LF power is well characterized—LF power generally reflects the activity of the baroreflex in response to vasomotor tone. This is broadly accepted consequential to the classical demonstrations of the close correspondence between blood pressure waves and sympathetic modulation (Guyton and Harris, 1951), which are reflected in the heart period by the compensation of the baroreflex. This interpretation is not in dispute; a comprehensive summary is given in Berntson et al. (1997).

THE REDUNDANCY OF NORMALIZED UNITS AND LF/HF RATIO

Normalized HRV values (LFnu, HFnu) are calculated from the raw values of either short-term frequency band (LF or HF) divided by the total spectral power (typically LF + HF), with the value of this typically expressed as a percentage or decimal. These variables have a long history (e.g., Lombardi et al., 1987) in quantifying HRV, and have been used to quantify proportional sympathetic and parasympathetic activity respectively (e.g., Pagani et al., 1986). They are of particular interest in reviewing the available literature as they provide a degree of interpretability between studies, as proportional change between defined frequency bands can be seen as roughly equivalent regardless of the spectral method used. Unlike raw power, this allows direct comparison between frequency and autoregressive methods for calculating spectral power, between spectral power expressed as ms^2 or bpm^2, and between different algorithms for calculation, windowing methods, time periods, etc. These differences often result in baseline spectral values which are multiple orders of magnitude apart between studies (Sandercock, 2007).

However, the typical use of normalized units presents a series of significant redundancies. Firstly, LFnu and HFnu are trivially equivalent, as LFnu = 1-HFnu. This implies that calculations cannot be duplicated, as LFnu calculations are perfectly linearly related (i.e., computationally identical) to HFnu (Chemla et al., 2005). Reporting both values provides no additional information over reporting one, and change in one is identical to change in the other. In this manner, it is necessarily incorrect to refer to HFnu and LFnu as separate concepts. Instead, this model must describe a single autonomic continuum along which individual points represent the admixture of low and high frequency power.

Furthermore, reporting calculations where only one normalized value is significant should be considered inconsistent.

There are exceptions to the above. Firstly, when normalized values are calculated from an expanded power spectrum; occasionally, Very Low Frequency (VLF; 0.003–0.04 Hz) may be included in the denominator of normalized units (i.e., LFnu = LF/VLF + LF + HF), likewise power about the HF cutoff (i.e., >0.4 Hz), or the total power of the observed spectrum (TP; 0–0.5 Hz) may be used as the denominator (i.e., LFnu = LF/TP; this is sometimes called LF%). However, in short recordings, the inclusion of these longer timescales is a significant problem as the contribution from very low frequencies is undersampled in the manner described below, and the Nyquist criterion prevents any meaningful contribution at frequencies higher than HF.

Secondly, when the autoregressive method is used to quantify spectral bands, often, the individual components identified for LF and HF bands sum to less than the measure of "total power"—the additive model minus the component at VLF. In this case, LFnu + HFnu will be less than 1, but most likely very close to it. As far as I am aware, there is no evidence to indicate that this establishes LFnu and HFnu as separate theoretical entities rather than measurement error. If the autoregressive model is a poor fit for the available data, then LFnu + HFnu may be significantly less than 1.

In addition, it is trivial to transform the LF/HF ratio as directly proportional to a normalized value of either spectral band (Burr, 2007):

$$
\begin{aligned}
&If\ \frac{LF}{HF} = \alpha,\\
&i.e.\ HF = \frac{LF}{\alpha}\\
&then,\\
&LFnu = \frac{LF}{LF + HF}\\
&\quad = \frac{LF}{LF + \frac{LF}{\alpha}}\\
&\quad = \frac{1}{1 + \frac{1}{\alpha}}\\
&i.e.\ LFnu = \frac{1}{1 + \left(\frac{LF}{HF}\right)^{-1}}\\
&and\ HFnu = \frac{1}{1 + \left(\frac{LF}{HF}\right)}
\end{aligned}
$$

Graphically, the function above is shown in **Figure 3A**—it is monotonically increasing at all positive non-zero values, non-linear, and well approximated by logarithmic regression over a typically observed range ($r^2 > 0.99$). As the distribution of the LF/HF ratio is often positively skewed, it is frequently log-transformed to meet criteria of normality (e.g., Kobayashi et al., 2012). In this case, the non-linear relationship becomes significantly attenuated and very closely approximates linearity (**Figure 3B**)—thus a linear regression has an identical coefficient, constant term and r^2-value.

In this manner, any given value of LFnu or HFnu has a directly equivalent LF/HF value. It should be emphasized that this is not a conceptual similarity but an equivalence at the level of definition—for example, an LF/HF ratio of 0.6 is precisely equivalent to LFnu = 37.5% or HFnu = 62.5%. Consequently, individual normalized values contain no more information than individual LF/HF ratio values, and on this basis it is unclear how "sympathetic balance" (LFnu) is mathematically different to "parasympathetic modulation" (HFnu) or how either is conceptually different to "sympathovagal balance" (LF/HF).

Similarly, due to the non-normal distribution of typical data, HRV variables are occasionally presented as median and interquartile range. As rank order is preserved in a monotonic increasing relationship, medians and inter-quartile values should remain direct transformations of each other, and statistical calculations on rank order should be identical between normalized and ratio values; a full description of this and other redundancies can be seen in Burr (2007).

However, due to the moderate non-linearity, mean (LF/HF) is not identical to mean (LFnu). This relationship is explored in

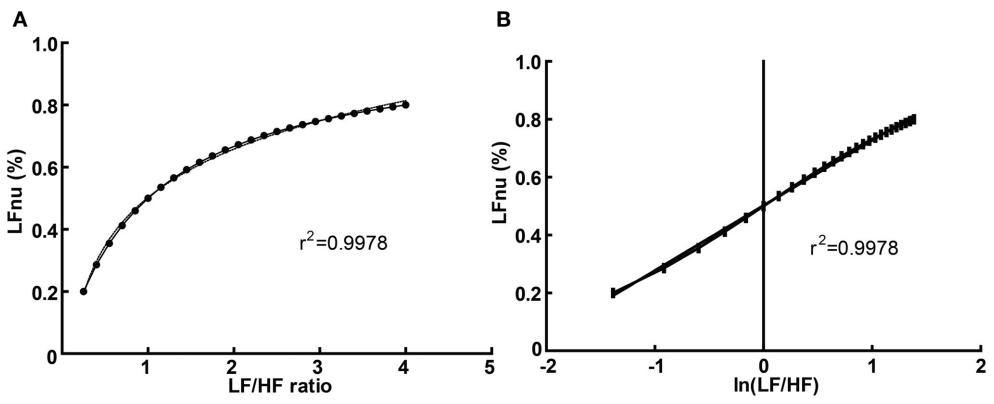


FIGURE 3 | The direct equivalence of normalized to ratio values with logarithmic regression (A), and of normalized to log-ratio values with linear regression (B). Values drawn from LFnu 0.2 to 0.8, $n = 25$.

Figure 4, where LFnu and LF/HF values from realistic artificial samples reveal a convergence toward the central value of LFnu with larger sample size, and a consistent predictive value between means. Thus, it is likely that statistical comparisons under standard parametric assumptions for LF nu and LF/HF would be similar without being identical.

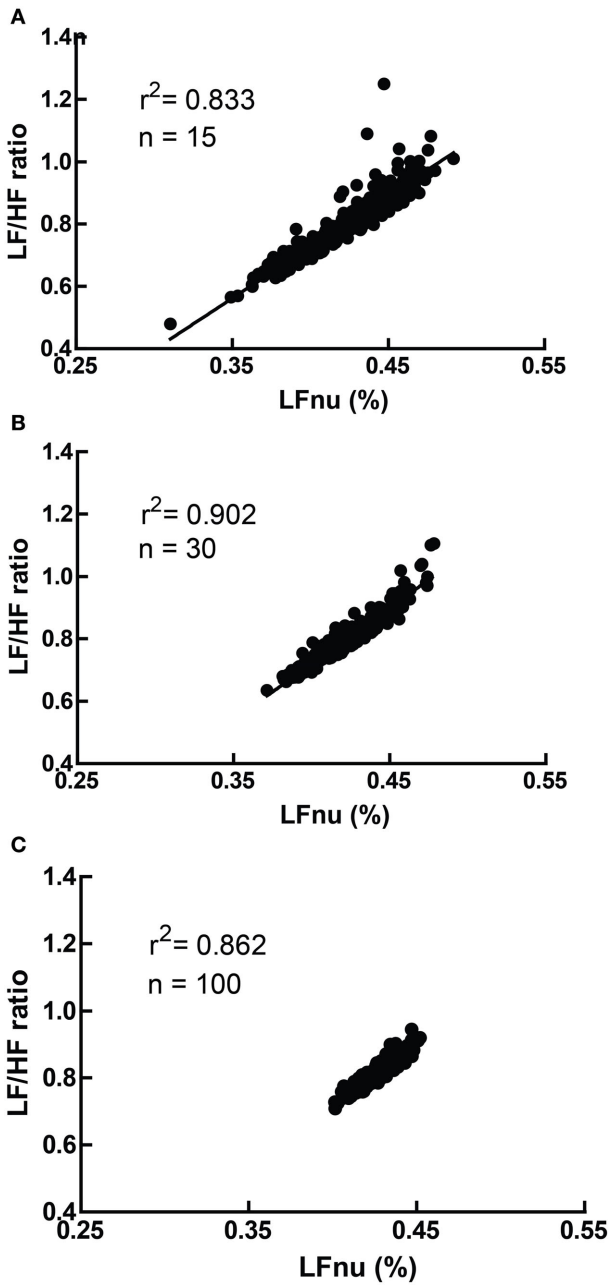


FIGURE 4 | A comparison of LFnu vs. LF/HF sample means, with (A) *n* = 15, (B) *n* = 30, (C) *n* = 100. Points are means derived from approximately typical pseudorandom (Mersenne Twister) normally distributed values of LF ms^2 (mean = 600, *SD* = 200) and HF ms^2 (mean = 800, *SD* = 200). The values are distributed near the point of mean equivalence, LFnu = 0.429. r^2-values range from 0.833 to 0.902.

INTERPRETING NORMALIZED UNITS IN THE ABSENCE OF RAW POWER

Normalized units, which report frequency power proportional to the total observed power, possess an additional problem—that several different patterns of change in individual spectral bands may result in identical changes in proportion. This is illustrated in **Figure 5**, where a hypothetical participant with a baseline LFnu = 0.33 increases to LFnu = 0.5 after experimental intervention. This change in normalized units therefore represents not one possible change, but a continuum of possible changes which variously encompass (1) an increase, decrease or no change in (2) either total power, raw LF or raw HF power. Any point on the line of identity described in **Figure 5** fulfills the criteria of LFnu = 0.5, but the individual points represent entirely different outcomes (Billman, 2013).

In other words, the reporting of HRV solely as a proportion directly obscures the underlying interpretation. It is precisely this form of interpretability which the seminal Task Force paper (Malik, 1996) sought to preserve within normalized values by recommending that research should always report both normalized and raw values for clarity.

This is not merely a hypothetical scenario, and one of our recent papers illustrates this clearly (Krygier et al., 2013). In this study, comparisons of HRV metrics are drawn from a sample of meditators at rest and during Vipassana meditation, and both before and after an intense intervention—around 100 h of intensive training over 10 days. While the overall interaction was not significant, an intriguing and significant increase in HFnu was observed, as reported in previous research on similar forms of meditation (e.g., Sarang and Telles, 2006; Wu and Lo, 2008; An et al., 2010). A naïve characterization might be that a beneficial change representing an "increase in vagal tone" or a "favourable autonomic balance" was introduced by meditative

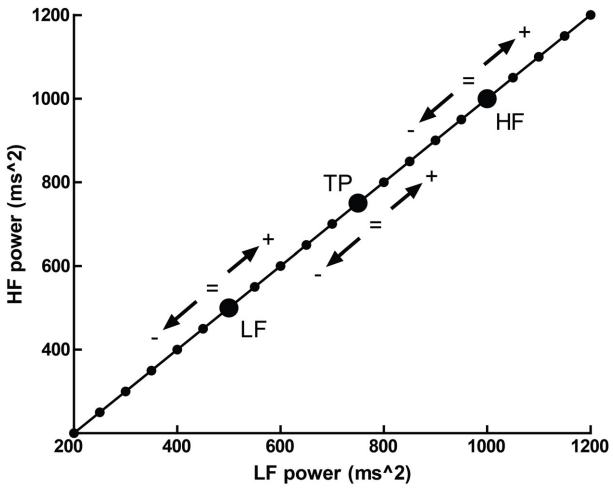


FIGURE 5 | The outcome of a hypothetical experiential effect—a participant with LFnu = 0.33 (LF ms^2 = 500, HF ms^2 = 1000) increases to LFnu = 0.5, which is defined by any point on the line of identity (i.e., LF ms^2 = HF ms^2). The arrows and operations on the line designate where values may be smaller, equal to, or greater for the corresponding spectral regions (LF, low frequency; HF, high frequency; and TP, total power).

training, but follow-up analyses revealed that normalized change was specifically mediated by (a) a profound increase in HRV at breathing frequency during meditation in untrained participants, and (b) a profound decrease in HRV at Mayer wave frequency during meditation when trained (**Figure 6**).

These changes precisely mirror the subjective reports of how meditative practice proceeds. Naïve practitioners of Vipassana, instructed to observe the breathing cycle rather than alter it, invariably "over-breathe," which typically corresponds to an increased tidal volume and reduced respiratory rate. Within the lower portion of the HF spectra, this increases observed HF power (Hirsch and Bishop, 1981; Brown et al., 1993). However, this problem is mastered within a few days as participants practice the ability to passively observe normal respiratory cycles.

The above is a single unreplicated finding, and due to the nature of the task, breathing could not be consciously controlled (a potential confound, as breath has its own relationship to attention; see Vlemincx et al., 2012). Thus, while the above explanation is speculative, two points remain regardless: (1) the reference to individual frequency bands has greater explanatory power than the original naïve interpretation, especially considering changes in respiratory parameters, mood, attention, etc. are reliably predicted by spectral power in individual frequencies, and (2) the changes described within individual frequency bands may be entirely inconsistent with, and obscured by, the reporting of lone normalized HRV values.

TIME RESOLUTION OF LF POWER

While an RR series does not consist entirely of cyclical processes (Peng et al., 1995), frequency analysis approximates the action of autonomic outflow to the heart by quantifying cyclical information present. In doing so, the number of times a cyclical frequency can be observed during an electrocardiographic recording varies linearly with the length of the recording, and inversely with the period of the frequency.

Consequently, HF HRV may be successfully recorded over periods of time as short as 60 s (Malik, 1996) as this gives adequate resolution to cycles within the heart period driven by respiratory sinus arrhythmia, typically around 0.25 Hz at rest. LF HRV requires a longer period in order for the spectral information to be reliably present. In short recordings, these frequencies may be insufficiently sampled—a signal at 0.04 Hz (i.e., with a period of 25 s) is observed 2.4 times per minute.

A heuristic rule which has been occasionally stated requires the sampling period to contain 10 complete cycles of the lowest observed frequency in order for the underlying information to be successfully approximated (Malik, 1996; Berntson et al., 1997) but there appears to be no analytical exposition of this. This has loosely translated into an accepted standard of a 5 min recording to measure short-term HRV, as a 5 min recording by this definition can resolve frequencies down to 0.033 Hz. Consequently, power from the LF spectrum down to 0.04 Hz is necessarily included in both normalized and LF/HF ratio calculations of HRV. Thus, both measurements should be taken over a minimum of 5 min.

EXTRANEOUS VARIABLES TO RECORDING BASELINE HEART PERIOD

Studies which measure variables that may be broadly affected by incidental day-to-day factors are usually carefully controlled. In human populations, research is often conducted specific to age group, experimental environment, time of day, medication status, environmental stimulants (i.e., caffeine or other methylxanthines), and so on. In longer studies or those requiring strenuous activity, standardized food and drink is provided. Studies in HRV are especially subject to these concerns—due to the autonomic innervation of the viscera, there are several instances where

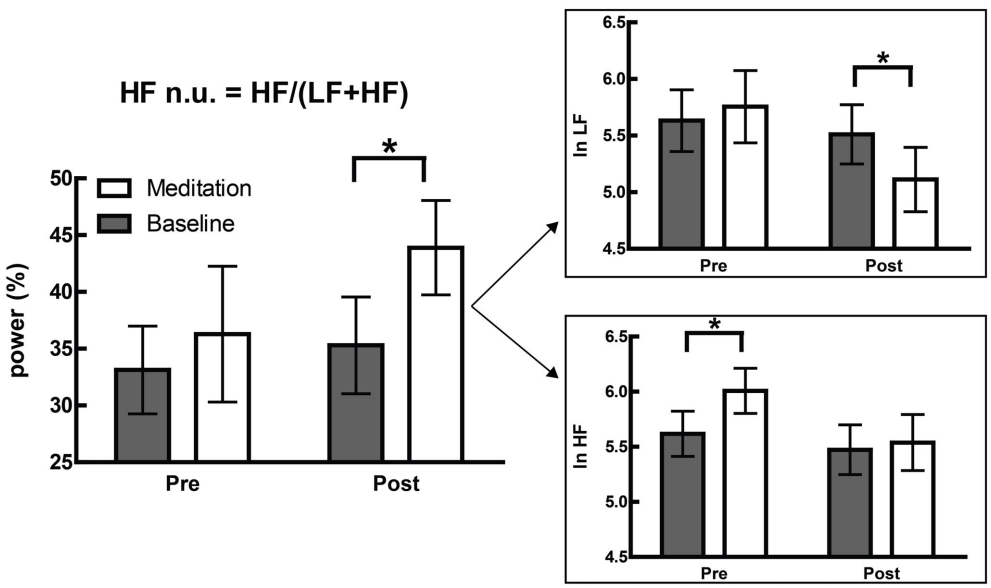


FIGURE 6 | Adapted from Krygier et al. (2013), Figure 1, with permission. The devolution of the normalized results (top panel) into raw power (bottom panel) reveals two specific effects inconsistent with the overall interpretation of an alteration in autonomic balance. $^*p < 0.05$.

artifacts to short-term HRV measurement at rest may reliably arise from demographic variables, and the normal activities of daily living. Of course, controlling daily activity is not possible or even desirable in some patient groups, especially if long term monitoring is required (i.e., if measured over 24 h) but in laboratory or naturalistic experiments, it is ideal to observe potential changes in autonomic activity with as few confounding variables present as possible.

These variables are occasionally recognized; most research, for instance, is aware that HRV declines with age (O'Brien et al., 1986), is broadly affected by cardiovascular, vasoactive and psychotropic medication (e.g., beta-blockers; Sandrone et al., 1994), and is affected by both circadian rhythm (e.g., Massin et al., 2000) and wakefulness (Walker et al., 2009). Less frequently recognized is the finding that the autonomic innervation of the viscera means the consequences of feeding (i.e., the acute consumption of food and water, gastric distension and bladder filling) directly affect HRV.

Of these, the most attention has been paid to water consumption (May and Jordan, 2011) subsequent to the finding that patients with severe hypotension due to autonomic failure derived a significant reduction in symptoms from drinking water, and this subjective improvement was observed parallel to substantial increases in blood pressure (Jordan et al., 2000). A similar effect can be observed when the baroreflex loop is opened in sinoaortically denervated mice (McHugh et al., 2010).

In normal participants, the same presumed pressor effect takes place, and can be observed in muscle sympathetic outflow (Scott et al., 2001), but changes in blood pressure are immediately buffered by the efferent vagal baroreflex, and the immediate consequence is a moderate to large compensatory increase in heart period and HF-HRV. Healthy participants approximately double baseline HF-HRV, while the effects on HR are significant within 10 min after ingestion, peak at around 15–20 min and return to baseline by 45 min (Routledge et al., 2002). Recent work (Mendonca et al., 2013) has suggested that these effects only become negligent at VO2 maximum.

Eating and subsequent digestion have autonomic consequences which appear to be mediated both by gastric distention (Rossi et al., 1998) and by exposure to food-related stimuli (Nederkoorn et al., 2000). Mechanical and electrical stimuli to the stomach are both powerful hypotensive stimuli (Pozo et al., 1985), and this effect is abolished by vagotomy (Liu et al., 2004). In addition, the digestive process provokes vagal withdrawal as measured by HRV for at least 60 min after a meal (Lu et al., 1999), and increases sympathetic outflow to the skeletal muscles but not the heart (Fagius and Berne, 1994; Cox et al., 1995). Due to the relationship between the thermic effect of food and sympathetic outflow, this response is heavily affected by macronutrient composition (Welle et al., 1981; Schwartz et al., 1985).

Finally, bladder distension has been observed to provoke a robust series of pressor-mediated responses in humans (Fagius and Karhuvaara, 1989), where bladder distention predicts an increase in muscle sympathetic nerve outflow and blood pressure. Ben-Dror et al. (2012) subsequently delineated a linear rise in lnLF power with acute bladder filling in healthy controls drinking water. While this may have been confounded with the osmopressor effect (as above), a similar effect was observed using filling cystometry (i.e., causing bladder distension without drinking; Mehnert et al., 2009).

REVIEW PARAMETERS

In order to confirm both the nature and the extent of the problems outlined above, a substantial body of work is drawn from the recent HRV literature (i.e., from 2012). This allows the possibility of (a) sufficiently characterizing HRV research as it is presently performed with reference to the methodological issues raised, (b) confirming the presence and relevance of the mathematical relationships defined above, and (c) observing the extent of experimental controls currently employed.

METHODS

A non-systematic review was conducted: Google Scholar and PubMed databases were searched using the terms "heart rate variability" or "HRV" through either the title or abstract, with a date restriction of 01/01/12 through 31/12/12. Full text articles were obtained.

REVIEW PROCESS

Non-English language journals, 24 h studies (title/abstract: "Holter," "24 hr"), animal (title/abstract: "mouse," "rat," "dog," etc.), developmental (title/abstract: "neonatal," "infant," "child," etc.), geriatric (title/abstract: "elderly," "geriatric," etc.), and conference abstract, qualitative or discussion papers (title/abstract: "editorial," "conference," "review," etc.) were excluded, as were papers which were formally published in 2011 or 2013 ($n = 293$). The remaining papers ($n = 573$) were superficially reviewed to set initial criteria for inclusion.

SELECTION CRITERIA

Age

Pre-natal, infant, child and youth (mean age <18 years) samples, and elderly/geriatric samples (mean age > 65) were excluded.

Time period

Consistent recording for more than 1 h was not considered short-term and excluded. 24 h or Holter monitor studies were included only if a short-term period was additionally analyzed and reported to the daily record.

Descriptive work

Reviews, meta-analyses, position papers or commentaries, correspondence, etc. were excluded if descriptive of HRV phenomena instead of primary research, and included if they reported data from novel primary research.

Breathing

Paced breathing at speeds above 0.15 Hz was included. Breathing protocols slower than 0.15 Hz likely to affect the fundamental distribution of spectral power were excluded.

Healthy baseline condition

If plural baseline conditions were included within-subjects over one or multiple sessions, the first criteria reported—either by time, or if unclear, by listed order—was considered the baseline.

If plural conditions were averaged to make a global value, this was considered equal to the total recorded time. If a baseline included plural subsequent measurement periods, i.e., two recordings of 3 min separated by task, then the first was used. Subsequent periods (i.e., "first 5 mins, second 5 mins") were recorded as a single value if given otherwise the first period was used. Studies combining the averages of multiple time periods (i.e., the average of spectral values from two 3 min periods) were not recorded. Baselines immediately before surgery requiring general anesthesia were not considered resting, due to anticipatory anxiety. Multiple healthy groups from the same study were included if (a) listed separately at all points, and (b) were taken from baselines administered before random assignment into groups, or after assignment in benign circumstances. If sub-clinical groups from healthy populations were defined (i.e., "high normal" anxiety vs. "low normal" anxiety) then the low pathology group was used. Unless specifically stated as standing or supine, it was assumed that participants or patients were seated.

RECORDED INFORMATION

LF power

The genesis of LF power provided was classified as being either (a) sympathetically mediated, (b) resulting from "both parasympathetic and sympathetic modulation," (c) representing the gain of the baroreflex, or (d) other (parasympathetically mediated/not stated). Studies specifically measuring the LF response to graded tilt or postural change were taken as implying a relationship between LF and baroreflex outflow, as this is an orthostatic manipulation. If the basis of LF was derived from a reference without an explicit statement of what LF power was to represent, the interpretation within the reference was used according to the above criteria.

CONTROL OF EXTRANEOUS VARIABLES

Circadian

Circadian factors were considered controlled if both between and within subject comparisons were identical within a 24hr period, and confined to an hour or a time window of up to 4 h (i.e., "9 am to 1 pm" or "beginning in the early morning").

Illness/Medication

Work addressing serious, debilitating, psychiatric or other chronic illness, or any illness whose primary etiology was cardiovascular or circulatory, was included only if a control group was available, as baseline HRV level or collection/analysis technique may be affected. Non-life threatening illness treatable with standard pharmacotherapy (such as asthma) or post-treatment groups which did not require major pharmacotherapy or surgery (e.g., recovered phobics) were included. The exclusion or statistical control of any medication apart from the contraceptive pill or unscheduled analgesics (e.g., Paracetamol, Ibuprofen) was considered controlled.

Food/Water

Meals were regarded as controlled either if participants were recorded during a fasted state, or if a standard meal was provided or prescribed for study inclusion, likewise water. A fasted state was assumed for participants measured at baseline before tilt-table testing. Water provided *ad libitum* was not considered controlled.

Bladder

Bladder emptying was only recorded if it was explicitly stated, as no pre-surgical population was included.

Content

With the exclusion criteria as above, the review proceeded pseudo-randomly (i.e., sequentially in alphabetical order by the surname of the first author) until 100 samples were recorded.

ANALYSIS

Comparisons between values were modeled respectively as the regressions HFnu = a/(b + c.(LF/HF)), LFnu = a/(b + c.(HF/LF)); all used the least-squares method and assumed initial conditions of any nominal constant = 1. The relationship between LFnu and HFnu was modeled by linear regression.

Relative standard errors (RSE; the standard error of the mean divided by the mean) were taken as measures of adjusted reliability for individual studies, and calculated from LF/HF ratios which were given in milliseconds squared, LFnu and HFnu values.

All calculations were performed in GraphPad Prism 5.

RESULTS

From $n = 378$ papers, $n = 97$ papers were accepted ($n = 3$ studies contained multiple baseline groups which met inclusion criteria), to give a total of $n = 100$ records of HRV at baseline. The list of these papers is included as supplementary material. If data was provided, participant age, sample size, HFnu mean and standard deviation (calculated from SEM if necessary), LFnu (likewise), or median and inter-quartile range were recorded separately. LF% and HF% were not recorded, as the inclusion of VLF power within short term calculations is problematic. All forms of spectral analysis (i.e., autoregressive method, FFT/DFT, Lomb-Scargle Periodogram, wavelet analysis etc.) were included as equivalent spectral analytical methods, as normalized units and/or LF/HF ratio were the recorded variables. The characterization of the acceptance/rejection criteria and use of spectral power is shown in **Tables 1**, **2**.

Extraneous controls varied substantially between measures: of the 97 separate studies accepted, 81% controlled for medication or health status, 76% for nicotine use, 58% for time of recording, 45% controlled for food intake, 23% controlled for water intake, and 4% for micturition. Of the above $n = 97$ studies, 91 (94%) analyzed some version of LF power, and 74 (76%) reported at least one normalized or ratio unit measure. 50 papers specifically reported the LF/HF ratio: 13/50 (26%) reported log-corrected units and 37/50 (74%) reported uncorrected units.

The time periods used for HRV recording were primarily 5 min ($n = 40$; 41%), or 10 min ($n = 20$; 21%). Recording times under 5 min were uncommon ($n = 13$; 13%), with $n = 10$ (10%) of these using a measure of LF power.

Remaining figures are descriptive of the parameters of review; **Figure 7** describes the primary interpretation given to ratio or

Table 1 | Inclusion and exclusion criteria for reviewed studies.

375	Reviewed	
278	Excluded	
	26	24 h or Holter monitor study
	6	Animal
	1	Duplicate record in database
	11	Elderly, geriatric, or palliative sample
	7	Elite or high level athletes
	86	Exclusive to patient population
	1	Incorrect calendar year (i.e., published 2013)
	32	Infant, child or teenage sample
	50	Letter, review, commentary, etc.
	23	No resting baseline given
	17	Non-linear, non-standard, etc. measures
	13	Time domain measures only
	5	Unavailable at the time of review
97	Included	
	97	Met criteria
	3	Multiple or duplicate usable records

Table 2 | Reporting of raw vs. adjusted values, single vs. multiple normalized or ratio units.

	Reported raw values	No raw values	
Single nu/Ratio unit	26	4	30
Multiple nu/Ratio units	26	18	44
	52	22	$n = 74$

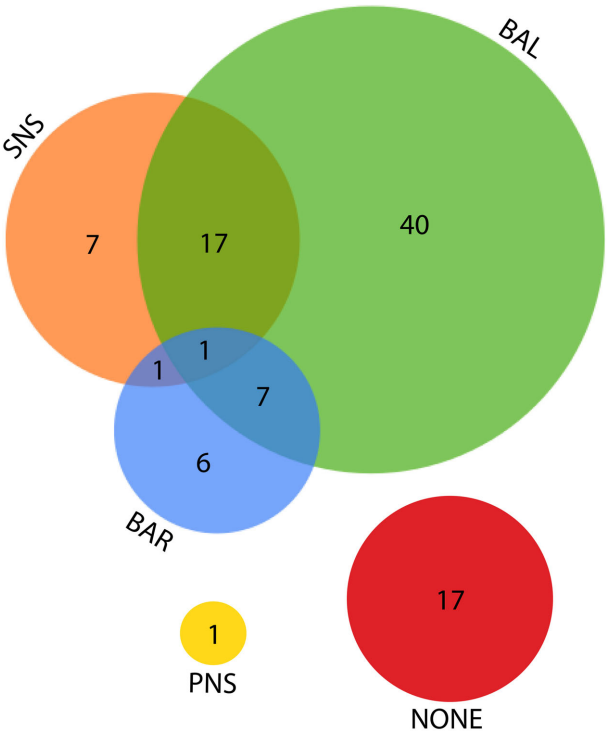


FIGURE 7 | Venn diagram for the various explanations given as the source of LF power. SNS, sympathetic nervous system; BAL, a balance of parasympathetic and sympathetic influences; BAR, the activity of the baroreflex; PNS, parasympathetic nervous system.

normalized power. The number of points available for each individual comparison below is noted separately per figure. **Figure 8** shows the means and relevant interquartile values assumed to be precisely equivalent due to equal rank order with the inverse-term regression relating LF/HF and nu units overlaid. **Figure 9** describes the sum and interrelationship of normalized values assumed to be precisely equal to unity. The relationship between mean normalized units and mean LF/HF ratio is shown in **Figure 10**, and their precision is shown in **Figure 11**.

DISCUSSION

Overall, the use of frequency analysis over short-term heart rate recordings to characterize autonomic state or sympathovagal balance is problematic. Relevant research frequently truncates or fails to explain the source of HRV power. Commonly co-investigated variables are reported as separate concepts, but are mathematically redundant as predicted. This redundancy is precise between individual values and moderate between group means. Time periods employed for recording are generally sufficient. Confounding variables which have the potential to substantially alter between- and within-subject variance are infrequently controlled.

OVERALL PRECISION AND EXPERIMENTAL CONTROL

The control of extraneous factors affecting recording in participants is perhaps the most problematic of the results here, because it may irreparably affect the veracity of between-subjects experimental models. Of course, depending on the circumstances, it may not be possible or even desirable to control all the listed variables—for instance, patient populations must remain on medication, opportunistic recording at any time of day is necessary to observe an episodic phenomenon, etc. However, the fact remains that circadian rhythm, medication, health status, food, water and bladder filling all potentially possess the ability to modify the variance of a normative group, even if only problematic in a minority of participants. Some of these external factors (medication, health status, and nicotine use) are well controlled, but a minority of work controlled for gastric or bladder filling. The amount that this affects a normative sample of HRV needs to be determined experimentally.

For experimentation within subjects, the situation is a lot less clear. Obviously, if within-subject measurement involves an intervention over multiple recording periods in time, the potential contamination presents precisely as it would between subjects. However, if a task effect is being observed in sequential recording periods during the same experiment, the problem may be substantially reduced. That is, in the presence of a strong artifact, the absolute or proportional change in HRV in response to a drug, task, intervention etc. may occur reliably but simply from an altered baseline.

Say, for instance, that gastric activity subsequent to feeding increases LF spectral power in an experimental participant

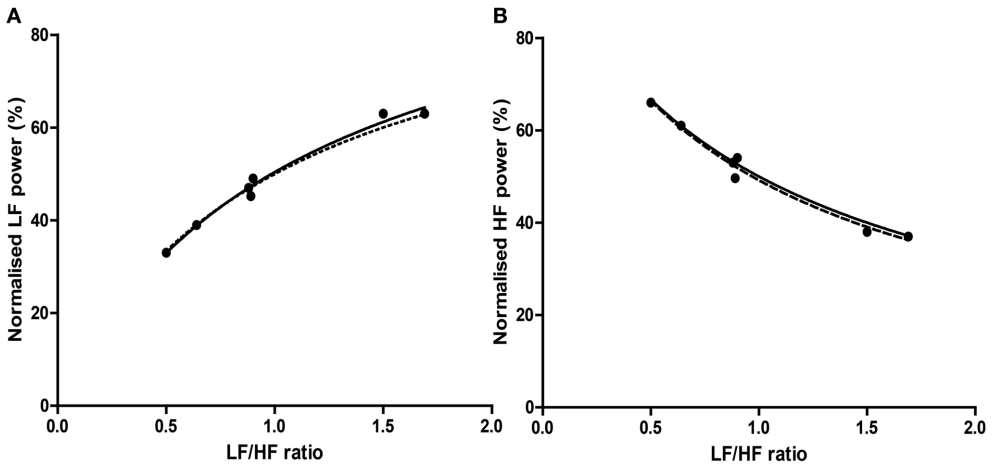


FIGURE 8 | The relationship of precisely equivalent values, i.e., median and interquartile range (IQR) between LFnu (A) or HFnu (B) and LF/HF ratio. Regression estimates: **(A)** $a = 1.00$, $b = 0.94$, $c = 1.06$, $r^2 = 0.985$, $n = 7$; **(B)** $a = 1.00$, $b = 0.98$, $c = 1.05$, $r^2 = 0.981$, $n = 7$. Values were uncommon as few studies reported both normalized and LF/HF ratio values in median/IQR format. The dashed line represents the mathematical identity as previously defined.

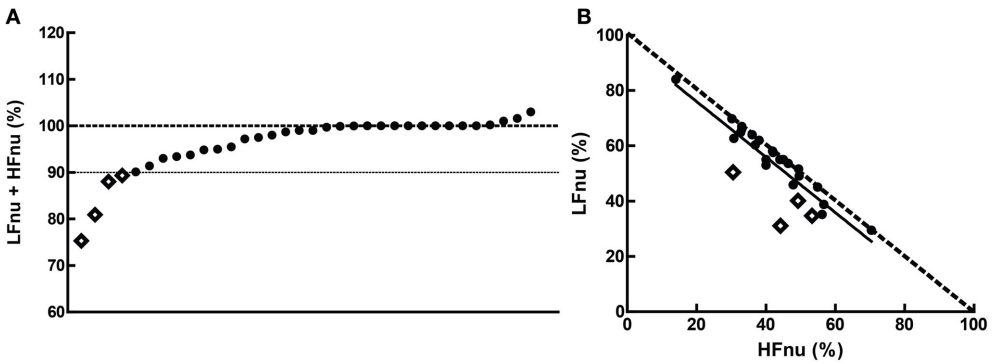


FIGURE 9 | The cumulative sums of the normalized components when (A) both were specified, and (B) their interrelationship (slope $= -1.003 \pm 0.1103$, $r^2 = 0.761$, $n = 20$). The dashed line in **(B)** represents the mathematical identity as previously defined. Points marked as diamonds are LFnu + HFnu <90%, and marked where relevant on **Figure 10**.

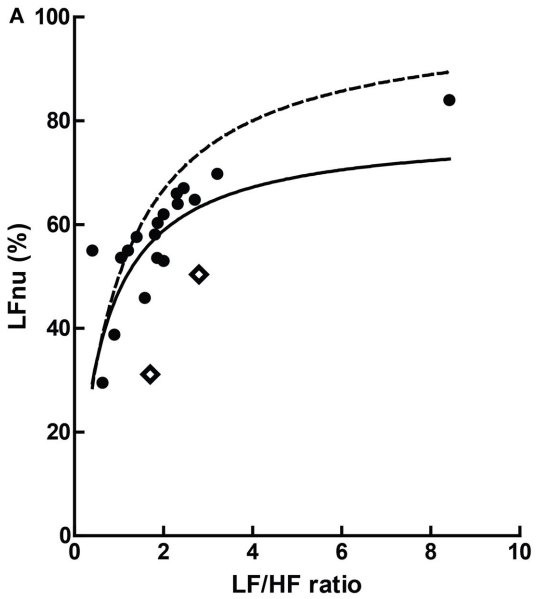
who is then subjected to social stress, which is also expected to increase LF power. If that power rises to a proportional level, i.e., rises by the same absolute or proportional amount that it otherwise would in the absence of feeding, then any potential source of error has been substantially ameliorated by the design.

The problem in this instance would be amplified if there was an interaction between the altered baseline and task. If the response is attenuated or amplified, i.e., there is an interaction between the task effect and the source of artifact, then the situation is concerning, doubly so if a small sample is being used. To a small sample with normative values (e.g., Nunan et al., 2010; $LF = 519\,ms^2$, $HF = 657\,ms^2$), a mean increase in HF ms^2 subsequent to drinking (Routledge et al., 2002; HF $+686\,ms^2$) has the potential to destroy the fidelity of an entire measurement at baseline. If this change interacts with any given task-related effect, the sample quickly runs the risk of becoming uninterpretable.

NORMALIZED AND LF/HF VARIABLES; CO-REPORTING AND EQUIVALENCE

Co-reporting of equivalent ratio values is reasonably common, observed in over half (59%) of the studies which employed normalized units or LF/HF. The definition of these measures as redundant is borne out by the results. The argument might be made that this is not problematic, as HRV studies typically employ a range of time and frequency domain measures which are multicollinear. This is impossible to avoid, as most HRV methods some manner of apportioning a meaning to some quantity of the available variance in a heart period series, and cross from time to frequency domain readily as the integral of the total power spectrum is equal to the variance. These interrelationships are often very high—Massin et al. (1999) report, for example, that RMSSD, pNN50 and HF power are mutually correlated above 0.9.

However, there are several problems with this line of argument when applied to multiple ratio measures. It is generally accepted that multiple similar measures of HRV might be employed to the



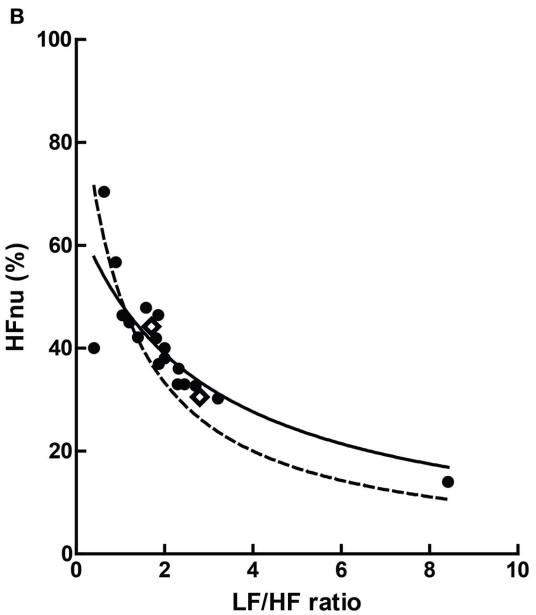


FIGURE 10 | The relationship of mean ratios to approximate mean normalized values, where (A) LF $r^2 = 0.363$; (B) HF $r^2 = 0.685$, $n = 20$. The dashed lines represent the mathematical identities as previously defined.

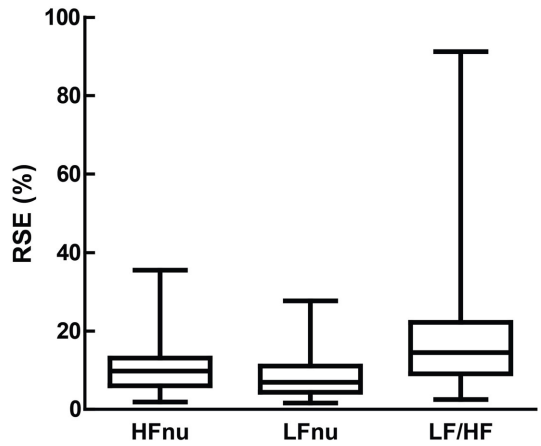


FIGURE 11 | Relative standard error (i.e., the standard error divided by the mean, which forms the sample-sized adjusted coefficient of variation) for LFnu ($n = 29$), HFnu ($n = 30$) and LF/HF ($n = 30$), shown here by median, interquartile range and min/max.

same end to address the same phenomenon may differ slightly—for example, in a group undergoing an experimental intervention, RMSSD may be significantly increased by task and HF power not. Were this the case, the result would be taken as equivocal support for a change in cardiac vagal modulation, and the difference in the result between the similar calculatory methods would be addressed. Alternatively, if LFnu was significant and LF/HF not, this might be interpreted as a change in relative sympathetic activity, but no change in sympathovagal balance. Secondly, other measures which may be closely correlated attempt to measure the same phenomenon but use entirely disparate methods. However, normalized and ratio values are mathematically, not theoretically, related. There is no equivalent transform which might imply, say, an RMSSD value from a value of HF spectral power.

Thirdly, it is unclear which of the ratio measures best represents the comparison they are both attempting to capture. For instance, normalized units are more likely to obey parametric assumptions, but LF/HF may exhibit significant skew and kurtosis (Kobayashi et al., 2012). This is directly confirmed in **Figure 11**, as the relative standard error of both normalized units is substantially lower than LF/HF. If the relationship is mathematically equivalent, and we also accept a degree of measurement error, how should we interpret an instance where one value is significant and the other not? Is it inconsistent to report both if one works? If a sample of LF/HF obeys standard parametric assumptions, should it still be log-transformed?

Lastly, it is by no means uncommon for normalized spectral bands and the LF/HF ratio to form the entirety of an analysis in an attempt to measure relative sympathetic and parasympathetic contributions, and their interrelationship. This is rarely the case with other interrelated variables.

NORMALIZED VARIABLES AND UNITY

Overall, the predictions from the mathematical equivalence in the introduction were borne out—the curvilinear relationship between normalized and ratio figures were observed. In some comparisons, however, slight to significant departure can be seen contingent on normalized values adding to unity—this was the case for a minority of studies observed, with 4 of the 34 observed sums of LFnu and HFnu below 90%. These departures are reflected in **Figure 9**, and are marked as diamonds between all graphs for continuity.

There are multiple, non-mutually exclusive possibilities for this discontinuity which are not simply calculatory error. The first is the use of an alternative definition of adjusted LF power, i.e., LF/(total power). As the contribution from VLF power is usually

significant, this may explain the larger error but not the preponderance of values from 95 to 99% of the sum LFnu + HFnu. The second is that there are small but significant contributions to spectral power above 0.4 Hz, which are included in total power but not included as part of the HF frequency band—this might explain the frequent values close to 1, but not the significant deviations from it. The third is confusion in the calculation of the autoregressive method between the dominant power components in each spectral band which frequently overlap into the other segments, and the power spectral density of all components but strictly within the defined power band.

This non-equivalence is responsible for the shift in the distribution that can be seen in **Figure 9**—the bulk of the points are distributed as expected, but lie rightwards of the line of definition, where the ratio value is slightly bigger than predicted. The regression of the data conforms to this.

It cannot be concluded precisely what this difference represents. The best case scenario for the use of normalized units would be that this difference is borne of the fact that the individual spectral powers retain some statistical independence, and describe a portion of the relevant variance in their spectral bands without absolute covariation.

The worst case scenario is that this is simply a calculatory curiosity which has no specific meaning, borne of the arbitrary distinction between the whole spectral element (say, the power under the peak which provides the bulk of LF power) and the truncated version (say, the PSD from precisely 0.04 to 0.15 Hz of all components). If the "true" sum of LFnu and HFnu is always 1, then their statistical equivalence is complete—in comparisons, the metrics they return for continuous or directional comparison (e.g., Spearman's r, Student's t) to other variables will differ by sign, and *F*-values not at all. If this is the case, then this error has previously allowed the precise equivalence of LFnu or HFnu to be partially obscured, and normalized/ratio units substantially obscured, by providing values which are somewhat divergent and giving the appearance of independence.

DISPERSION OF RATIO VALUES

LF/HF ratio shows an obvious decrease in precision over either normalized variables (**Figure 11**). This is likely due to the volatility of the LF/HF ratio during normal sympathetic dominance as HF approaches zero, as recently suggested (Billman, 2013). Two examples from the sample set reviewed demonstrate this potential volatility. Muralikrishnan et al. (2012) report a range of autonomic measures on Ishant Yoga practitioners vs. normal controls. At supine rest, the normal sample was described thus: $n = 14$, $\mu = 1.86$, and $SD = 6.35$. As LF/HF ratio cannot be less than zero, this sample must contain one or more participants with a ratio of 15 or more, most likely due to HF power being minimal (a common occurrence when breathing rates are slow; see Saboul et al., 2014). As a consequence, the description of this sample by mean and standard deviation is unintelligible, as the distribution has profound positive skew.

Similarly, Chen et al. (2012) compared HRV metrics of resuscitated cardiac arrest patients, patients with sepsis and healthy controls. The raw LF and HF power of healthy controls ranged between approximately 12–100 times greater than all patient groups. For instance, post-cardiac arrest patients had both LF and HF spectral power of approximately 5 ms^2, and healthy controls approximately 100 ms^2. As a consequence, both of these groups had a median LF/HF ratio of 1. Alternatively, a difference was found between non-surviving (LF/HF = 0.2) and surviving (LF/HF = 3.1) cardiac arrest patients. However, none of the four spectral powers involved in this calculation had a median above 7.6 ms^2.

None of the values above defined by ratio would be meaningful by themselves, and in the context of the original papers are appropriately reported and interpreted with both measures of the raw spectral power and total power. But as seen in **Table 2**, this is not the case for approximately 30% of published work.

LIMITATIONS

There are several limitations to the present work, the most obvious of which is that it makes no attempt to propose a method by which spectral power *should* be assessed. There are a profound amount of variables to consider regarding such a question; whether spectral assumptions are appropriate in the first instance, which variant of spectral analysis is sufficient or optimal, how heartbeat series should be interpolated (if at all), how the series should be corrected (if at all) or windowed, and so forth.

On the same basis, this work records neither outcomes nor differences between free and paced breathing, specific time of day of recording, or participant age. The data set as reviewed is incapable of sustaining the scale of such a meta-analysis—for instance, Nunan et al. (2010) initially reviewed over 3000 individual pieces of research to draw a sample of $n = 44$ in which different methods of spectral analysis and the values they return within LF and HF bands could be compared, a requirement to meta-analytically compare regular measures of HRV spectral power to normalized or ratio variables. This relationship would almost certainly interact with the use of HRV to predict, investigate or stratify clinical conditions, as HRV values may be profoundly affected especially by autonomic and circulatory diseases. As a consequence, this work cannot speak to whether normalized or ratio units are capable of sustaining conclusions which are similar to those from raw values. Regardless of the basis on which they are characterized, or their internal consistency, or the manner of their usage, they may still reliably report the same or similar conclusions to other methods.

Finally, this work cannot determine the dispersion of values over the time of day or lifespan, or any relationship between these variables and the methodology used. This would be a worthy topic of future investigation, as HRV is used differentially within particular fields which are defined by time or age at recording (for instance, chronobiology or antenatal care), and the methodology between them is rarely compared.

CONCLUSION

This review has concentrated on commonly used methodology, and hence the internal and external consistency, for collecting HRV by frequency analysis over the short term. In general, the nature of commonly used HRV metrics are not well understood, and these measurement are intimately related both on a mathematical level and in practice. Regardless of this, they

are frequently treated as independent concepts and deployed redundantly. Additionally, insufficient attention is paid to the environment of data collection. None of these are trivial concerns; rather, they call into question the accuracy of the existing literature on HRV and warrant the re-establishment of an authoritative source for correct methodology and practice.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fphys.2014.00177/abstract

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The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance

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Power spectral analysis of the beat-to-beat variations of heart rate or the heart period (R–R interval) has become widely used to quantify cardiac autonomic regulation (Appel et al., 1989; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Denver et al., 2007; Thayler et al., 2010; Billman, 2011). This technique partitions the total variance (the "power") of a continuous series of beats into its frequency components, typically identifying two or three main peaks: Very Low Frequency (VLF) <0.04 Hz, Low Frequency (LF), 0.04–0.15 Hz, and High Frequency (HF) 0.15–0.4 Hz. It should be noted that the HF peak is shifted to a higher range (typically 0.24–1.04 Hz) in infants and during exercise (Berntson et al., 1997). The HF peak is widely believed to reflect cardiac parasympathetic nerve activity while the LF, although more complex, is often assumed to have a dominant sympathetic component (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Billman, 2011). Based upon these assumptions, Pagani and co-workers proposed that the ratio of LF to HF (LF/HF) could be used to quantify the changing relationship between sympathetic and parasympathetic nerve activities (i.e., the sympatho-vagal balance) (Pagani et al., 1984, 1986; Malliani et al., 1991) in both health and disease. However, this concept has been challenged (Kingwell et al., 1994; Koh et al., 1994; Hopf et al., 1995; Eckberg, 1997; Houle and Billman, 1999; Billman, 2011). Despite serious and largely under-appreciated limitations, the LF/HF ratio has gained wide acceptance as a tool to assess cardiovascular autonomic regulation where increases in LF/HF are assumed to reflect a shift to "sympathetic dominance" and decreases in this index correspond to a "parasympathetic dominance." Therefore, it is vital to provide a critical assessment of the assumptions upon which this concept is based.

The hypothesis that LF/HF accurately reflects sympatho-vagal balance rests upon several interrelated assumptions as follows (modified from Eckberg, 1997): (1) cardiac sympathetic nerve activity is a major, if not the exclusive, factor responsible for the LF peak of the heart rate power spectrum; (2) cardiac parasympathetic is exclusively responsible for the HF peak of the heart rate power spectrum; (3) disease or physiological challenges provoke reciprocal changes in cardiac sympathetic and parasympathetic nerve activity (i.e., increases in cardiac parasympathetic nerve activity are always accompanied with corresponding reductions in cardiac sympathetic nerve activity and *vice versa*); and (4) there is a simple linear interaction between the effects of cardiac sympathetic and cardiac parasympathetic nerve activity on heart rate variability (HRV).

As previously noted, frequency domain analysis of HRV usually reveals two or more peaks, a lower frequency (<015 Hz) and a higher frequency peak (>0.15 Hz) that are often assumed to correspond to cardiac sympathetic and cardiac parasympathetic neural activity, respectively (Pagani et al., 1984, 1986; Malliani et al., 1991). However, accumulating evidence clearly demonstrates that this assumption is naïve and greatly oversimplifies the complex non-linear interactions between the sympathetic and the parasympathetic divisions of the autonomic nervous system (Berntson et al., 1997; Eckberg, 1997; Parati et al., 2006; Billman, 2009, 2011). This is particularly true with regards to the relationship between LF power and cardiac sympathetic regulation (Randall et al., 1991; Ahmed et al., 1994; Kingwell et al., 1994; Hopf et al., 1995; Eckberg, 1997; Houle and Billman, 1999; Parati et al., 2006; Billman, 2009, 2011).

The LF peak of the heart rate power spectrum is reduced by at least 50% by either cholinergic antagonists or selective parasympathectomy (Akselrod et al., 1981; Randall et al., 1991; Houle and Billman, 1999). Importantly, this peak is not completely eliminated by the combination of selective denervation and beta-adrenoceptor blockade (Randall et al., 1991); ∼25% of the peak remains after this treatment. As a consequence, LF/HF often actually increases from baseline values when both parasympathetic and adrenergic nerve activity have been blocked. For example, using the data reported by Randall and co-workers (Randall et al., 1991), LF/HF increased from a baseline value of 1.1–8.4 when selective parasympathetic denervation was combined with beta-adrenergic receptor blockade, falsely suggesting a major shift to sympathetic dominance! In a similar manner, interventions that would be expected to increase cardiac sympathetic activity, such as acute exercise or myocardial ischemia, not only failed to increase LF power but actually provoked significant reductions in this variable (Houle and Billman, 1999), once again yielding LF/HF values that are difficult to interpret. Indeed, despite large increases in heart rate, LF/HF ratio was largely unaffected by either acute myocardial ischemia, exercise, or the cholinergic antagonist atropine sulfate (Houle and Billman, 1999). Finally, direct recording of sympathetic nerve activity failed to correlate with LF power in either healthy subjects or patients with heart failure (Hopf et al., 1995; Notarius and Floras, 2001; Jardine et al., 2002; Moak et al.,

2007; Piccirillo et al., 2009), a condition known to increase cardiac sympathetic drive (Hasking et al., 1986; Saul et al., 1988; Watson et al., 2007). Thus, the LF component of HRV does not provide an index of cardiac sympathetic drive but rather reflects a complex and not easily discernible mix of sympathetic, parasympathetic, and other unidentified factors with parasympathetic factors accounting for the largest portion of the variability in this frequency range. As a consequence, the physiological basis for LF/HF is difficult to discern.

Although the vast majority of the clinical and the experimental studies demonstrate a strong association between HF power and cardiac parasympathetic activity (Katona et al., 1970; Appel et al., 1989; Billman and Hoskins, 1989; Billman and Dujardin, 1990; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Billman, 2009, 2011; Thayler et al., 2010), this concept has also been challenged (Kollai and Mizsei, 1990; Goldberger et al., 1994; Hedman et al., 1995; Taylor et al., 2001; Parati et al., 2006). Unlike LF power and sympathetic nerve activity, a strong correlation between HF power and direct recordings of cardiac parasympathetic activity has been reported (Chess et al., 1975; Piccirillo et al., 2009). However, just as parasympathetic activation exerts profound influences on the LF component of HRV, sympathetic neural activity may modulate the HF component of the R–R interval variability (Taylor et al., 2001; Cohen and Taylor, 2002). Taylor et al. (2001) found that cardioselective beta-adrenergic receptor blockade (drugs that should not indirectly alter vagal outflow via action within the central nervous system) increased the amplitude of the respiratory sinus arrhythmia over a wide range of respiratory frequencies (i.e., the increases were not restricted to lower frequencies, <0.15 Hz). They concluded that "*cardiac sympathetic outflow can oppose vagally mediated R-R interval oscillations and sympathetic blockade removes this effect*" (Cohen and Taylor, 2002). Based upon these data, sympathetic nerve activation may alter the HF peak by perhaps as much as 10%. Thus, differences in cardiac sympathetic activation during a physiological challenge (e.g., exercise or postural changes) in healthy subjects or that occur as the consequence of cardiovascular disease (following myocardial infarction) could restrain vagally mediated changes in HRV. These data suggest that HF power cannot be solely attributed to changes in cardiac vagal efferent nerve traffic, further compromising an accurate interpretation of the LF/HF ratio.

Accurate interpretation of LF/HF ratio also depends upon the assumption that physiological interventions always elicit reciprocal changes in parasympathetic and sympathetic nerve activity. However, following the termination of exercise sympathetic activation remains high despite the rapid re-activation of cardiac parasympathetic drive (Smith et al., 2005; Billman and Kukielka, 2007; Billman, 2009). Furthermore, chemoreceptor activation by carbon dioxide provokes parallel reductions in sympathetic and parasympathetic nerve activity (Eckberg, 1997) while facial emersion in cold water (activating the so-called "diving reflex") increased sympathetic nerve activity yet elicited a profound bradycardia (Eckberg et al., 1984; Fagius and Sundlof, 1986). The observation that heart rate declines, despite increases in sympathetic nerve activity, highlights the complex non-linear interactions of the sympathetic and parasympathetic nervous system, providing an example of "accentuated antagonism" (Levy, 1971; Stramba-Badiale et al., 1991; Uijtdehaage and Thayer, 2000), the dominance of parasympathetic over sympathetic influences on cardiac rate. Finally, reciprocal changes in parasympathetic and sympathetic nerve activity do not always occur even during the activation of the baroreceptor reflex (Eckberg, 1997). Eckberg and co-workers have shown that, although small changes in arterial pressure typically provoke reciprocal changes sympathetic and parasympathetic nerve activity, large increases in arterial pressure only provoke increases in parasympathetic nerve activity without altering the prevailing sympathetic activity (Eckberg, 1980; Rea and Eckberg, 1987). Furthermore, autonomic response to baroreceptor reflex activation depends on whether the pressure changes occur near the threshold or the saturation point of the response curve; the same change in pressure can elicit larger or smaller autonomic responses depending on how close the prevailing pressure lies to the threshold (larger) or saturation (smaller) portion of the stimulus-response curve (Eckberg, 1980, 1997). As previously noted, changes in heart rate do not result from the simple algebraic summation of the sympathetic and parasympathetic nerve activity. Rather, parasympathetic nerve activation can completely override even maximal sympathetic nerve stimulation, provoking large reductions in heart rate in the face of sympathetic nerve activation as was previously noted for the diving reflex. Thus, physiological interventions can elicit either complex non-linear reciprocal or parallel changes in either division of the autonomic nervous system. These complex interactions can profoundly influence the calculation and the interpretation of LF/HF.

Mathematical considerations can also influence LF/HF values. Similar LF/HF values can be obtained via either exclusive changes in the numerator (i.e., LF), or the dominator (i.e., HF), or by some combination of the two, as is illustrated in **Table 1**. For example, a doubling of parasympathetic activity against maintained sympathetic nerve activation yields the identical LF/HF value as a 50% reduction in sympathetic nerve activity against a constant background parasympathetic regulation. Based upon the literature, one can conclude that parasympathetic nerve activation contributes to at least 50% of the LF variability while sympathetic activity, at best, only contributes 25% to this variability (Randall et al., 1991). A substantial portion of the variability in the LF band also results from other unidentified factors. In a similar fashion, sympathetic nerve activity could contribute to perhaps as much as 10% of the HF variability (Taylor et al., 2001; Cohen and Taylor, 2002). As a consequence, the effects of changing sympathetic and parasympathetic activity on the LF/HF are quite variable and not intuitively obvious, as is illustrated in **Figure 1**. This figure was constructed using the following formula that was based upon a synthesis of the literature (particularly, Randall et al., 1991; Taylor et al., 2001; Cohen and Taylor, 2002), LF = 0.5 parasympathetic + 0.25 sympathetic activity while HF = 0.9 parasympathetic + 0.1 sympathetic nerve activity. The nerve activity was varied from baseline (1 arbitrary unit each) increasing or decreasing by up to a

Table 1 | Examples of the effects of varying cardiac sympathetic and parasympathetic nerve activity on LF/HF.

Parasympathetic nerve activity	Sympathetic nerve activity	LF	HF	LF/HF
1	1	0.75	1	0.75
2	1	1.25	1.9	0.66
0.5	1	0.5	0.55	0.91
1	2	1	1.1	0.91
1	0.5	0.625	0.95	0.66
2	2	1.5	2	0.75
2	0.5	1.125	1.85	0.61
0.5	2	0.75	0.65	1.15
0.5	0.5	0.375	0.5	0.75

These numbers were generated using the following formula (derived from a synthesis of the literature, particularly Randall et al., 1991; Taylor et al., 2001; Cohen and Taylor, 2002): LF/HF = (0.5 parasympathetic + 0.25 sympathetic nerve activity)/(0.9 parasympathetic + 0.1 sympathetic nerve activity). The nerve activity is reported as arbitrary units where at baseline sympathetic and parasympathetic nerve activity were normalized as 1 arbitrary unit each. The data shown are for baseline and various combinations of doubling (2 × baseline) or halving (0.5 × baseline) the autonomic nerve activity.

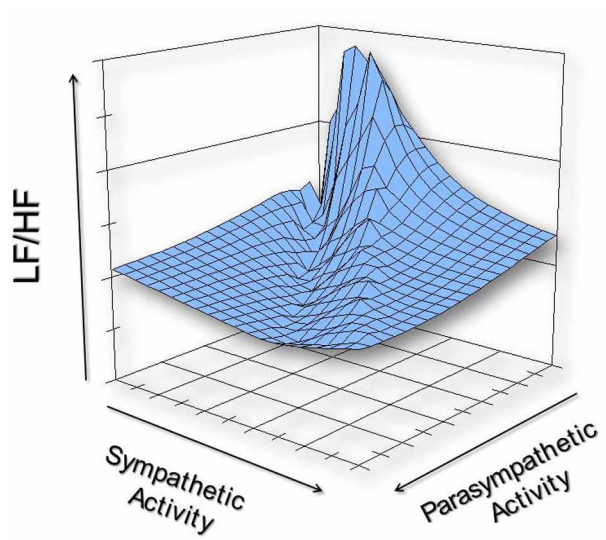

FIGURE 1 | An illustration of the possible non-linear effects of varying cardiac sympathetic and cardiac parasympathetic nerve activity on LF/HF. This graph was constructed using the following formula (derived from a synthesis of the literature, particularly Randall et al., 1991; Taylor et al., 2001; Cohen and Taylor, 2002): LF/HF = (0.5 parasympathetic + 0.25 sympathetic nerve activity)/(0.9 parasympathetic + 0.1 sympathetic nerve activity). The nerve activity was varied from baseline (1 arbitrary unit each) increasing or decreasing by up to a factor of 10 (i.e., from 0.1 to 10 units).

factor of 10. Due to the substantial contribution (accounting for up to 25% of the variability) (Randall et al., 1991) from non-neural factors to LF power, very distorted values of LF/HF can be obtained when both sympathetic and parasympathetic nerve activity are minimal. If for example, one assumes that LF = 0.5 parasympathetic + 0.25 sympathetic + 0.25 other factors and both parasympathetic and sympathetic nerve activity are reduced to 1/100 the baseline values, the calculated LF/HF becomes $(0.005 + 0.0025 + 0.25)/(0.009 + 0.001) = 25.75$! Despite the almost complete absence of cardiac autonomic regulation, this value could be inappropriately interpreted as a major shift toward sympathetic dominance. Furthermore, LF/HF cannot be determined if both sympathetic activity and parasympathetic nerve activity were to be abolished completely (i.e., when the dominator is zero). Finally, mathematical complications also arise due to the non-linear relationship between R–R interval and heart rate; similar changes in heart rate elicit much greater variability in R–R interval at lower than at higher heart rates (Sacha and Pluta, 2008). As a consequence of this non-linear relationship, it is difficult to separate the changes in HRV that arise from direct action of cardiac autonomic nerves from those changes that result indirectly from neurally induced changes in average heart rate. This observation led Sacha and Pluta (2008), to propose that HRV has both physiological and mathematical influences that can be corrected by the division of HRV by average R–R interval. Thus, the physiological basis for changes in LF/HF is not readily discernible and spurious values for LF/HF can result as a consequence of the mathematical manipulations of the data.

It should also be noted, that HRV (and thereby LF/HF) is affected by respiratory parameters and mechanical events independent of changes in cardiac autonomic nerve activity. The contribution of mechanical factors (due to stretch of the atria that results from both changes in cardiac filling and the changing thoracic pressure that occur during respiration) to changes in HRV was first proposed by Bainbridge (1930). This conclusion is supported by the observation that heart transplant patients, despite the absence of cardiac nerves, still exhibit small (∼2–8% of normal) change in R–R interval associated with the respiratory cycle (Bernardi et al., 1989). Taylor et al. (2001) further demonstrated that atrial stretch can exert significant influences on R–R interval in subjects with complete autonomic blockade. They found that after combined cholinergic and adrenergic receptor blockade slow deep breathing could still provoke oscillations of ∼120 ms in healthy human subjects (Taylor et al., 2001). In similar manner, mechanical distortion (stretch) of the sinoatrial nodal stretch in pigs without functional autonomic innervation (vagal nerve section combined with propranolol

treatment) reduced the HF component of HRV (Horner et al., 1996).

Respiratory parameters can also profoundly alter heart rate and R–R interval variability independent of changes in cardiac autonomic regulation (i.e., against a constant background level of automatic regulation) (Angelone and Coulter, 1964; Davies and Neilson, 1967; Hainsworth, 1974; Melcher, 1976; Hirsch and Bishop, 1981; Brown et al., 1993; Van De Borne et al., 2001). It is now well established that increases in respiratory frequency reduce the amplitude of heart rate oscillations (Angelone and Coulter, 1964; Melcher, 1976; Hirsch and Bishop, 1981; Brown et al., 1993) while either increases in tidal (Davies and Neilson, 1967; Melcher, 1976; Hirsch and Bishop, 1981; Eckberg, 1983; Kollai and Mizsei, 1990; Brown et al., 1993) or static lung volume (Hainsworth, 1974) provoke increases in the R–R interval variability. The facts are in direct opposiiton to the assumptions. Conversely, reductions in respiratory frequency increase HRV (Angelone and Coulter, 1964; Melcher, 1976; Hirsch and Bishop, 1981; Brown et al., 1993) while decreases in tidal volume lead to reductions in the R–R interval variability (Davies and Neilson, 1967; Melcher, 1976; Hirsch and Bishop, 1981; Eckberg, 1983; Kollai and Mizsei, 1990; Brown et al., 1993). Thus, it is critical to control breathing (paced or timed breathing) in order to interpret HRV data accurately. For obvious reasons, it is much more difficult to control respiratory parameters in conscious animal than in human studies. However, these respiratory parameters frequently are not controlled even in human studies (Brown et al., 1993). Brown and co-workers (Brown et al., 1993), reviewed the human literature and found that only about 51% controlled respiratory rate, and even fewer studies controlled for tidal volume (11%). They further reported that respiratory parameters not only altered HF power but also strongly influenced the LF components of the R–R interval power spectrum, a component that previously was viewed to vary independently of changes in respiration (Brown et al., 1993).

Finally, prevailing heart rate can also influence HRV. There are a number of studies that report a strong positive correlation between mean R–R interval and various time domain indices of HRV (e.g., the standard deviation of normal beats, SDNN) (Kleiger et al., 1987; Van Hoogenhuyze et al., 1991; Fleiss et al., 1992) such that HRV was greater during longer mean R–R intervals (slower heart rates) than at shorter mean R–R intervals (faster heart rates). Frequency domain analysis of HRV is similarly affected by mean heart rate. Sacha and Pluta (2005) found that LF was directly related, while HF was indirectly related, to the average heart rate of the subject. As a consequence, they further report that LF/HF varied depending on heart rate, lower at slower and higher at faster heart rates. Thus, heart rate *per se* can influence LF/HF independent of changes cardiac autonomic nerve activity.

As we have seen, the hypothesis that LF/HF quantifies "sympatho-vagal balance" depends upon four interrelated assumptions, all of which can be proven to be false. The facts are in direct opposition to the assumptions. In particular, the complex nature of LF power, its exceedingly poor relationship to sympathetic nerve activation, and the non-linear (and often non-reciprocal) interactions between sympathetic and parasympathetic nerve activity that are confounded by the mechanical effects of respiration and prevailing heart rate, make it impossible to delineate the physiological basis for LF/HF with any degree of certainty. Thus, the LF/HF sympatho-vagal balance hypothesis has been disproven—the preponderance of evidence confirms that LF/HF data cannot accurately quantify cardiac "sympatho-vagal balance" either in health or disease.

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Clinical application of heart rate variability after acute myocardial infarction

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Heart rate (HR) variability has been extensively studied in patients surviving an acute myocardial infarction (AMI). The majority of studies have shown that patients with reduced or abnormal HR variability/turbulence have an increased risk of mortality within few years after an AMI. Various measures of HR dynamics, such as time-domain, spectral, and non-linear measures of HR variability, as well as HR turbulence, have been used in risk stratification of post-AMI patients. The prognostic power of various measures, except of those reflecting rapid R–R interval oscillations, has been almost identical, albeit some non-linear HR variability measures, such as short-term fractal scaling exponent, and HR turbulence, have provided somewhat better prognostic information than the others. Abnormal HR variability predicts both sudden and non-sudden cardiac death after AMI. Because of remodeling of the arrhythmia substrate after AMI, early measurement of HR variability to identify those at high risk should likely be repeated later in order to assess the risk of fatal arrhythmia events. Future randomized trials using HR variability/turbulence as one of the pre-defined inclusion criteria will show whether routine measurement of HR variability/turbulence will become a routine clinical tool for risk stratification of post-AMI patients.

Keywords: mortality, coronary artery disease, sudden cardiac death

The purpose of this mini-review is to discuss the role of measurement of heart rate (HR) variability in patients after an acute myocardial infarction (AMI), especially its potential or proven clinical utility, by itself or in combination with other variables. Studies will be reviewed that employed a large variety of HR variability measures, with the majority derived from easily obtainable 24-h recordings.

Clinical utility is, of course, a broad concept. In this review, we will consider a measure to have clinical utility, if it identifies patients at higher risk of an adverse outcome, differentiates those with more advanced from those with less advanced disease, identifies those who would benefit from interventions or identifies those who are benefiting from or being harmed by an intervention. HR variability has been applied in a huge number of studies. We have chosen to limit our review to main studies focusing on clinical application of HR variability measurements from 24-h Holter recordings in patients who have survived an AMI.

BACKGROUND FOR ABNORMAL HR VARIABILITY

Heart rate variability is a term that encompasses a large number of measures of different types that have been described in details in other articles of this Special Topic of the journal. Time-domain measures reflect "how much" HRV there is. In general, if such measures are extremely low, it can be assumed that there is true autonomic dysfunction, but higher values for these various measures could reflect more healthy autonomic function or an unhealthy, highly irregular HR pattern (erratic rhythm). Frequency domain measures have the same properties. Extremely low values are associated with a lack of autonomic modulation of HR, but higher values, without examination of their underlying organization using power spectral plots or other graphical methods, cannot be assumed to reflect better HR variability. Non-linear measures like the short-term fractal scaling exponent and the power–law slope, appear to better differentiate between healthy and unhealthy organization of the heart's rhythm and have proved to be more sensitive for risk stratification that some of the standard measures (Bigger et al., 1996; Huikuri et al., 2000). HR turbulence, which was covered recently in detail (Bauer et al., 2008), also appears to be sensitive to autonomic dysfunction, especially to impaired baroreflex function. It is likely that the optimal characterization of normal and abnormal HR variability would be based on a combination of these different HR variability parameters rather than on a single one.

PROGNOSTIC SIGNIFICANCE OF HR VARIABILITY AFTER AMI

The prognostic significance of HR variability has been extensively studied in patients who have survived an AMI. **Table 1** summarizes the results of main studies assessing the prognostic significance of HR variability measurements from 24-h recordings. As early as Wolf et al. (1978) showed that patients with a low magnitude of short-term HR variability (no sinus arrhythmia on 30 consecutive R–R intervals on admitting ECG) had a poor prognosis after AMI. In the late 1980s, the multicenter post-infarction project (MPIP) confirmed the predictive value of reduced HRV by measuring 24-h SD of N–N intervals (SDNN) over 24 h following AMI (Kleiger et al., 1987). Adjustment for covariates did not explain

Table 1 | Summary of main studies assessing the prognostic significance of heart rate variability after acute myocardial infarction.

Study	HRV measurement	Number of patients	Mean follow up time	Primary endpoint	Main results	Comment
Kleiger et al. (1987)	SDNN	808	34 months	All-cause mortality	Increased mortality, if SDNN < 50 ms	Landmark study
Bigger et al. (1992)	Frequency domain measures	715	2.5 years	All-cause death, cardiac death, arrhythmic death	All frequency domain measures of HRV predicted an increased risk of primary endpoint	VLF spectral power was the strongest predictor
Bigger et al. (1993)	Frequency domain measures	331	3 years	All-cause mortality	All frequency domain measures predicted the risk of primary endpoint	Holter recording were performed 1 year after AMI
Fei et al. (1996)	SDNN and HRV index	700	1 year	All-cause mortality	Geometric HRV index predicted mortality better than SDNN	
Bigger et al. (1996)	Power–law behavior	715	3 years	All-cause mortality	Power–law regression between log(power) and log(frequency) predicted mortality	Power–law regression analyzed from ULF and VLF spectral areas
La Rovere et al. (1998)	SDNN	1284	21 months	Cardiac death and non-fatal cardiac arrest	SDNN < 70 ms predicted increased cardiac mortality	Multicenter study
Huikuri et al. (2000)	Spectral, fractal and time-domain measures	446	685 days	All-cause mortality	Short-term fractal scaling exponent was the most powerful predictor of mortality	Post-AMI patients with depressed ejection fraction
Mäkikallio et al. (2005)	Spectral, fractal and, time-domain measures	2130	1012 days	Sudden cardiac death, non-sudden cardiac death	All HRV measures predicted sudden and non-sudden cardiac death	HRV predicted equally both sudden and non-sudden cardiac death. Largest HRV study
Stein et al. (2005)	Spectral, fractal, and time-domain measures	740	362 days	All-cause mortality	Fractal measures predicted all-cause mortality	Short-term fractal scaling exponent was more powerful predictor than other HRV measures
Perkiömäki et al. (2008)	Spectral, fractal and time-domain measures	675	30 months	Non-fatal coronary events	Several measures of HRV predicted primary endpoint	
Huikuri et al. (2009)	Spectral, fractal and time-domain measures	312	2 years	ECG documental VT or VF	Many HRV measures predicted the primary endpoint	VLF power was the strongest predictor
Erdogan et al. (2008)	SDNN	412	4.3 years	All-cause mortality	SDNN < 50 ms predicted mortality	Positive predictive power of SDNN was low

AMI, acute myocardial infarction; ECG, electrocardiogram; HRV, heart rate variability; SDNN, SD of N–N intervals; ULF, ultra-low frequency; VF, ventricular fibrillation; VLF, very-low frequency; VT, ventricular tachycardia.

this association. Other studies (including a reanalysis of MPIP) have shown that spectral measures of HR variability, mainly the very-low and low frequency spectral components, are reduced in survivors of AMI and that decreased values are associated with an increased risk of all-cause mortality (Bigger et al., 1992).

Because accurate Holter scanning is relatively labor intensive, the group at St. George's hospital in London experimented with global, geometric indices of HR variability (HR variability index) that were derived from the histogram of N–N intervals (Malik et al., 1989). Decreased values for these measures were also found to be associated with higher risk of mortality (Fei et al., 1996). In addition, this group suggested that markedly reduced SDNN for a single, stable 5-min period could pre-select patients in whom 24-h Holter recordings using geometric methods would provide significant risk stratification.

The autonomic tone and reflexes after myocardial infarction (ATRAMI) trial was a multicenter observational study performed about 10 years after MPIP. The ATRAMI investigators confirmed

that reduced 24-h SDNN (<70 ms in this case) is associated with an increased mortality during 21 months of follow up (La Rovere et al., 1998). Furthermore, the combination of low SDNN and left ventricular ejection fraction <35% carried a high risk of mortality.

Early studies focused on measurement of HR variability relatively early after AMI. Measures of HR variability appeared to be more depressed at the early phase of AMI with substantial improvement during recovery (Jokinen et al., 2003). Despite this autonomic recovery, measurement of HR variability has been shown to yield similar prognostic information when the 24-h HRV measurements were performed late after AMI (Bigger et al., 1993; Jokinen et al., 2003).

The post-MI studies performed in the 1980s and 1990s used 24-h time and frequency domain as well as geometric measures of HR variability for risk stratification of patients after AMI. Although all statistical, geometrical, and spectral measures of HR variability differ in their manner of computation and analysis, these methods are fundamentally based on moment statistics and describe the magnitude of HR variability or of its underlying components. It is therefore not surprising that most of these measures that have been shown to have prognostic value have relatively close mutual correlation, and that there are only minor differences in prognostic power between them. It must be kept in mind though that measures of beat-to-beat HR variability which are supposed to reflect respiratory related vagal control of HR (HF power, pNN50, rMSSD) have rarely shown a strong association with outcome. This is likely because of the high prevalence of erratic rhythm in these populations resulting in higher measures for these parameters that do not reflect better parasympathetic function. One study showed that when erratic short-term R–R intervals was excluded from the analysis, HR variability related to respiratory cycles predicted sudden cardiac death even better than the standard HR variability indexes (Peltola et al., 2008).

More recent studies have applied methods based on HR turbulence, non-linear dynamic, and maximum deceleration capacity of R–R intervals, which provide very different and perhaps complementary information on HR dynamics compared to traditional statistical methods (Mäkikallio et al., 2002; Bauer et al., 2006, 2008). Methods based on non-linear dynamics and HR turbulence have provided somewhat better prognostic information than that obtained by traditional methods (Bauer et al., 2006, 2008; Perkiömäki et al., 2008). In particular, decreased short-term fractal scaling exponent by the DFA method (called DFA1 or $\alpha 1$), a measure of greater randomness in HR patterns, has turned out to be a powerful non-linear index in risk stratification, a more powerful prognostic tool than other HR variability indexes in post-AMI populations (Huikuri et al., 2000; Mäkikallio et al., 2002). In the DIAMOND-MI trial, reduced short-term fractal scaling exponent identified post-AMI patients at a relative high risk of mortality and was more strongly related to outcome than traditional time and frequency domain measures (Huikuri et al., 2000). Moreover, reduced scaling exponent was related to both arrhythmic and non-arrhythmic mortality (Huikuri et al., 2000). In another study of consecutive patients with acute MI, both reduced short-term fractal scaling exponent and power–law slope were independently associated with recurrent non-fatal coronary events (Perkiömäki et al., 2008). Another measure of increased randomness of HR patterns, such as the ratio of the axes of a Poincare plot fitted to the plot of each N–N interval vs. the next, also predicted mortality in the CAST trial (Stein et al., 2005). Patients in the CAST were at variable times post-MI but were selected for having significant ventricular arrhythmias.

One study in the era of modern therapy showed a very-low incidence of severely depressed HR variability (SDNN <50 ms; Erdogan et al., 2008). All patients in the study were treated with direct percutaneous coronary angioplasty within 12 h of their event. Although the 4-year survival was significantly higher in the low SDNN group, the positive predictive value was low, suggesting that the predictive power of HR variability may not be as high as earlier in patients receiving optimal medical and revascularization therapy. However, results were based on traditional time-domain HR variability measures and there was no report of results for non-linear measures or HR turbulence in this population.

HR VARIABILITY AS A PREDICTOR OF ARRHYTHMIC EVENTS/SUDDEN CARDIAC DEATH

The studies assessing the prognostic power of HR variability after AMI have usually used all-cause mortality as the primary endpoint (see **Table 1**). Some studies, using various definitions of arrhythmic events in their designs, have suggested that reduced or altered HR variability may be specifically related to arrhythmic events and sudden cardiac death. However, the clinical applicability of measurement of HR variability as a predictor of fatal arrhythmic events was challenged in a randomized prophylactic implantable cardioverter-defibrillator (ICD) trial, the defibrillator in AMI trial (DYNAMIT) which, however, used reduced SDNN combined with reduced left ventricular ejection fraction measured early (within 2 weeks) after AMI as an inclusion criterion for the trial. The trial could not show any mortality benefit from ICD therapy in these presumably high risk patients (Hohnloser et al., 2004). The more recent multicenter cardiac arrhythmias and risk stratification after myocardial infarction (CARISMA) study showed that reduced HR variability measured relatively late (6 weeks after AMI) rather than early after AMI, especially the very-low frequency spectral component, was the most powerful index among many other non-invasive risk markers in predicting the fatal or near-fatal arrhythmic events diagnosed by implantable arrhythmia devices (Huikuri et al., 2009). A further sub analysis of the CARISMA and another recent similar study (Risk estimation after infarction, non-invasive evaluation, REFINE) study confirmed that HR variability and HR turbulence yield more powerful prognostic information for arrhythmic events when measured late (6–10 weeks after AMI) rather than early (within 2 weeks) after AMI in the current treatment era (Huikuri et al., 2010). Thus the lack of recovery from reduced autonomic function after AMI was a stronger predictor of adverse events than decreased autonomic function early after AMI. A large study including more than 2000 post-AMI patients (FINGER-study) showed that reduced HR variability and HR turbulence predicted sudden cardiac death better among those with a left ventricular ejection fraction >40% than in those with ejection fraction <40% (Mäkikallio et al., 2005).

HR VARIABILITY AS A PREDICTOR OF NON-SUDDEN CARDIAC DEATH

Observational studies using various end-points have shown that reduced HR variability also predicts non-sudden cardiac death. The largest study in this field showed that reduced HR variability/turbulence is in fact a stronger predictor of non-sudden than sudden cardiac death (Mäkikallio et al., 2005). In this study, the majority of patients were treated according to current guidelines, i.e., with primary coronary interventions, beta-blocking medication, and angiotensin converting enzyme inhibitors.

Considered together, the available data show that abnormal HR variability, measured by various time and frequency domain measures, non-linear measures, and turbulence/deceleration capacity measures from 24-h R–R interval recordings is a general risk marker for common modes of cardiac death: arrhythmic, vascular, and hemodynamic after AMI. HR variability/turbulence measured early after AMI seems to provide more powerful information on the risk of early non-sudden cardiac death, especially due to progressive heart failure. Because of remodeling of the arrhythmia substrate after AMI, early measurement of HR variability to identify those at high risk of early mortality should likely be repeated later in order to assess the true risk of fatal or near-fatal arrhythmia events.

Often forgotten, however, in this focus on risk stratification, is the negative predictive value of HR variability measures. Patients with normal HR variability measures, even if they are post-MI, are at very-low risk of mortality. However, as has been found with other non-invasive risk markers, the positive predictive accuracy and sensitivity of abnormal HR variability for adverse outcomes has remained low in most observational studies. The sensitivity, specificity, and predictive values of HR variability in predicting future events depend partly on defining the cutoff points of abnormal HR variability measures. The cutoff points vary between the studies and depend on the study populations, such as post-AMI patients with and without heart failure, usage of beta-blocking medication etc., which influence the HR variability measures, and there is no universal consensus on the ideal cutoffs for different measures. Age and presence of diabetes and even clinical depression are other factors influencing HR variability and should be considered in defining the cutoff of abnormal measures. HR variability has been combined with other risk markers in order to improve risk stratification in some studies. The problem with combining various risk markers, however, is the overall loss of sensitivity despite an improved positive predictive accuracy.

EFFECT OF INTERVENTIONS ON HR VARIABILITY

Finally, little is known about the effect of interventions on HR variability in post-AMI patients and about whether interventions that are associated with improvements in HR variability are associated with better outcomes or vice versa. One intervention, coronary artery bypass surgery (CABG) is associated with markedly decreased HR variability which slowly improves over weeks and months (Stein et al., 2004). It is likely that any predictive value for long-term outcomes contained in traditional time and frequency domain HR variability is lost once the patient has undergone CABG surgery. However, two studies have shown that measurements of non-linear HRV parameters after CABG surgery did identify those at higher risk of a complicated post-surgical course (Laitio et al., 2000; de Godoy et al., 2009). Only one trial, the BHAT (Beta-Blocker Heart Attack Trial) examined change in HR variability with therapy and the subsequent relationship of HR variability to mortality (Lampert et al., 2003). In BHAT, patients randomized to propranolol had a significantly greater increase in HF power at 6-weeks and after 21 months of follow up, higher HR variability at 6 weeks and assignment to the beta-blocker arm were independent predictors of a combined endpoint. On the other hand, anti-arrhythmic therapies that proved to be associated with reduced survival have been associated with negative effects on HR variability. In a meta-analysis of the effect of pharmacologic, behavioral, and exercise strategies on HR variability, Nolan et al. (2008) concluded that these secondary preventive strategies have a moderate but significant positive effect on HR variability. However, none of the large-scale studies on post-MI patients conducted so far have shown that improvement of HR variability by any of the therapeutic interventions would be associated with better outcome.

FUTURE CHALLENGES AND RECOMMENDATIONS

Future research in this field should focus on randomized trials that take into consideration the timing of HR variability/turbulence measurement after AMI in their study designs. Information from such trials may identify the populations in which the clinical applicability of routine measurement of HR variability is warranted. Measurement of the evolution of HR variability over time in association with prescribed therapies, perhaps on an individual basis, may also add to the clinical value of HR variability. Measures of HR variability should be combined with other clinical risk variables or biomarkers, in future studies to create risk scoring for post-AMI patients based on multiple risk factors. Currently, ongoing study (REFINE–ICD), where patients with reduced HR turbulence, abnormal time-domain T-wave alternans, and left ventricular ejection fraction between 35 and 50% measured 2–14 months after AMI are being randomized to ICD therapy (ICD) and standard therapy (personal communication). If mortality benefit is observed in the ICD arm of this trial, measurement of HR turbulence will become a routine clinical tool for a large number of post-AMI patients. Before the completion of the trial or other similar trials, patients with low HR variability/turbulence after AMI should receive the best available medical and invasive therapy, rehabilitation, and follow-up, even if routine measurements of HR variability/turbulence cannot yet be recommended in clinical practice guidelines.

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Heart rate turbulence as risk-predictor after myocardial infarction

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Heart rate turbulence (HRT) is the baroreflex-mediated short-term oscillation of cardiac cycle lengths after spontaneous ventricular premature complexes. HRT is composed of a brief heart rate acceleration followed by a gradual heart rate deceleration. In high risk patients after myocardial infarction (MI) HRT is blunted or diminished. Since its first description in 1999 HRT emerged as one of the most potent risk factors after MI. Predictive power of HRT has been studied in more than 10,000 post-infarction patients. This review is intended to provide an overview of HRT as risk-predictor after MI.

Keywords: autonomic function, heart rate turbulence, myocardial infarction, risk stratification, sudden death

INTRODUCTION

Despite significant advances in interventional and medical therapy late mortality after myocardial infarction (MI) is still high. A substantial number of these late deaths occur suddenly, potentially preventable by an implantable cardioverter-defibrillator (ICD). Randomized multicenter trials have shown that mortality can be reduced in post-infarction patients at high risk for death by 20–54% (Moss et al., 1996, 2002; Buxton et al., 1999; Bristow et al., 2004; Bardy et al., 2005). Current guidelines recommend ICD implantation in patients characterized by a compromised left ventricular ejection fraction (LVEF 30–35%) which is considered to be the gold standard in risk prediction (Gregoratos et al., 2002; Zipes et al., 2006).

However, clinical studies have consistently shown that the criterion of a reduced LVEF is neither sensitive nor specific. It lacks of sensitivity as approximately 2/3 of deaths in post-infarction patients occur in patients with LVEF >35% who are not covered by the criterion of reduced LVEF (Myerburg et al., 1997, 1998; Huikuri et al., 2001; Buxton, 2003). It also lacks of specificity as 11 ICDs have to be implanted to save one life (Camm et al., 2007). The majority of ICD recipients will never receive an adequate shock. Therefore, development of additional risk stratification strategies is urgently needed.

Twelve years ago, an electrocardiographic phenomenon later on termed "heart rate turbulence (HRT)" has been described (Schmidt et al., 1999). At that time, it has been firstly recognized that in healthy persons spontaneous ventricular premature complexes (VPC) are followed by a characteristic short-term oscillation of heart rate. The oscillation is composed of a brief heart rate acceleration followed by a gradual heart rate deceleration before returning to baseline. As post-ectopic changes of cycle lengths are in the range of milliseconds and masked by heart rate variability of other origin it can only be visualized by signal averaging.

Very early it became clear that post-infarction patients at increased risk for adverse events showed different post-extrasystolic patterns of heart rate. In high risk patients the typical HRT response is blunted or entirely missing. Within the last decade, HRT emerged as one of the most potent ECG based risk predictors. Several large-scale studies have demonstrated its strong prognostic power in post-infarction patients. This review is intended to provide a review of HRT as risk-predictor in post-infarction patients.

MEASUREMENT OF HEART RATE TURBULENCE

Heart rate turbulence is obtained from standard 24-h Holter recordings with a minimum temporal resolution of 128 Hz which allow for accurate determinations of RR intervals and beat classifications. In contrast to other techniques such as T-wave alternans no specific electrodes or other equipments are needed. The technical assessment of HRT has been described in details elsewhere (Bauer et al., 2008). Briefly, the RR intervals surrounding spontaneous VPCs are averaged in order to obtain a so-called local tachogram demasking the average pattern of sinus RR intervals surrounding VPCs (**Figure 1**). VPCs used for HRT computation need to fulfill certain criteria with respect to prematurity and compensatory pause. Details regarding these filter criteria have been described elsewhere (Bauer et al., 2008).

The two phases of HRT are quantified by the two parameters turbulence onset (TO) and turbulence slope (TS).

Turbulence onset is calculated as follows:

$$\mathrm{TO} = \frac{(\mathrm{RR}_1 + \mathrm{RR}_2) - (\mathrm{RR}_{-2} + \mathrm{RR}_{-1})}{(\mathrm{RR}_{-2} + \mathrm{RR}_{-1})} \times 100[\%]$$

whereas RR_{-2} and RR_{-1} are the two RR intervals immediately before the VPC coupling interval. RR_1 and RR_2 are the

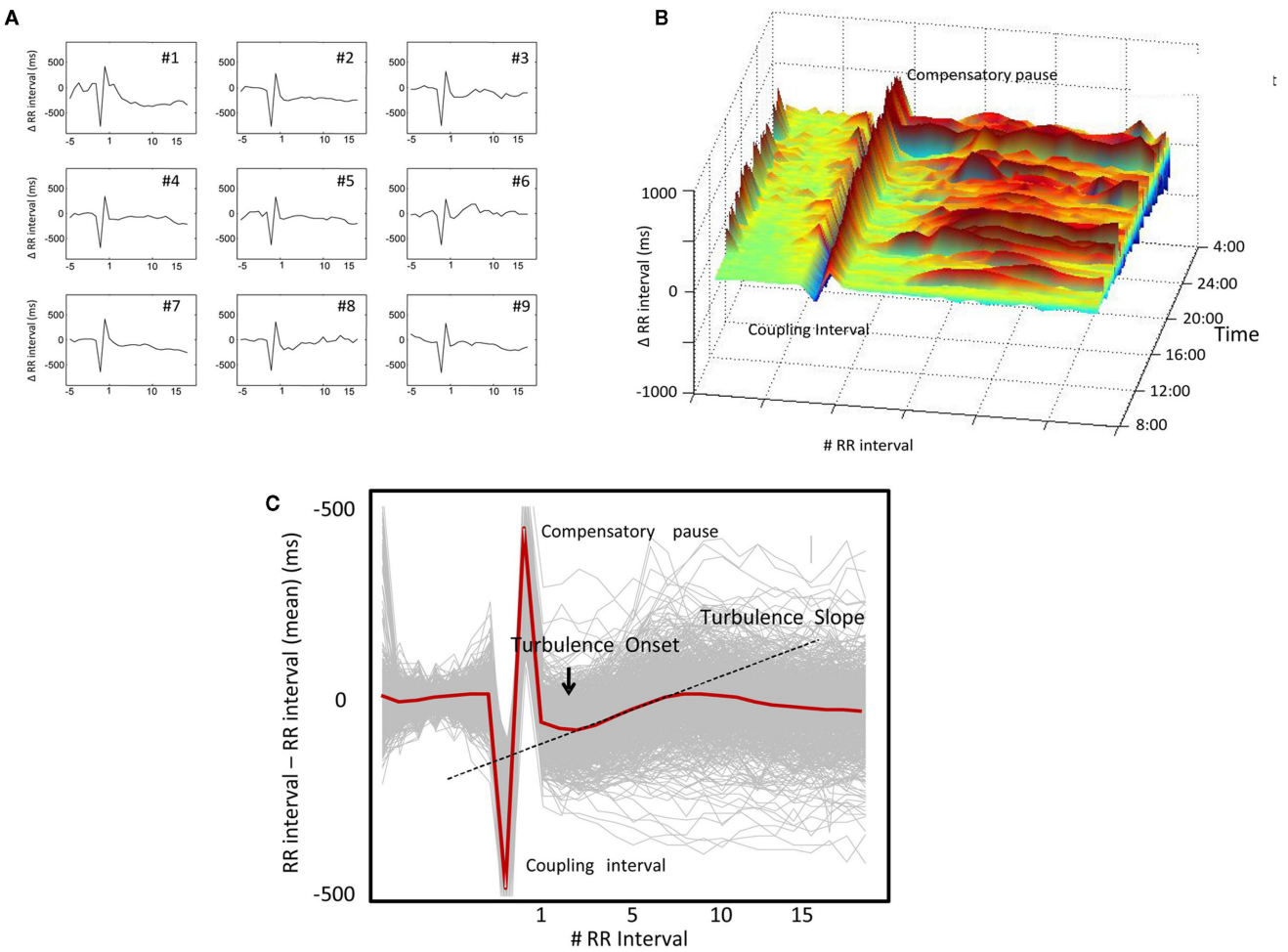

FIGURE 1 | Assessment of heart rate turbulence (HRT) from 24-h Holter recordings: (A) high variability in single "local tachograms" surrounding randomly selected ventricular premature complexes (VPCs); (B) high circadian variability in single local tachograms observed over 24 h; although typical HRT response can be suggested from this graph; (C) smooth HRT pattern (red curve) after signal averaging of single tachograms (gray curves). Quantification of HRT by turbulence onset and turbulence slope (see text for explanation).

two RR intervals which immediately follow the compensatory pause. TS is defined as the maximum positive regression slope assessed over any five consecutive sinus RR intervals within the first 15 RR intervals following the VPC. Hence, in normal subjects, the initial acceleration of sinus rate after the VPC is characterized by negative TO and the following heart rate deceleration is characterized by positive TS. TO <0% and TS >2.5 ms/RR interval are considered normal (Bauer et al., 2008).

For use of risk stratification in different patient populations, HRT is usually divided into three categories: (1) HRT category 0 is defined as normal TO *and* normal TS, (2) HRT category 1 means that either TO *or* TS are abnormal, and (3) HRT category 2 is characterized by *both* abnormal TO and TS. If no sufficient VPC tachograms are recorded and patients are otherwise in sinus rhythm, HRT is classified as category 0 since those patients were shown to have equally good prognosis as patients with normal HRT (Barthel et al., 2003). As this was only shown for post-infarction patients, this approach might not be valid if other pathologies (e.g., heart failure) are considered.

The HRT software is commercially available on GE and Getemed Holter systems. However, as the algorithms have been published in detail (www.h-r-t.com) HRT can also be obtained from the series of RR intervals by a custom-made software.

PHYSIOLOGY OF HEART RATE TURBULENCE

When the first clinical studies of HRT in risk prediction have been published the exact physiological mechanisms behind HRT were largely unknown (Schmidt et al., 1999; Bauer and Schmidt, 2007). The (patho)physiological mechanisms behind HRT are complex and involve both branches of the autonomic nervous system. In their work, Wichterle et al. (2006) provide an excellent review of HRT physiology. The VPC induces a transient drop of arterial blood pressure which leads to an activation of the baroreceptors. Vagal activity is abruptly withdrawn resulting in an almost

immediate shortening of RR interval cycle lengths (as measured by TO). However, also the sympathetic system reacts (Segerson et al., 2007). Increased sympathetic activity results in a gradual increase of vascular resistance and systolic arterial blood pressure. As consequence, vagal activity reestablishes and cycle lengths prolong (as measured by TS). Importantly, HRT requires an intact interplay of both, vagal and sympathetic systems. Absence of normal HRT can be caused by an alteration in one of the systems (Wichterle et al., 2006).

HRT STUDY POPULATIONS

Evidence of HRT as risk-predictor in post-infarction patients is based on five retrospective and five prospective studies including a total of more than 10,000 patients. Study characteristics are summarized in **Tables 1** and **2**.

Heart rate turbulence was originally developed in a small dataset comprising of 100 patients suffering from coronary artery disease and subsequently validated in a blinded fashion in the cohorts of the MPIP study ($n = 577$) and the placebo arm of the EMIAT study ($n = 614$; Schmidt et al., 1999). Two years later, Ghuran et al. (2002) tested the predictive power of HRT in the dataset of the ATRAMI study ($n = 1{,}212$) which was originally designed to assess the prognostic power of baroreflex sensitivity. Another 3 years later, predictive power of HRT was also tested in the dataset of the CAST study ($n = 744$; Hallstrom et al., 2005). The FINGER study combined a Finish and German post-infarction population (Barthel et al., 2003; Huikuri et al., 2003) to specifically address the question whether HRT predicts sudden death (Makikallio et al., 2005).

In 2003, the results of the first prospective study ISAR-HRT ($n = 1{,}455$) were published which was designed to validate the prognostic value of HRT in a large cohort of post-infarction patients receiving contemporary treatment (Barthel et al., 2003). The REFINE study ($n = 322$) published 2007 was designed to assess the predictive value of a combination of several risk predictors including HRT as well as the time of their assessment after acute MI (Exner et al., 2007). In 2009, the results of the largest prospective HRT study were published. ISAR-RISK tested the prognostic value of a combination of HRT and deceleration capacity (Bauer et al., 2006a) in post-infarction patients with preserved LVEF (Bauer et al., 2009a). Deceleration capacity is an integral measure of all deceleration related modulations of heart rate observed over 24 h and thus, most presumably, a measure of tonic vagal activity. The CARISMA study ($n = 312$) deserves special attention as loop recorders have been implanted in all patients to specifically address the endpoint of severe arrhythmic events (Huikuri et al., 2009). Very recently, the results of ISAR-SWEET have been published

Table 1 | Retrospective studies (or sub-studies) investigating heart rate turbulence as a post-infarction risk-predictor.

	MPIP (Schmidt et al., 1999)	EMIAT (Schmidt et al., 1999)	ATRAMI (Ghuran et al., 2002)	CAST (Hallstrom et al., 2005)	FINGER (Makikallio et al., 2005)
Number of patients	577	614	1,212	744	2,130
Year of publication of original study	1983	1997	1998	1991	2003
Inclusion criteria*	MI ≤4 weeks, age ≤70 years	MI ≤4 weeks, age ≤75 years, LVEF ≤40%	MI ≤4 weeks, age ≤80 years	MI ≥6 VPC/h	MI ≤4 weeks, age ≤75 years
Follow-up (months)	22	21	20	55	33
Endpoint	Mortality	Mortality	Cardiac mortality†	Mortality	Sudden death
Endpoints reached (%)	13	14	4	29‡	2
Time of HRT assessment after MI	2nd week	2nd to 3rd week	2nd to 4th week	10 weeks	2nd week
Treatment of acute MI	None	60% Lysis	63% Lysis	28% Lysis	70% PCI, 14% lysis
Mean LVEF (%)	45	30	49	37	Not specified
Betablockers (%)	55	32	20	30	94
UNIVARIATE ANALYSIS					
HRT category 2	5.0 (2.8–8.8)	4.4 (2.6–7.5)	6.9 (3.1–15.5)	Not specified	4.6 (2.6–8.1)‖
LVEF ≤30%	4.0 (2.5–6.4)	2.2 (1.4–3.5)	4.7 (2.6–8.3)	Not specified	4.5 (2.5–8.0)#
MULTIVARIATE ANALYSIS					
HRT category 2	3.2 (1.7–6.0)	3.2 (1.8–5.6)	4.1 (1.7–9.8)	20.4 (10.2–30.6)**	2.9 (1.6–5.5)
LVEF ≤30%	2.9 (1.8–4.9)	1.7 (1.1–2.7)	3.5 (1.8–7.1)	Not specified	Not specified

Sinus rhythm was inclusion criterion in all studies.

†*Cardiac Mortality included fatal and non-fatal cardiac arrest.*

‡*Cumulative mortality rate only presented for total study population of CAST after 5 years.*

‖*Relative risks presented for turbulence slope ≤2.5 ms/RR interval.*

#*LVEF was dichotomized at 35%.*

***Log of Turbulence Slope corrected for heart rate and VPC count (optimized in CAST data).*

Table 2 | Prospective studies (or sub-studies) investigating heart rate turbulence as a post-infarction risk-predictor.

	ISAR-HRT (Barthel et al., 2003)	REFINE (Exner et al., 2007)	ISAR-RISK‡‡ (Bauer et al., 2009a)	CARISMA (Huikuri et al., 2009)	ISAR-Sweet‡‡ (Barthel et al., 2011)
Number of patients	1,455	322	2,343	312	481
Inclusion criteria*	MI ≤4 weeks, age ≤75 years	MI, LVEF <50%	MI ≤4 weeks, age ≤75 years	MI <21 days, LVEF ≤40%	MI ≤4 weeks, age ≤80 years, diabetes
Follow-up (months)	22	47	60	24	60
Endpoint	Mortality	Cardiac death†	Mortality	VF/sustained VT on loop recorder	Mortality
Endpoints reached (%)	5	9	8	8	17
Time of HRT assessment after MI	2nd week	2nd to 4th and 10th to 14th week	2nd week	1st and 6th week	2nd week
Treatment of acute MI	90% PCI, 6% lysis	45% PCI, 21% lysis	92% PCI, 3% lysis	14% PCI, 34 lysis	89% PCI
Mean LVEF (%)	56	47	55	31	51
Betablockers (%)	93	92	94	96	94
UNIVARIATE ANALYSIS					
HRT category 2	11.4 (5.7–22.8)	2.9 (1.1–7.5)††	7.5 (5.3–10.7)	2.8 (1.1–7.2)‖	6.6 (3.9–11.0)
LVEF ≤30%	7.1 (4.2–12.1)	3.3 (1.4–7.6)	6.1 (4.2–8.7)	1.3 (0.5–3.0)#	4.7 (2.8–7.8)
MULTIVARIATE ANALYSIS					
HRT category 2	5.9 (2.9–12.2)	Not specified	3.1 (2.1–4.6)	Not specified	4.1 (2.3–7.2)
LVEF ≤30%	4.5 (2.6–7.8)	Not specified	3.0 (2.0–4.4)	Not specified	2.4 (1.4–4.1)

**Sinus rhythm was inclusion criterion in all studies.*

†Cardiac mortality included fatal and non-fatal cardiac arrest.

‖Relative risks presented for turbulence slope ≤2.5 ms/RR interval.

#LVEF was dichotomized at 35%.

††HRT category ≥1 vs. 0 tested; HRT was assessed 10–14 weeks after MI.

‡‡ISAR-RISK primarily tested the combination HRT category 2 and abnormal deceleration capacity (Bauer et al., 2006a).

which tested the combination of abnormal HRT and deceleration capacity in diabetic post-infarction patients (Barthel et al., 2011).

RISK PREDICTIVE POWER OF HRT IN POST-INFARCTION PATIENTS

In all populations, abnormal HRT was a significant and independent predictor of adverse events yielding a relative risk of 2.8–11.4 on univariate and 3.1–5.9 on multivariate analysis.

The single HRT studies substantially differ with respect to study design (retrospective vs. prospective), the primary endpoint investigated (total mortality, cardiac mortality, and sudden death), time of follow-up, time after MI when Holter recordings have been performed, mean LVEF, and treatment of MI.

Heart rate turbulence is generally a very strong predictor in all studies which used total mortality as primary endpoint (which most of the studies did: MPIP, EMIAT, CAST, ISAR-HRT, ISAR-RISK, ISAR-SWEET). HRT was also a very strong predictor in the ATRAMI study which used a combined endpoint of cardiac mortality and fatal and non-fatal cardiac arrest. Two studies investigated sudden death as primary endpoint, namely the FINGER study and the CARISMA study. While in the FINGER study mode of death was determined anamnestically or with the use of medical recordings, CARISMA had a unique study design: all patients of the CARISMA study underwent implantation of a loop recorder which allowed for a definite assessment of the rhythm at time of death. While HRT was a strong predictor of sudden death in the FINGER study, predictive power of HRT in the CARISMA study was somewhat lower although still significant [relative risk of abnormal TS 2.8 (95% CI 1.1–7.2), $p = 0.038$]. It should be noted, however, that in CARISMA only 25 endpoints occurred during a follow-up of 2 years and that only patients with reduced LVEF (≤40%) have been included. Therefore, conclusions drawn from the CARISMA should not be directly extrapolated to unselected post-infarction populations with no restriction of LVEF.

Time of HRT assessment after acute MI is an important issue. In most HRT studies Holter recordings have been performed early after MI (usually within the first 4 weeks; MPIP, EMIAT, ATRAMI, FINGER, ISAR-HRT, ISAR-RISK, and ISAR-SWEET). In all of these studies, HRT was a strong and significant predictor of the primary endpoint. However, also in CAST, where risk assessment was performed later after MI, HRT was a strong predictor of death (Hallstrom et al., 2005). In two studies, namely the REFINE and the CARISMA study, risk assessment has been performed at two different time points. In REFINE risk assessment has been performed between the 2nd and the 4th week as well as between the 10th and the 14th week after MI. In CARISMA, risk assessment has been performed during the first week as well as during the sixth week after MI. In both studies, risk assessment later after MI was superior to risk assessment earlier after MI. It might therefore be concluded, that HRT assessed later after MI might be a better predictor than HRT assessed early after MI. These findings are in

agreement with the observation that autonomic dysfunction early after MI might recover, and that patients with sustained blunted autonomic function have the worst prognosis (Huikuri et al., 2010).

The single HRT studies cover the whole spectrum of MI treatment eras. In summary, neither acute treatment of MI (conservative, lysis, PCI) nor concomitant medical treatment including betablockers, ACE-inhibitors, or statins seem to affect risk predictive power of HRT. However, it should be noted that effective reperfusion strategies for acute MI which lead to increased myocardial salvage translate into improved autonomic function and better HRT (Bauer et al., 2009b).

Positive predictive accuracies and sensitivities of abnormal HRT for prediction of adverse events strongly depend on the populations and endpoints investigated. Generally, both positive predictive accuracy and sensitivity is in the range of that yielded by LVEF ≤30%. **Figure 2** exemplarily shows risk stratification by HRT in the largest post-infarction study, the ISAR-RISK study. Of the 2,343 patients studied, HRT category 2 identified a high risk group of 193 patients (8%) out of whom 56 patients died. 2,150 patients (92%) had normal (HRT category 0 and 1) out of whom 125 patients died. Probability of death within 5 years of follow-up of patients with abnormal HRT (HRT category 2) was 34%. In contrast, the 1,652 patients (71%) with completely normal HRT (HRT category 0) had a 5-year mortality of 6%. These figures translate into a positive predictive accuracy of 34% at a sensitivity level of 31%.

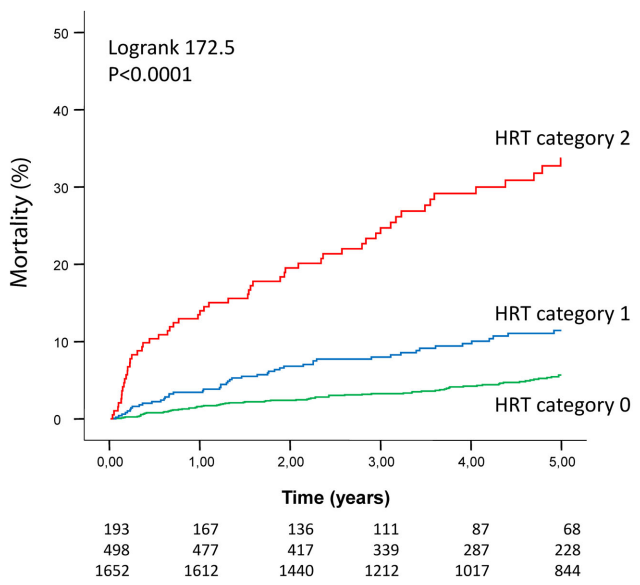

FIGURE 2 | Risk stratification by heart rate turbulence in the ISAR-RISK study (Bauer et al., 2009a). The number of patients of the individual groups involved in the analyses at 0, 1, 2, 3, 4, and 5 years are shown under the graphs. The top and the bottom row corresponds to the upper and bottom Kaplan–Meier curve respectively. For all three comparisons (HRT category 0 vs. HRT category 1, HRT category 0 vs. HRT category 2, and HRT category 1 vs. HRT category 2) the *p*-value was <0.0001. (Adapted by permission from Bauer et al., 2009a).

INDEPENDENCY FROM AND COMBINATION WITH OTHER RISK PREDICTORS

In all studies, predictive value of HRT was independent from that of other risk predictors tested. These included demographic factors and comorbidities (age, gender, presence of diabetes mellitus, and renal insufficiency; Barthel et al., 2011), markers of electrical instability [arrhythmias, T-wave alternans (Exner et al., 2007), late potentials (Bauer et al., 2005), QRS duration (Bauer et al., 2006b)], markers of structural damage (e.g., LVEF), and other markers of autonomic dysfunction (heart rate, heart rate variability, and deceleration capacity; Bauer et al., 2006a, 2009a).

In order to enhance risk predictive power, HRT should be combined with other risk predictors. The ISAR-RISK and the ISAR-SWEET studies investigated the combination of abnormal HRT (HRT category 2) with mildly abnormal deceleration capacity (≤4.5 ms) which is a measure of tonic autonomic activity and bases on the processing of RR interval time series by a new mathematical algorithm (Bauer et al., 2006c). For this combination, the term "severe autonomic failure (SAF)" has been introduced. In both, ISAR-RISK and ISAR-SWEET which included 2,343 and 481 patients respectively, SAF proved to be the strongest predictor of death. These findings were confirmed by the results of a recent metaanalysis which analyzed the combined populations of the MPIP, EMIAT, and MRFAT studies ($n = 2{,}594$; Bauer et al., 2009c). Also the combination of HRT with T-wave alternans is promising as both were independently associated with the primary endpoint in the REFINE study. However, available data do not allow for final conclusions.

Risk stratification by HRT is complementary to risk stratification by LVEF. Only a small proportion of patients with abnormal HRT (category 2) also have LVEF ≤30%. Therefore, the strength of HRT lies in the identification of high risk patients in the large group of patients with preserved LVEF (>30%). **Figure 3** shows complimentary risk stratification by HRT and LVEF in the ISAR-RISK study (Bauer et al., 2009a). The small proportion of patients with both, abnormal HRT and impaired LVEF ($n = 40$; 1.7% of the study population) had the worst prognosis. Patients with either abnormal HRT ($n = 153$; 6.5% of the study population) or impaired LVEF ($n = 80$; 3.4% of the study population) had equally poor prognosis. In contrast, patients with normal HRT (category 0 or 1) and LVEF >30% ($n = 2{,}070$; 88.3% of the study population) had an excellent prognosis. As mentioned above risk stratification by HRT can be further improved by combination with deceleration capacity (Bauer et al., 2009a).

LIMITATIONS OF HEART RATE TURBULENCE

Several limitations need to be recognized when using HRT as risk-predictor. Firstly, assessment of HRT requires the presence of sinus rhythm. Patients with other rhythms than sinus rhythm such as atrial fibrillation have been excluded in all HRT studies but might be at increased risk. In the Global Utilization of Streptokinase and Tissue plasminogen activator for Occluded coronary arteries (GUSTO-I) trial, 10.5% of the 41,021 patients had atrial fibrillation during hospitalization (Crenshaw et al., 1997). Further, most HRT studies also excluded elderly patients (age >75 years or age >80 years). It is well known from the ATRAMI study that autonomic function loses some of its predictive value with increasing

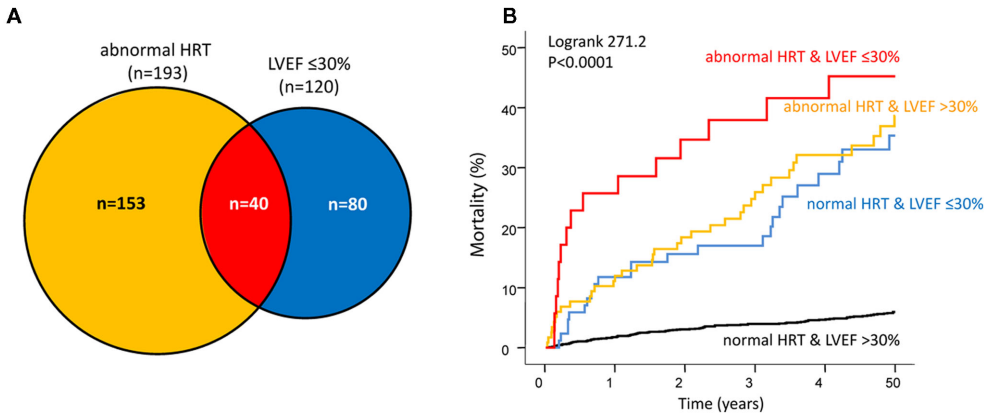

FIGURE 3 | Complimentary risk stratification by HRT and LVEF in the ISAR-RISK study (Bauer et al., 2009a). (A) Distribution of patients with abnormal HRT but LVEF >30% ($n = 153$, orange area), abnormal HRT and LVEF ≤ 30% ($n = 40$, red area), and normal HRT but LVEF ≤30% ($n = 80$, blue area). **(B)** Corresponding 5-year mortality rates of the three groups. Abnormal HRT defined as HRT category 2 (adapted by permission from Bauer et al., 2009a).

age (La Rovere et al., 1998). Similar observations have been made for HRT in the ISAR-HRT study (Barthel et al., 2005). In contrast, HRT was a significant predictor for sudden death in the population based Cardiovascular Health Study which included adults ≥65 years (average 73 years; Stein et al., 2010). Assessment of HRT also requires presence of VPCs. In most studies, patients without VPCs have therefore been excluded from the analysis (e.g., MPIP, EMIAT, ATRAMI). As shown in the ISAR-HRT study post-infarction patients without VPCs have equally good prognosis as patients with normal HRT (Barthel et al., 2003). Therefore, these patients might be treated as having normal HRT (HRT category 0). HRT is usually assessed from full 24-h Holter recordings. Whether HRT derived from shorter recordings provides similar predictive value needs further investigations. A retrospective analysis of HRT in the Multicenter Automatic Defibrillator Implantation Trial 2 that used only 10-min recordings showed the inappropriateness of very short recordings (Berkowitsch et al., 2004). All post-infarction HRT studies included patients early after MI. A large-scale study which investigates the prognostic value of HRT in patients with remote infarction is still missing. This might be of substantial importance as prophylactic ICD implantation in the acute phase of infarction has been generally questioned by the negative results of two randomized ICD trials (Hohnloser et al., 2004; Steinbeck et al., 2009). It should be noted, however, that both studies, the DINAMIT ($n = 674$) and the IRIS study ($n = 898$), used entry criteria that selected only a very small proportion of post-infarction patients at very high risk. For instance, the IRIS study selected 898 patients out of 62,944 patients (1.4%). Conclusions drawn from these two studies should therefore not be generalized and extrapolated to other risk stratification strategies.

CONCLUSION

Heart rate turbulence is easily applicable from routine 24-h Holter recordings. In all post-infarction studies HRT was a strong and independent predictor of adverse events which included death of any cause, cardiac death, and sudden death. In all post-infarction studies, predictive value of HRT was independent from other risk factors tested. For purpose of identifying high risk individuals who might benefit from prophylactic ICD implantation, HRT should be combined with other independent predictors. Potential candidates include impaired LVEF, abnormal deceleration capacity, and/or T-wave alternans. The combination of abnormal HRT and abnormal deceleration capacity termed "SAF" has been tested in the ISAR-RISK study and provides strong prognostic value also in post-infarction patients with preserved LV-function. However, only future interventional trials can finally answer the question whether high risk patients identified by abnormal HRT benefit from prophylactic therapy.

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Heart rate variability and non-linear dynamics in risk stratification

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The time-domain measures and power–spectral analysis of heart rate variability (HRV) are classic conventional methods to assess the complex regulatory system between autonomic nervous system and heart rate and are most widely used. There are abundant scientific data about the prognostic significance of the conventional measurements of HRV in patients with various conditions, particularly with myocardial infarction. Some studies have suggested that some newer measures describing non-linear dynamics of heart rate, such as fractal measures, may reveal prognostic information beyond that obtained by the conventional measures of HRV. An ideal risk indicator could specifically predict sudden arrhythmic death as the implantable cardioverter-defibrillator (ICD) therapy can prevent such events. There are numerically more sudden deaths among post-infarction patients with better preserved left ventricular function than in those with severe left ventricular dysfunction. Recent data support the concept that HRV measurements, when analyzed several weeks after acute myocardial infarction, predict life-threatening ventricular tachyarrhythmias in patients with moderately depressed left ventricular function. However, well-designed prospective randomized studies are needed to evaluate whether the ICD therapy based on the assessment of HRV alone or with other risk indicators improves the patients' prognosis. Several issues, such as the optimal target population, optimal timing of HRV measurements, optimal methods of HRV analysis, and optimal cutpoints for different HRV parameters, need clarification before the HRV analysis can be a widespread clinical tool in risk stratification.

Keywords: heart rate, heart rate variability, non-linear methods, mortality, sudden death

INTRODUCTION

Heart rate variability (HRV) describes the complex regulatory system between heart rate and the autonomic nervous system. There are several methods to measure HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The traditional statistical methods and the power–spectral analysis of HRV are classic methods to measure HRV and are most widely used. The conventional measures of HRV have been shown to provide prognostic information in several patient populations (Kleiger et al., 1987; Bigger et al., 1992, 1993; Fei et al., 1996; Zuanetti et al., 1996; Nolan et al., 1998). The conventional measures of HRV cannot reveal delicate changes in heart rate beat-to-beat dynamics. Therefore several non-linear methods for measuring heart rate dynamics have been developed (Saul et al., 1987; Goldberger, 1990b, 1996; Skinner et al., 1993; Pincus and Goldberger, 1994; Peng et al., 1995; Voss et al., 1998; Tuzcu et al., 2006; Norris et al., 2008a). Few of these newer methods of HRV, such as the fractal-like scaling property and the complexity, have been tested in well-designed studies, which have included a relevant number of patients and have had well-defined endpoints. Some of these studies have suggested that some of the non-linear measures of HRV work better than the traditional measures of HRV in predicting future adverse events in various patient groups. The physiological background of the non-linear measures of HRV is much less well understood than that of the conventional measures of HRV. The non-linear methods of HRV assess qualitative properties rather than the magnitude of the heart rate dynamics.

Several other factors than the type of the parameter influence the prognostic value of HRV measurements. The timing of the HRV measurement after an acute myocardial infarction (AMI) has a direct influence on the prognostic significance of HRV due to substantial electrical and mechanical remodeling after AMI (Exner et al., 2007; Huikuri et al., 2009a). The prognostic value of HRV variables is also dependent on the left ventricular function and the severity of heart failure (Mäkikallio et al., 2001a, 2005). HRV parameters analyzed from short-term recordings obtained during controlled conditions yield somewhat different prognostic information than the HRV variables analyzed from long-term ambulatory 24-h recordings. It is also important to select appropriate preprocessing methods for editing premature depolarizations and irrelevant oscillations from RR interval time series in order to obtain reliable and reproducible prognostic data for clinical purposes (this is dealt in another review in the present issue). It is noteworthy that HRV parameters work prognostically differently in patients with different diseases and healthy subjects. The predictive power of HRV variables varies also depending on whether total mortality, different modes of mortality, or other adverse events are selected as endpoints. Several novel methods to describe heart rate dynamics, such as heart rate turbulence, have been developed

(Schmidt et al., 1999). Their role in risk stratification is discussed in other reviews in this issue as is the influence of exercise on heart rate dynamics.

During the past two decades the number of publications dealing with HRV has exploded reaching at least over 14,000 at the moment. The present review is focusing on some of these studies, of which the majority have relevant number of patients and well-defined endpoints, and which elucidate the value of conventional and non-linear methods of HRV in risk assessment.

HEART RATE VARIABILITY IN RISK EVALUATION

CLASSIC STUDIES APPLYING CONVENTIONAL METHODS OF HEART RATE VARIABILITY ANALYSIS

The prognostic significance of the conventional measures of HRV in post-AMI patients is well established. Schneider and Costiloe (1965) first proposed that HRV is reduced in patients with AMI and is associated with adverse prognosis. Wolf et al. (1978) found a significantly lower in-hospital mortality rate among the patients with AMI, who had more pronounced sinus arrhythmia. Kleiger et al. (1987) published the cornerstone study, where they showed that decreased HRV measured by the SD of all normal-to-normal RR-intervals (SDNN) analyzed from 24-h electrocardiographic recordings predicts mortality in post-infarction patients. The combination of reduced HRV and the occurrence of late potentials was found to have a sensitivity of 58%, a positive predictive accuracy of 33%, and a relative risk of 18.5 for arrhythmic events in post-AMI patients (Farrell et al., 1991). In the study by Cripps et al. (1991), which included 177 patients after AMI, the relative risk of sudden death or symptomatic sustained ventricular tachycardia during a median follow-up of 16 months was found to be seven times greater in those with low baseline values of the triangular index of HRV. In another study, after adjustment for relevant clinical risk markers, the total, ultralow-frequency and very-low-frequency powers of HRV remained a significant and powerful predictor of mortality after AMI (Bigger et al., 1992). In 433 survivors of first AMI, Odemuyiwa et al. (1994) observed that HRV was an independent predictor of sudden death and total cardiac mortality only during the first 6 months of follow-up. The results of the study including 226 consecutive patients with AMI confirmed the previous observations concerning the association between decreased HRV and mortality after AMI and suggested the importance of disturbance in sympathovagal regulation unrelated to left ventricular function or infarct location as a mechanism of high-risk (Vaishnav et al., 1994). Reduced HRV has been found to be related to both arrhythmic and non-arrhythmic death in post-AMI patients (Hartikainen et al., 1996). Fei et al. (1996) showed in their study including 700 consecutive patients after AMI that although the predictive accuracy of SDNN analyzed from 5-min short-term RR interval data for 1-year total cardiac mortality was lower than that of the HRV index analyzed from 24-h period, the SDNN short-term data could be used in preselection of high-risk patients. Bigger et al. (1993) studied the predictive value of the power–spectral measures of HRV obtained from short from 2 to 15 min electrocardiographic recordings in 715 post-AMI patients. They concluded that the power–spectral measures of HRV obtained from short recordings are remarkably similar to those obtained from 24-h recordings and predict all-cause mortality and sudden cardiac death. Zuanetti et al. (1996) evaluated the predictive value of several time-domain measures of HRV for total and cardiovascular mortality in 567 patients with AMI, who were treated with thrombolytic therapy and followed up for 1000 days. They concluded that time-domain indexes of HRV retain their independent prognostic significance even in post-AMI patients of all ages treated with fibrinolysis. Heart rate and HRV analyzed from predischarge 24-h electrocardiographic recordings were found to be more powerful predictors of mortality than ejection fraction in post-AMI patients in the study by Copie et al. (1996). Disturbed autonomic modulation of heart rate seems to be specifically related to susceptibility to ventricular fibrillation, but not to stable monomorphic ventricular tachycardia, suggesting that autonomic influences modify the presentation of malignant ventricular tachyarrhythmia after previous myocardial infarction (Perkiömäki et al., 1997).

The analysis of HRV can give an insight into various pathophysiological processes including the progression of focal coronary atherosclerosis (Huikuri et al., 1999a) and the angiographic severity of coronary artery disease (Hayano et al., 1990). Patients with coronary artery disease may have decreased vagal activity (Airaksinen et al., 1987) and abnormal circadian rhythm of HRV (Huikuri et al., 1994). Furthermore, ischemia may be preceded by increased sympathetic tone (Bernardi et al., 1988).

It is noteworthy that the HRV counterparts of autonomic drives are very different in patients with heart failure and in normal subjects (van de Borne et al., 1997). However, there are a lot of publications, which show that HRV is decreased in patients with congestive heart failure (Saul et al., 1988; Casolo et al., 1989, 1991; Kienzle et al., 1992; Nolan et al., 1992; Mortara et al., 1994). Saul et al. (1988) observed decreased vagal, but relatively well preserved sympathetic modulation of heart rate in patients with chronic stable congestive heart failure. On the contrary, reduction of all spectral components of HRV were found in patients with congestive heart failure in the study by Casolo et al. (1991). Brouwer et al. (1996) observed in 95 patients with mild to moderate heart failure that abnormal Poincare plots were independent predictors of all-cause and sudden cardiac death.

Hypertensive patients have been found to have increased low-frequency components and reduced high-frequency components of HRV (Guzetti et al., 1988). On the other hand, lower values of low- and high-frequency power of HRV were observed in hypertensive patients with left ventricular hypertrophy than in controls, while ultralow- and very-low-frequency components were similar in the groups (Petretta et al., 1995). Left ventricular hypertrophy is a well known risk indicator, and HRV has been shown to be inversely related to left ventricular mass index (Mandawat et al., 1994, 1995; Petretta et al., 1995). However, not all studies have confirmed this finding (Perkiömäki et al., 1996).

Disturbances in cardiac autonomic regulation assessed by HRV have been observed in several other conditions. Patients with diabetic neuropathy have decreased HRV (Wheeler and Watkins, 1973; Pfeifer et al., 1982; Smith, 1982). A reduced HRV in patients with diabetes still yields long-term prognostic information (Wheeler et al., 2002). In post-AMI patients the association between decreased HRV and long-term mortality has been observed to be at least as strong in diabetic patients as in

non-diabetic patients (Whang and Bigger, 2003). However, there are also some data to suggest that including patients with diabetes decreases the association between HRV and mortality after AMI (Stein et al., 2004). The measurement of HRV offers also prognostic information in an elderly population beyond that provided by the assessment of conventional risk factors (Tsuji et al., 1994). Patients with chronic renal failure (Axelrod et al., 1987; Cloarec-Blanchard et al., 1992) have decreased HRV. Patients with different neurological conditions, such as Parkinsonism (Kuroiwa et al., 1983), multiple sclerosis (Neubauer and Gundersen, 1978), and quadriplegia (Inoue et al., 1990), have also been observed to have reduced HRV. Decreased HRV has been found in heart transplant recipients, but allograft rejection may lead to increased HRV (Sands et al., 1989).

STUDIES APPLYING NON-LINEAR METHODS OF HEART RATE VARIABILITY AND NEWER STUDIES

The fractal measures are the non-linear measures of HRV, whose prognostic significance has most widely been assessed in clinical studies including relevant number of patients and using well-defined endpoints. The fractal measures of HRV analysis have been shown to provide incremental prognostic information compared with the conventional measures of heart rate fluctuations. A basic feature of a fractal system is scale-invariance, i.e., same features repeat themselves on different measurement scales (Goldberger, 1996). Healthy subjects´ erratic fluctuations of sinus rhythm have fractal-like characteristics (Denton et al., 1990; Goldberger, 1990a).

Power-law HRV analysis is as a qualitative measure of the power spectrum in the region of the ultralow- and very-low-frequency bands obtained from long-term recordings. A plot of spectral power and frequency on bi-logarithmic scale shows linear portion between 10^{-4} and 10^{-2} Hz, and the slope of this relationship reflects long-term fractal-like scaling characteristics of HRV (Saul et al., 1987). Healthy subjects have power-law exponent values around -1 (Saul et al., 1987; Bigger et al., 1996), and the value of this slope has been observed to decrease with advancing age (Pikkujämsä et al., 1999). The finding that denervated hearts have a substantially steeper power-law slope emphasizes the important role of the autonomic nervous system in determining the steepness of the slope (Bigger et al., 1996). A steep power-law slope has been shown to be a better predictor of all-cause mortality or arrhythmic death than the traditional power–spectral bands in post-AMI patients (Bigger et al., 1996), and a better predictor of mortality than the traditional measurements of HRV in the elderly (Huikuri et al., 1998).

Detrended fluctuation analysis (DFA) quantifies intrinsic fractal-like correlation properties of dynamic systems (Peng et al., 1994). Peng et al. (1995) have described the details of this method. Briefly, the RR interval variability in relation to a local trend is analyzed in observation windows of different sizes in preprocessed and integrated RR interval time series. The RR interval variability is shown on a log–log scale as a function of the observation window size. In the presence of scaling, this relationship has a linear portion. The short-term scaling exponent (DFA1; for window sizes <11 beats) describes short-term scaling properties, and the intermediate-term scaling exponent (DFA2; for window sizes >11 beats) longer term scaling properties of the signal. Healthy middle-aged subjects have the short-term scaling exponent values somewhat over or around 1 (Pikkujämsä et al., 2001). The values of the short-term scaling exponent are determined by a complex interplay of the parasympathetic and sympathetic autonomic nervous system. Concomitant activation of both vagal and sympathetic outflow has been shown to decrease the short-term fractal scaling exponent resulting in a random heart rate behavior (Tulppo et al., 2005). The physiological background of the short-term scaling exponent has been discussed in detail elsewhere (Huikuri et al., 2009b). Healthy elderly subjects may have changes in the fractal correlation properties of heart rate dynamics (Iyengar et al., 1996; Pikkujämsä et al., 1999), and the short-term scaling exponent has also been observed to predict cardiac death in the elderly (Mäkikallio et al., 2001b).

There is some evidence that the short-term fractal-like scaling properties of heart rate dynamics analyzed by the DFA technique can yield prognostic information beyond that obtained by the conventional measures of HRV. Studies in post-AMI patients have suggested that decreased short-term scaling exponent is a better predictor of mortality than the conventional measurements of HRV (Mäkikallio et al., 1999a; Huikuri et al., 2000) and that decreased short-term scaling exponent values are associated with vulnerability to ventricular tachycardia (Mäkikallio et al., 1997), ventricular fibrillation (Mäkikallio et al., 1999b), arrhythmic death, and non-arrhythmic cardiac death (Huikuri et al., 2000). The prognostic value of the short-term scaling exponent has also been shown in a general post-AMI population without a marked left ventricular dysfunction and high proportion of patients on beta-blocking medication (Tapanainen et al., 2002). Fractal HRV has been observed to retain its prognostic value even when the vast majority of the patients were taking beta-blockers after AMI (Jokinen et al., 2003). Among the autonomic risk markers, the short-term scaling exponent has been found to be the strongest predictor of recurrent non-fatal coronary events after AMI (Perkiömäki et al., 2008). Disturbed cardiac autonomic regulation represented by reduced values of the short-term scaling exponent has been found to predict perpetuating ventricular tachyarrhythmias, but not self-terminating ventricular tachyarrhythmias in post-AMI patients with moderate left ventricular dysfunction suggesting that perpetuating and self-terminating ventricular tachyarrhythmias may have differences in factors, which modify arrhythmias (Perkiömäki et al., 2011; **Figure 1**). **Figure 2** shows two typical cases of RR interval behavior in high-risk patients with low short-term fractal scaling exponent and one case with normal fractal scaling exponent.

Heart failure patients show loss of fractal organization in heart rate dynamics (Peng et al., 1995), and this is associated with the risk of death (Ho et al., 1997). Reduced short-term scaling exponent is more closely related to the risk of mortality in patients with less severe than in those with more advanced heart failure (Mäkikallio et al., 2001a). The short-term scaling exponent has also been shown to predict long-term risk for heart failure hospitalization after AMI (Perkiömäki et al., 2010).

The spontaneous onset of atrial fibrillation in patients without a structural heart disease is preceded by altered short-term fractal-like scaling properties of HRV (Vikman et al., 1999), and the short-term scaling exponent changes toward more random direction in

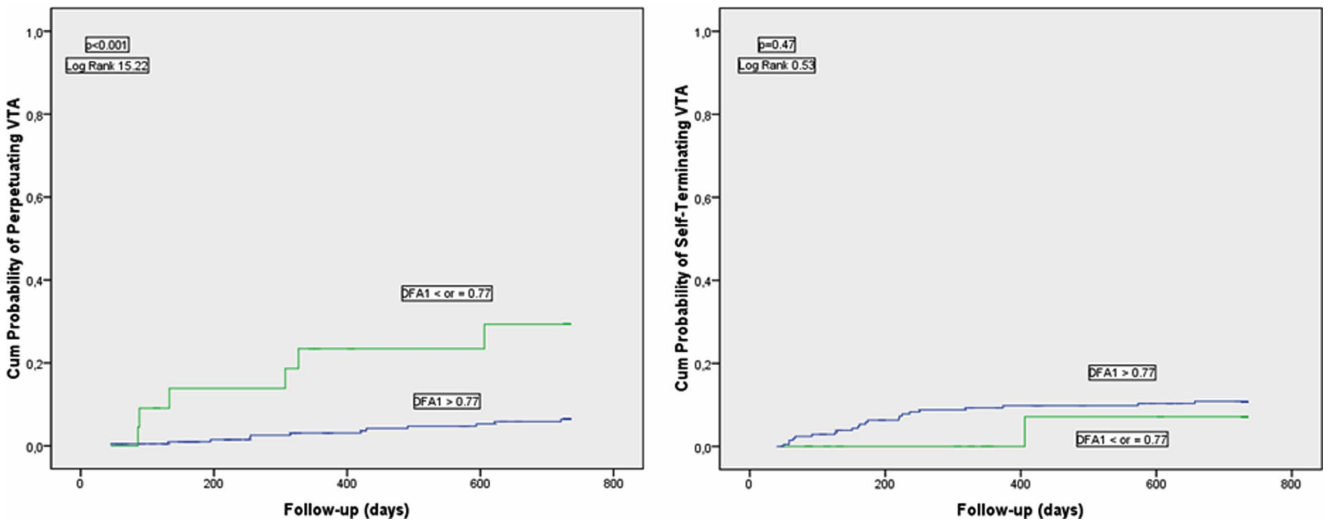

FIGURE 1 | Cumulative proportional probability of perpetuating (left chart) and self-terminating (right chart) ventricular tachyarrhythmias (VTA) for patients with the short-term scaling exponent obtained by the detrended fluctuation analysis (DFA1) ≤ or >0.77. Reproduced with a permission from Perkiömäki et al. (2011).

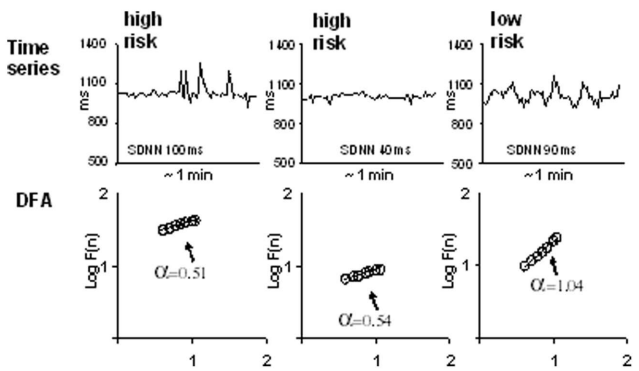

FIGURE 2 | RR interval tachograms in two patients at high-risk for cardiac mortality who have low short-term fractal scaling exponent (α; left and middle) and one patient with low risk and normal short-term fractal scaling exponent. Reproduced with a permission from Huikuri et al. (2009b).

ectopic tachycardia reflecting disturbances in autonomic regulation or in ectopic atrial pacemakers (Huikuri et al., 1999b).

Some of the non-linear measures of HRV, such as the short-term scaling exponent, have some advantages over the conventional measures of HRV considering risk stratification purposes. These advantages include: less dependency on heart rate, less inter-individual and intra-individual variation (Perkiömäki et al., 2001c; Pikkujämsä et al., 2001; Maestri et al., 2007), smaller relative changes of individual values over time after AMI (Perkiömäki et al., 2001c), and relatively good comparability of individual values between long-term and short-term electrocardiographic recordings (Perkiömäki et al., 2001b).

The cardiac arrhythmias and risk stratification after acute myocardial infarction (CARISMA) study included patients, who had left ventricular ejection fraction ≤40% measured from 3 to 21 days after AMI. Several HRV measures, when analyzed from 24-h ECGs recorded at 6 weeks after the AMI, predicted the primary endpoint of ventricular fibrillation or symptomatic sustained ventricular tachycardia. However, the short-term scaling exponent was the only HRV measure, when analyzed from 24-h ECGs recorded at 1 week after the AMI, which significantly predicted the primary endpoint. It is noteworthy that the arrhythmic endpoint events were objectively detected using implantable loop recorders in the CARISMA study (Huikuri et al., 2009a), whereas in most of the previous studies arrhythmic events/sudden arrhythmic cardiac death have been evaluated on clinical basis. In the recent non-invasive risk assessment early after a myocardial infarction (REFINE) study (Exner et al., 2007), which included post-AMI patients with somewhat better preserved left ventricular function than the CARISMA study, HRV measured from 10 to 14 weeks after the AMI tended to predict the primary endpoint of cardiac death or resuscitated cardiac arrest, but had no association with the primary endpoint when measured from 2 to 4 weeks after the AMI. These notions underline the importance of timing for measurement of HRV after AMI.

The data on the prognostic significance of the complexity measures of HRV are limited (Huikuri et al., 2009b). Decreased multiscale entropy of heart rate has been observed to predict mortality in trauma patients (Norris et al., 2008a,b). There has been shown to be association with reduced complexity in heart rate dynamics and postoperative complications after vascular surgery (Fleisher et al., 1993). Decreased complexity in heart rate behavior measured by approximate entropy has been found to precede spontaneous episodes of atrial fibrillation in patients without structural heart disease (Vikman et al., 1999) and in patients after coronary artery bypass surgery (Hogue et al., 1998). Furthermore, the complexity of heart rate dynamics has been observed to reduce during 1 year follow-up after coronary artery bypass operation (Laitio et al., 2006).

USEFULNESS OF HEART RATE VARIABILITY IN CLINICAL DECISION MAKING AND FUTURE PERSPECTIVES

Despite a large body of evidence documenting the predictive value of various HRV indices, none of these methods are in widespread clinical use at the moment. If patients would be risk stratified to a therapy based on a HRV measurement, the therapy should improve the patients' outcome. Therefore it should be evaluated in well-designed prospective randomized studies, whether the selection of the patients to the therapy based on potentially useful HRV measurements improves the prognosis. An ideal risk stratifier should specifically predict sudden arrhythmic death as an implantable cardioverter-defibrillator (ICD) therapy is effective in preventing such events. The patients with severely depressed left ventricular function are at highest risk for life-threatening ventricular arrhythmias (Huikuri et al., 2001), however, it has been found that HRV measurements work prognostically better in patients with more preserved left ventricular function (Mäkikallio et al., 2005) than in those with more severe left ventricular dysfunction. Additionally, there is evidence to support the concept that disturbed HRV predicts cardiac death in general and not specifically sudden arrhythmic death in patients with severe left ventricular dysfunction (Perkiömäki et al., 2001a; Zareba et al., 2003). On the other hand, there are numerically more sudden deaths among lower risk post-AMI patients with better preserved left ventricular function (Huikuri et al., 2001). Furthermore, as described above the results of the CARISMA study show that measures of HRV analyzed from electrocardiographic recordings obtained several weeks after AMI predict life-threatening ventricular tachyarrhythmias in post-AMI patients with moderately depressed left ventricular function (Huikuri et al., 2009a). Taken together, the post-AMI patients with moderate/mild left ventricular dysfunction would be the best target population for future prospective studies aiming to assess whether an intervention based on the assessment of HRV improves the outcome. In the CARISMA study, according to the receiver operator characteristics curve analysis, e.g., the short-term scaling exponent analyzed from 24-h ECGs recorded at 6 weeks after the AMI had the area under the curve of order of 0.75 for predicting life-threatening ventricular tachyarrhythmias. This can be considered potentially useful accuracy for risk stratification purposes. However, the accuracy of this level does not simultaneously allow both an excellent specificity and sensitivity for any selected cutpoint. Therefore it would be important to try to find additional good risk markers, which could in combination with HRV measurements increase the accuracy in predicting sudden arrhythmic death. This is particularly important, if ICD therapy is considered based on this risk stratification strategy as ICD implantation and therapy suffer from potential complications and discomfort and are relatively expensive. Severely depressed left ventricular function is considered to be a sufficient risk indicator for prophylactic ICD implantation in post-AMI patients with acceptable numbers needed to treat values for getting a benefit (Moss et al., 2002; Bardy et al., 2005). However, most of the post-AMI patients with better preserved left ventricular function at high-risk for sudden arrhythmic death do not get a primary prophylactic ICD therapy. Taken together, it would therefore be justified to consider to apply a cutpoint with compromised sensitivity of a HRV measurement to get high specificity, high positive predictive accuracy, and a low number needed to treat value in future prospective randomized prophylactic ICD studies in post-AMI patients with moderate/mild left ventricular dysfunction.

Despite many advancements, the analysis of HRV is still far from routine clinical use. Before it can be a useful tool for clinicians, at least the following questions need clarification: what would be the optimal timing for HRV analysis after AMI? What would be the optimal target population for the use of HRV analysis as a risk stratifier? What method(s) should be used for HRV analysis in clinical settings? What would be the optimal preprocessing method for editing premature depolarizations for different HRV parameters in different clinical settings? What would be the recommendable cutpoints of selected HRV measurements for risk stratification purposes in different cardiac conditions for different endpoints, in a same cardiac condition with different degree of left ventricular dysfunction and heart failure, and in different age and gender groups? What would be a recommendable length of ECG recording? Under what kind of conditions should the ECG recordings be done? What is the accuracy of selected HRV measures in predicting different adverse events in different cardiac conditions? What would be the expected benefits of HRV analysis to patients' further evaluation and treatment? What would be the number needed to treat value, e.g., to get benefit from ICD therapy? Could the HRV analysis be used in the patients' follow-up, etc.? Hopefully, after further developments and standardization the HRV analysis alone or with other risk indicators will soon serve as a useful tool for risk stratification.

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Association of heart rate variability and inflammatory response in patients with cardiovascular diseases: current strengths and limitations

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Many experimental and clinical studies have confirmed a continuous cross-talk between both sympathetic and parasympathetic branches of autonomic nervous system and inflammatory response, in different clinical scenarios. In cardiovascular diseases, inflammation has been proven to play a pivotal role in disease progression, pathogenesis and resolution. A few clinical studies have assessed the possible inter-relation between neuro-autonomic output, estimated with heart rate variability analysis, which is the variability of R-R in the electrocardiogram, and different inflammatory biomarkers, in patients suffering from stable or unstable coronary artery disease (CAD) and heart failure. Moreover, different indices derived from heart rate signals' processing, have been proven to correlate strongly with severity of heart disease and predict final outcome. In this review article we will summarize major findings from different investigators, evaluating neuro-immunological interactions through heart rate variability analysis, in different groups of cardiovascular patients. We suggest that markers originating from variability analysis of heart rate signals seem to be related to inflammatory biomarkers. However, a lot of open questions remain to be addressed, regarding the existence of a true association between heart rate variability and autonomic nervous system output or its adoption for risk stratification and therapeutic monitoring at the bedside. Finally, potential therapeutic implications will be discussed, leading to autonomic balance restoration in relation with inflammatory control.

Keywords: heart rate variability, inflammation, autonomic nervous system, coronary artery disease, cardiovascular disease, mortality

INTRODUCTION

Systemic inflammation is a normal response to altered homeostasis and has an important role in several pathophysiological processes, such as infection or trauma. It is characterized by the endocrine release of different cytokines, such as tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1), IL-4, IL-6, IL-10, and many others, normally confined to paracrine regulation of a local inflammatory response (Koj, 1997; Sporn, 1997). Apart from their involvement in local and systemic inflammation, cytokines may induce activation of brain-derived neuroendocrine immunomodulatory responses. Neuro-endocrine pathways, such as hypothalamo-pituitary-adrenal (HPA) axis and both the sympathetic and parasympathetic divisions of the autonomic nervous system (ANS) are powerful modulators of inflammation, typically through an anti-inflammatory balancing mechanism (Reichilin, 1993; Webster et al., 2002).

Recently, it has been demonstrated that subclinical inflammation and the con-centration of inflammatory markers, such as cytokines, correlate strongly to cardio-vascular mortality and morbidity in both healthy subjects and in those with known coronary artery disease (CAD) (Phillips et al., 1992; Ridker et al., 1997). Furthermore, vascular inflammation plays a critical role in the initiation, evolution, and rupture of atherosclerotic plaque (Ross, 1993).

In the healthy state there is some degree of stochastic variability in physiologic variables, such as heart rate (heart rate variability). This variability is a measure of complexity that accompanies healthy systems and has been suggested to be responsible for their greater adaptability and functionality related

Abbreviations: ACE, angiotensin-converting enzyme; ACTH, adrenocorticotropin hormone; ANS, autonomic nervous system; ATRAMI, Autonomic Tone and Reflexes After Myo-cardial Infarction Study; CAD, coronary artery disease; CHF, congestive heart failure; CNS, central nervous system; CRP, C-reactive protein; DHA, docasah-exaenoic acid; DMN, dorsal motor nucleus of the vagus; DVC, dorsal vagal complex; ECG, electrocardiogram; EPA, eicosapentaenoic acid; FFT, Fast Fourier trans-formation; HPA, hypothalamo-pituitary-adrenal axis; HRV, heart rate variability; HF, high frequency; LF, low frequency; LVEF, left ventricular ejection fraction; MODS, multiple organ dysfunction syndrome; NYHA, New York Heart Association; NTS, nucleus tractus solitaries; pNN50, proportion derived from dividing NN50 (number of interval differences of successive intervals greater than 50 ms) by the total NN intervals; PSD, power spectrum density; PUFA, polyunsaturated fatty acids; PVN, paraventricular nucleus; RMSSD, square root of the mean squared differences of successive intervals; RVLM, rostral ventrolateral medulla; TNF-α, tumor necrosis factor α; SAN, sinus atrial node; SDNN, standard deviation of the normal-to-normal intervals which is the square root of the variance; SD, standard deviation; SNS, sympathetic nervous system; THS, Twins Heart Study; ULF, ultra low frequency; VLF, very low frequency; WBC, white blood cell count.

to pathologic systems (Buchman, 2002). Studying physiological signals of patients can easily identify "hidden" information concerning inherent dynamics and overall variability within a time series. Recognition that physiologic time series contain such information defies traditional mechanistic approaches based on conventional biostatistical methodologies and has fueled growing interest in applying techniques from statistical physics for the study of living organisms (Seely and Christou, 2000). Through those techniques different "physiomarkers" can be estimated that fulfill the requirements of contemporary medicine for better and more accurate early warning signs, since they are based on high-frequency measurements and are much easier to measure at the bedside (Seely and Christou, 2000). In this respect, a number of international databases and different processing methods of heart rate signals have been developed with free access from different investigators, such as the Web Site Physionet (www.physionet.org).

On the contrary, it has been repeatedly demonstrated that various "biomarkers" such as cytokines, exhibit marked interdependence, pleiotropy (multiple effects) and redundancy (multiple cytokines with the same effect) (Friedland et al., 1996). At the same time, their plasma concentrations fluctuate from day to day and correlate poorly with classic physiologic variables in different groups of patients (Friedland et al., 1996; Seely and Christou, 2000; Buchman, 2002). Furthermore, biomarkers are difficult to obtain routinely at the bedside. In addition, the financial cost of various immunoassay techniques for their detection in blood samples tends to become an inhibiting factor for their extensive use, as diagnostic or even prognostic tools, in many Medical Centers.

NEURO-IMMUNOLOGICAL CROSS-TALK AND HEART RATE VARIABILITY ANALYSIS

The pathophysiological link between the communication between the Central Nervous System (CNS) and the immune-regulated inflammation is the capability of the brain to monitor and to affect at the same time the immune status. The first mechanism relies upon activation of vagus nerve afferent fibers that signal the brain that inflammation is occurring. Different kind of mediators such as cytokines can activate visceral vagus afferent fibers which terminate within the dorsal vagal complex (DVC) of the medulla oblongata. The DVC consists of the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus (DMN) and the area postrema (AP) (Berthhoud and Neuhuber, 2000). Ascending projections from the NTS reach hypothalamic paraventricular nucleus (PVN), which is associated with the synthesis and release of corticotropin releasing hormone (CRH). This factor induces the production of adrenocorticotropin hormone (ACTH) from the anterior pituitary, which is the main inducer of the synthesis of immuno-suppressive glucocorticoids from the adrenal cortex. Projections from NTS are connected to the DMN and to rostral ventrolateral medulla (RVLM). This region increases firing of the noradrenergic preganglionic neurons in the spinal cord (Tracey, 2007).

The brain can affect the immunological status through the activation of the HPA axis and increased outflow of sympathetic (SNS) and parasympathetic nervous system. The SNS activation during the early stages of stress induces local inflammatory response through α_2–subtype adrenoreceptor stimulation by norepinephrine (NE), whereas stimulation of β_2-subtype adrenoreceptor-cAMP-protein kinase A pathway is associated with an inhibition of pro-inflammatory cytokines' production (van der Poll et al., 1996; Elenkov et al., 2000; Zhou et al., 2001). It seems that SNS activation protects the organism from the detrimental effects of pro-inflammatory cytokines, while it can increase local inflammatory response (Chrousos, 1995). In addition to the SNS, a link between the parasympathetic part of the ANS and immune-regulatory processes has been suggested (Tracey, 2002). It has been demonstrated that acetylcholine decreases TNF-α production by endotoxin-stimulated human macrophage cultures, through α7-subunit of the nicotinic acetylcholine receptor (Wang et al., 2003; de Jonge et al., 2005). The vagus nerve cholinergic signaling interacts with the above receptor on immune cells in the spleen and inhibits TNF-α production and release into the circulation (Huston et al., 2006). Acetylcholine is also effective in suppressing other pro-inflammatory cytokines such as IL-1β, IL-6, and high mobility group box 1 (HMGB1) protein (Wang et al., 2004). This "cholinergic anti-inflammatory pathway" is responsible for a "hard-wired" connection between the nervous and immune systems and is considered, as the primary component of the "immuno-reflex." A more complete understanding of these reflexes can yield insight into both pathophysiological pathways and therapeutic strategies in many pathological processes, including infections, sepsis and cardiovascular diseases (Tracey, 2002, 2007).

In conclusion, there is strong evidence that CNS controls body's systemic response to inflammation. Recently, different clinical studies investigating a possible association between ANS outflow and various inflammatory indices in patients with heart diseases have appeared in the literature (Aronson et al., 2001; Malave et al., 2003; Janszky et al., 2004; Shehab et al., 2004; Hamaad et al., 2005; Lanza et al., 2006; Madsen et al., 2007; Nolan et al., 2007; Psychari et al., 2007; von Känel et al., 2011). The aim of these studies was to measure ANS activity through a set of different "physio-markers" and correlate them with various biomarkers that can indirectly assess inflammatory response in different clinical scenarios, such as CAD (Janszky et al., 2004; Hamaad et al., 2005; Lanza et al., 2006; Madsen et al., 2007; Nolan et al., 2007; Psychari et al., 2007; von Känel et al., 2011) and heart failure (Aronson et al., 2001; Malave et al., 2003; Shehab et al., 2004). Moreover, the prognostic value of such measurements was tested in different groups of patients with cardiovascular diseases, in terms of mortality and risk of rehospitalization.

The best "physiomarkers" are obtained from analysis of heart rate variability (HRV); that is, the variability of R-R series in the electrocardiogram (ECG), and its frequency components (Lombardi et al., 1987; Task Force, 1996). Beat-to-beat fluctuations reflect the dynamic response of the cardiovascular control systems to a host of naturally occurring physiological perturbations. A variety of animal and human research has established two clear frequency bands in heart rate signals. These bands include high frequency oscillations, between 0.15 and 0.4 Hz that are associated with respiration, and bands with a lower frequency range, below 0.15 Hz (Task Force, 1996). Akselroad et al. (1981)

introduced power spectrum analysis of heart rate fluctuations in order to quantify beat-to-beat cardiovascular control. Power spectrum density (PSD) analysis provides the basic information of how power (variance) distributes as a function of frequency (Akselroad et al., 1981; Malik and Camm, 1993). In 1996, the Task Force of the European Society of Cardiology and the Northern American Society of Pacing and Electrophysiology published guidelines regarding standardization of nomenclature, specification of methods of measurement, definition of physiological and pathophysiological correlates, description of clinical applications and identification of different areas for future research.

The association of higher risk of post-infarction mortality with reduced HRV was first shown by Wolf et al. (1978). The clinical importance of HRV became appreciated in the late 1980s, when it was demonstrated that low HRV was a strong and independent predictor of mortality after an acute myocardial infarction (MI) (Kleiger et al., 1987; Lombardi et al., 1987).

MEASUREMENY OF HEART RATE VARIABILITY

The RR variations may be evaluated by a number of methods:

TIME DOMAIN METHODS

Time domain methods determine heart rate or RR intervals in continuous ECG records. Each QRS complex is detected and the normal-to-normal (NN) intervals (all intervals between adjacent QRS complexes) are calculated. Other time domain variables include the mean NN interval, the mean heart rate or the difference between the longest and the shortest NN interval, as well. The simplest of these metrics is the standard deviation of the NN intervals (SDNN), which is the square root of the variance. However, it should be emphasized that SDNN becomes less accurate with shorter monitoring periods. The most commonly used time domain methods are the square root of the mean squared differences of successive NN intervals (RMSSD), the number of interval differences of successive NN intervals greater than 50 ms (NN50) and the proportion derived from dividing NN50 by the total NN intervals (pNN50) (Akselroad et al., 1981; Task Force, 1996; **Table 1**).

FREQUENCY DOMAIN METHODS

Spectral analysis of heart rate partitions HRV into its frequency components. Most commonly used methods are Fast Fourier Transformation (FFT) and auto-regressive modeling. FFT displays in a plot the relative contribution (amplitude) of each frequency. This plot includes at least three peaks.Fast periodicities in the range 0.15–0.4 Hz [high frequency (HF)] are largely due to the influence of the respiratory phase on vagal tone. Low-frequency periodicities (LF), in the region of 0.04–0.15 Hz, are produced by baroreflex feedback loops, affected by both sympathetic and parasympathetic modulation of the heart, whereas very low frequency periodicities (VLF), in the frequency range between 0.003 and 0.04 Hz and ultra low frequencies (ULF, <0.003 Hz) have been variously ascribed to modulation by chemoreception, thermoregulation and the influence of vasomotor activity. The area under the power spectral curve (power) in a particular frequency band is considered to be a measure of HRV at that frequency, whereas the LF/HF ratio has been suggested as an indirect index of sympathovagal balance (Task Force, 1996). According to the report of the Task Force, the analyzed ECG signals must satisfy several technical requirements in order to obtain reliable information. The optimal sampling frequency range should be between 250 to 500 Hz. Ectopic beats, arrhythmic events, missing data and noise effects should be properly filtered and omitted. Frequency domain methods must be preferred in cases of short term investigations. The recordings should last for at least 10 times the wavelength of the lower frequency bound, thus recordings of ∼1 min can assess the HF component of HRV while 2 min are needed for the LF component. In conclusion, 5-min recordings are preferred, unless the aim of the study dictates a different design (Akselroad et al., 1981; Lombardi et al., 1987; Task Force, 1996; **Table 2**).

Table 1 | HRV metrics in time domain.

SDNN	Standard deviation of all N-N intervals
SDNN index	Average of the standard deviations of N-N intervals for each 5-min period
SDANN	Standard deviation of the average N-N intervals for each 5-min period over 24 h
NN50	Number of N-N intervals differing by >50 ms from the preceding interval
pNN50	Percentage of adjacent cycles that are >50 ms apart
RMSSD	Root mean square of successive differences in ms

ORIGIN OF HEART RATE VARIABILITY COMPONENTS

HIGH FREQUENCY OSCILLATIONS

The cyclic variations in intrathoracic pressure perturbate venous return, cardiac out-put and thus, blood pressure. These changes are sensed by baroreceptors and result in changes in autonomic activity to the heart. These perturbations are mediated via the vagus nerve as atropine administration abolishes high frequency oscillations in heart rate (Akselroad et al., 1981). It seems that a major cause of respiratory sinus arrhythmia (RSA) is a central coupling of respiratory drive to cardiac vagal motor neurons. However, the changes in vagal activity are partly induced by baroreceptor sensing of respiratory oscillations in blood pressure and reflect all components of the baroreflex loop (DeBoer et al., 1987). In addition, factors such as reduced respiratory capacity and body position may alter the amplitude of high frequency oscillations in blood pressure and subsequently the HF component of heart rate signals (Malpas, 2002). Thus, heart rate variability analysis cannot be used for comparisons between different patient groups as there is a need for controlling ventilation for both rate and depth. Moreover, and since there is marked inter-individual variation in the relationship between HRV and parasympathetic effect, differences in HRV between individuals may reflect differences in this relationship, as was postulated by Goldberger (Goldberger et al., 2001). In this respect,

Table 2 | HRV metrics in frequency domain.

ULF (ultra low frequency)	ms^2	24 h recordings ≤0.003 Hz
VLF (very low frequency)	ms^2	24 h and 5-min recordings −0.003–0.04 Hz
LF (low frequency)	ms^2	24 h and 5-min recordings −0.04–0.15 Hz
HF (high frequency)	ms^2	24 h and 5-min recordings −0.15–0.4 Hz

this relationship in humans was described by a quadratic function in which there is an initial ascending limb, where HRV increases in parallel with vagal effect until it reaches a plateau level. Beyond this level, HRV decreases with further augmentation of vagal tone, probably due to a saturated HRV response upon intense autonomic stimulation (Goldberger et al., 2001). Finally, age and sex-related differences have also been associated with this variability (Goldberger et al., 2001).

LOW FREQUENCY OSCILLATIONS

The LF component of HRV is probably the most contentious aspect with respect to cardiovascular variability. There are two opposing theories in the literature proposing different potential origins: (1) the central oscillator theory (Montano et al., 1996) and (2) the baroreflex feedback loop theory (Lanfranchi and Somers, 2002). According to the first theory, it is believed that LF oscillations reflect sympathetic tone and are generated by the brain stem circuits. In cats, Montano (Montano et al., 1996) analyzed the dis-charges of single sympathetic neurons located in the rostral ventrolateral medulla and caudal ventrolateral medulla. He observed activity at 0.12 Hz, which was positively correlated with heart rate and blood pressure variability. As the above oscillations remained after sino-aortic and vagal resection, it was assumed that the central nervous system is able to generate such oscillations.

According to the baroreflex feedback loop theory, a change in blood pressure is sensed by arterial baroreceptors, resulting in heart rate adjustment through the central nervous system and via both the fast vagal and the slower sympathetic actions (Lanfranchi and Somers, 2002). At the same time, baroreceptors induce a slow sympathetic withdrawal from the vessels. The delay in the sympathetic branch of the baroreflex in turn determines a new oscillation, which is sensed by the baroreflex and induces a new oscillation in heart rate. It has been also proposed that the LF oscillation arises from the interaction of slow sympathetic and fast vagal responses, where baroreflex buffering of the slow respiratory induced blood pressure oscillations results in resonant low frequency oscillations, due to the delay in the slow conducting sympathetic loop of the baroreflex (DeBoer et al., 1987).

In conclusion, it must be stressed that the low frequency oscillations of heart rate reflect the ability of the individual components of the baroreflex feedback loop to respond to different inputs that can alter the power of such oscillations and they are not just a measure of sympathetic nerve activity.

INTRACARDIAC ORIGIN OF HRV

The reasons for reduced HRV during cardiovascular diseases have been debated and two theories have been developed. The first theory focuses on reduction of vagal tone and has been introduced by Akselroad et al. (1981). The second theory developed by Goldberger and colleagues (2002) states that normal physiology has fractal-like properties with high levels of complexity that explain phenomena such as HRV. Its reduction during severe disease reflects a "de-complexification," mostly attributed to uncoupling between different restorative mechanisms (Godin and Buchman, 1996). In addition, accumulating evidence from both *in vitro* and *ex vivo* experiments support a potential third mechanism (Griffin et al., 2005), which is associated with an intracardiac origin of HRV. According to this hypothesis, sinus atrial node (SAN) cells can be viewed as an amplifier of various input signals (Zaza and Lombardi, 2001). During cardiovascular diseases, an unfavorable metabolic milieu could affect ion channel gating properties or membrane receptor densities, with significant impact upon level and variability of pacemaker activity. In addition, a possible reduced responsiveness of SAN cells to external stimuli could also negatively affect HRV (Zaza and Lombardi, 2001).

Moreover, different clinical studies in heart transplant recipients have found evidence for heart rate fluctuations originating from the heart itself (Hrushesky et al., 1984; Bernardi et al., 1990). Bernardi studied intrinsic mechanism regulating HRV in both transplanted and intact heart during exercise (Bernardi et al., 1990). He found that at peak exercise a non-autonomic mechanism, probably intrinsic to the heart muscle, may determine heart rate fluctuations in synchrony with ventilation, in transplanted as well as in intact hearts. Hrushesky and colleagues (1984) quantified respiratory sinus arrhythmia and found that individuals with a transplanted heart had resting RSA values similar to healthy subjects.

In conclusion, there is marked inter-individual variation between HRV response and different levels of autonomic stimulation. Basal autonomic activity, age and sex differences, alterations in expression of ion channel activity or autonomic receptors could be responsible for individualized curves, relating autonomic effects to HRV (Eckberg, 1997; Goldberger et al., 2001). In addition, LF/HF ratio has been criticized as an indirect measure of sympathovagal balance, reflecting rather autonomic fluctuations and not absolute measures of autonomic nerve traffic (Eckberg, 1997; Billman, 2013). Thus, interpretation of different studies investigating HRV alterations in different groups of patients should be cautious since variability in time of recordings and methods for HRV analysis, as well as heterogeneity of studying population, limit generalization of their findings.

CLINICAL IMPLICATIONS OF ALTERED HEART RATE VARIABILITY

The first large prospective population study that reported the significant prognostic value of low HRV after an acute myocardial infarction was the Autonomic Tone and Reflexes After Myocardial Infarction Study (ATRAMI) (La Rovera et al., 1998), and included 1284 patients with a recent (<28 days) myocardial infarction. A 24 h Holter recording was done to quantify HRV (using SDNN values) and ventricular arrhythmias. Low values of HRV (SDNN < 70 ms) carried a significant multivariate risk of cardiac mortality. Furthermore, the association of low SDNN with left ventricular ejection fraction (LVEF) <35% carried a relative risk of 6.7, compared with patients with LVEF above 35%. Investigators from the Framingham Heart Study (Tsuji et al., 1994) computed HRV time and frequency domain measures in 736 patients and correlated them with all-cause mortality during 4 years of follow-up. They concluded that HRV offers prognostic information independent of that provided by traditional risk factors.

During the Zutphen study (Dekker et al., 1997), 885 middle-aged (40–60 years old) and elderly Dutch men (aged 65–85)

were followed from 1960 until 1990, whereas SDNN was determined from the resting 12-lead ECG. It was shown that low HRV is predictive of mortality from all causes, indicating that it can be used as an index of compromised health in the general population. It seems that the predictive value of low HRV is independent of other factors, such as depressed left ventricular ejection fraction and presence of late potentials (Kleiger et al., 1987; Lombardi et al., 1987). In addition, retrospective ECG data analysis from 127 patients included in the Veterans Affairs' Survival Trial of Antiarrhythmic Therapy in Congestive Heart Failure (CHF) (Bilchick et al., 2002) demonstrated that CHF patients with SDNN <65.3 ms had a significantly increased risk of sudden death. Moreover, this study demonstrated that every 10 ms increase in SDNN conferred a 20% decrease in risk of mortality.

HEART RATE VARIABILITY AND INFLAMMATORY BIOMARKERS IN CARDIOVASCULAR DISEASES

The relationship between HRV and inflammation has been studied mainly in patients with acute or stable CAD, CHF and metabolic syndrome with impaired glucose tolerance (Brunner et al., 2002). The inflammatory biomarkers that were used included C-reactive protein (CRP), TNF-α, IL-6 and white blood cell count (WBC).

PATIENTS WITH CORONARY ARTERY DISEASE

Hamaad et al. (2005) tested the association between time and frequency domain indices of HRV and circulating IL-6, high sensitivity CRP (hs-CRP) and white cell counts, in a sample of 100 patients with proven acute coronary syndrome. In addition, they compared these metrics with healthy controls ($n = 49$) and estimated possible relationships on repeated measures at 4 months in recovery ($n = 51$). They found modest negative correlations between all inflammatory biomarkers and mainly SDNN, VLF and LF power. The strongest associations were seen between WBC and SDNN ($r = -0.351$). However, relationships did not persist on multivariate analyses after a 4-month period. According to the authors, the correlations were observed largely among HRV indices reflecting sympathetic activity, suggesting that the inflammatory response in acute coronary events may be associated with sympathetic activation instead of vagal withdrawal. Furthermore, leukocytosis observed in these patients seems to be a potential source of pro-inflammatory cytokines within the atheromatous plaque and might induce a potential rupture.

In another study, Lanza and colleagues (2006) assessed HRV and measured CRP serum levels within 24 h of admission in 531 patients with unstable angina pectoris. They found a significant negative correlation between CRP levels and all HRV metrics derived from both time and frequency domain, with the highest correlation coefficient with SDNN and VLF. After categorizing patients into 4 subgroups according to CRP quartile levels, significantly lower HRV values were found in the upper CRP quartile. The subsequent multivariate analysis revealed that SDNN and VLF were the most significant predictors of increasing CRP, whereas CRP was a strong predictor of impaired ANS activity as well.

In a study including patients with suspected CAD, Madsen et al. (2007) enrolled 269 subjects referred for elective coronary angiography. They found that SDNN of heart rate signals was significantly higher in the lower CRP quartile compared to the upper one, whereas associations were stronger for patients with a previous myocardial infarction and with significant coronary stenoses.

In a similar study (Nolan et al., 2007), a negative correlation between CRP and HRV frequency components was reported, whereas a decreased HF power (reflecting vagal tone) in the high CRP quartile, compared to the lowest one, was found. In the study by Janszky and colleagues (2004) that included only female patients who survived hospitalization for acute myocardial infarction and were evaluated 1 year after the event, levels of IL-6 showed an inverse relation with all HRV frequency measures, except for HF. However, this relationship, as well as the association between CRP levels and IL-1 receptor antagonist (IL-1ra) with HRV indices was non-significant. Psychari et al. (2007) also reported a strong inverse association between CRP and several HRV indices (SDNN, HF, and LF) in post-MI patients and after adjustment for left ventricular function.

Recently, von Känel et al. (2011) investigated the association between HRV measured in the time domain, CRP, IL-6 and fibrinogen, in a cohort of 862 subjects recruited from the Heart and Soul Study, which assessed health outcomes in 1.024 outpatients with stable CAD. They found that SDNN was inversely and significantly associated with inflammatory indices, after adjustment of all covariates.

PATIENTS WITH HEART FAILURE

In 2001, Aronson et al. (2001) evaluated for the first time the relationship between HRV metrics derived from both time and frequency domains and different biomarkers, such as IL-6, TNF-α, and serum levels of norepinephrine, in 64 patients admitted for decompensated chronic heart failure. TNF-α levels did not correlate with any of the HRV indices. However, IL-6 was inversely correlated with SDNN ($r = -0.36$), with total power of heart rate signals and ULF ($r = -0.37$ and $r = -0.43$, respectively). No correlation was found between IL-6 and time (pNN50 and RMSSD) or frequency domain (HF power) indices of vagal activity.

Malave et al. (2003) examined HRV in relation to circulating levels of TNF-α, TNF-α receptors and norepinephrine in 10 controls, 15 patients with mild CHF and 14 subjects with moderate heart failure. There was a significant inverse linear correlation between increased levels of all biomarkers and SDNN, LF and HF power among CHF patients. In addition, LF power was more closely correlated with circulating levels of TNF-α than was the HF component, whereas multiple linear regression analysis showed that TNF-α was a stronger predictor of reduced HRV than was the circulating levels of norepinephrine. The authors concluded that over-expression of TNF-α and subsequent loss of β-adrenergic responsiveness contributes to the decrease in HRV, observed in heart failure. According to findings from experimental studies (Chung et al., 1990), TNF-α might inhibit β-adrenergic signal transduction through either activation of Gi proteins or impairment of activation of Gs proteins, something that could be viewed as an adaptive mechanism in the early stages of CHF, protecting cardiac myocytes from the deleterious

actions of catecholamines. However, in the more advanced stages of the disease, this mechanism could become maladaptive, leading to a reduction in cardiac output (Mann et al., 1992).

Finally, in a small prospective study that included 34 patients with CHF followed for a 2-year period with monthly CRP measurements and 24-h Holter recordings, it was shown that five unexpected deaths that occurred were preceded by progressive increases in both CRP serum levels and autonomic dysfunction (low HRV indices) (Shehab et al., 2004).

HEALTHY CONTROLS

As a part of the Copenhagen Holter study, that assessed the value of 24-h Holter recording in the risk assessment of men and women aged 55, 60, 65, 70, and 75 years with no apparent heart disease, Sajadieh et al. (2004) investigated the associations between time domain components of HRV, CRP and WBC in 643 healthy men and women. They found that SDNN was negatively correlated with smoking, inflammatory indices, blood sugar, triglyceride concentration, female gender and diabetes. Moreover, in multivariate regression analysis, increased heart rate and reduced HRV were significantly related to white blood cell count and CRP. The reduction of SDNN was attributed to sympathetic predominance, whereas lack of any association between inflammation and pNN50, which is considered as a marker of vagal activity, indicates that reduced HRV is mainly due to increased sympathetic activity rather than vagal withdrawn.

From the Whitehall II cohort, a multicenter epidemiologic investigation of over 5000 subjects, two studies (Owen and Steptoe, 2003; Sloan et al., 2007) used sub-samples to examine the relation between HRV indices derived from the frequency domain and inflammation, in healthy subjects. In the first study, Sloan found in a sample of 757 people, an inverse correlation between CRP and IL-6 with both LH and HF components of HRV power spectrum. In the second study, Owen and Steptoe did not find any association between IL-6, TNF-α and time domain measures of HRV, in a group of 211 healthy adults.

Other investigators (Albert et al., 2002) reported a strong positive association between CRP levels and the long-term risk of sudden cardiac death, in case-control analysis among healthy individuals, followed for 17 years in the Physician's Health Study. Men in the upper CRP quartile had a 2.8 fold increased risk of sudden cardiac death compared to men in the lower quartile. According to the authors, a low-grade inflammation involved in atherosclerosis shifts ANS balance toward sympathetic activation, making individuals more prone to ventricular arrhythmias and sudden cardiac death.

The Twins Heart Study (THS) (Goldberg et al., 2002) was an investigation of psychological, biological and behavioral risk factors for subclinical cardiovascular diseases in 7.369 middle-aged male-male twin pairs, who served in the United States military during the Vietnam War. From this registry, a cohort of 264 twins free of symptomatic CAD was examined by Lampert et al. (2008), for assessing possible associations between HRV, CRP, and IL-6. They found an inverse relationship between frequency domain HRV metrics (except for HF) and both CRP and IL-6. These associations persisted after adjustment for other traditional CAD risk factors, such as smoking, hypertension, diabetes, high-density lipoprotein (HDL) and depression.

A significant confounding factor of HRV analysis that has to be considered in these studies includes the presence of depressive symptoms and anxiety. It has been estimated that ∼12–20% of hospitalized cardiac patients suffer from major depression, whereas 15% of subjects following acute myocardial infarction exhibit a posttraumatic stress disorder (Frasure and Lesperance, 2006; Garder and von Karel, 2006; Pizzi et al., 2008). In a 2-year follow up prospective observational study, Pizzi investigated the relation between time domain HRV indices, IL-6, TNF-α, CRP and depression in a cohort of 415 subjects free of CADs, with at least two CAD risk factors (age, male gender, current smoking, hypertension, dislipidaemia). All HRV and inflammatory indices were significantly associated with depression. Logistic regression further showed that depressive individuals were more likely to have a higher CRP and IL-6 and altered HRV (lower SDNN).

In a recent study of Kop and colleagues (2010) who recruited 908 patients, free of CAD, for a median follow-up period of 13.3 years, it was demonstrated that among depressed participants, HF power of HRV was negatively correlated with CRP ($r = -0.205$), IL-6 ($r = -0.233$) and WBC ($r = -0.292$). Moreover, depression was associated with high IL-6 serum levels and increased cardiovascular mortality risk. In conclusion, in patients without heart disease depression seems to be associated with HRV imbalance and inflammation.

Table 3 summarizes the majority of clinical studies that have evaluated the relationship between different inflammatory biomarkers and HRV metrics, in patients with different cardiovascular diseases.

HEART RATE VARIABILITY AND SYSTEMIC INFLAMMATION IN CRITICAL ILLNESS

The presence of sympathetic overactivity, autonomic dysfunction, inappropriately increased heart rate, insulin resistance and in some cases, cardiomyopathy with reduced cardiac contractility, has also been observed during severe sepsis and multiple organ failure (Muller-Werdan et al., 2006). However, in cardiac patients sympathetic activity dominates over vagal tone where in septic patients both branches of ANS are attenuated (Muller-Werdan et al., 2006). For these reasons, it has been hypothesized that during critical illness except for ANS impairment, a defective signal transduction at the level of pacemaker cells could also account for observed differences between cardiac and septic patients (Fairchild et al., 2009).

Alterations in HRV during septic shock and multiple organ dysfunction syndrome (MODS), have been reported from different research groups (Goldstein and Buchman, 1998; Goldstein et al., 1998; Seely and Christou, 2000). In this respect, Goldstein et al. (1998) found that both increased total variability and LF power were associated with recovery and survival, whereas a decrease in total power, LF/HF and LF power correlated with severity of illness and mortality in septic patients, 48 h after being admitted to the Intensive Care Unit. In an animal study of experimental endotoxemia, induced by administration of lipopolysacchraride (LPS, endotoxin derived from the cell wall of Gram-negative bacteria) Fairchild and colleagues demonstrated a

Table 3 | Summary of several clinical studies investigating a possible association between HRV indices and inflammation in patients with CAD, CHF and healthy individuals.

References	Study population	Duration and HRV measures	Inflammatory indices	Results
Hamaad et al., 2005	100 patients with acute CAD vs. 29 healthy controls	20 min time, time and frequency domain	CRP, IL-6	Negative correlation with SDNN, VLF and LF
Lanza et al., 2006	531 patients with unstable CAD	24-h time, time and frequency domain	CRP	Inverse correlation between CRP with SDNN and VLF
Madsen et al., 2007	269 patients with suspected CAD	24-h time, time domain	CRP	Upper CRP quartile negatively correlated with SDNN
Nolan et al., 2007	29 patients with CAD	5-min time, frequency domain	CRP	HF power decreased in high CRP group
Psychari et al., 2007	98 patients with acute CAD (post-MI)	24-h time, time and frequency domain	CRP	Inverse relation between CRP and SDNN, HF and LF power
von Känel et al., 2011	862 patients with CAD	24-h time, time domain	CRP, IL-6, fibrinogen	Inverse association between CRP, IL-6 and SDNN
Aronson et al., 2001	64 patients with CHF	24-h time, frequency domain	TNF-α, IL-6	IL-6 inversely correlated with SDNN and ULF power
Malave et al., 2003	10 healthy controls, 15 patients with mild CHF, 14 patients with moderate CHF	24-h time, frequency domain	TNF-α, TNF soluble type 1 and 2 receptors	Inverse correlation between inflammatory measures, SDNN, LF and HF
Sajadieh et al., 2004	643 subjects without CHF	24-h time, time domain	CRP, WBC	Inverse correlation between SDNN with CRP and WBC SDNN predictor of CRP
Sloan et al., 2007	757 young healthy adults	10-min time, frequency domain	CRP, IL-6	CRP and IL-6 inversely correlated with HF and LF
Owen and Steptoe, 2003	211 healthy subjects	20–30 min time, time domain	TNF-α, IL-6	No relation between both TNF-α and IL-6 with HRV
Lampert et al., 2008	264 healthy twins individuals	24-h time, frequency domain	CRP, IL-6	Inverse relation between CRP and IL-6 with all HRV frequency metrics (except for HF)

Abbreviations: CRP, C-reactive protein; CAD, coronary artery disease; CHF, congestive heart failure; MI, myocardial infarction; WBC, white blood cell count.

strong inverse correlation between SDNN and total power of RR time series and peak concentrations of different cytokines, 3–9 h post-LPS (Fairchild et al., 2009). The same results were found after administration of recombinant TNF-α. It was suggested that mechanisms responsible for decrease in HRV could be related with effects of LPS and/or cytokines on various ion channels.

Tateishi et al. (2007) investigated the relationships between HRV and interleukin 6 upon admission in a cohort of 45 septic patients and they found that IL-6 exhibited significant negative correlations with both LF and HF power values. These findings indicate a possible association between low HRV indices and hyper-cytokinemia.

In another study Papaioannou et al. (2009), we investigated possible associations between different HRV indices and various biomarkers of inflammation, in 45 septic patients and during the first 6 days of their stay in the ICU. We daily assessed HRV in time (SDNN) and frequency domain (LF, HF and LF/HF) and measured C-reactive protein, Interleukin 6 and 10 serum levels in two groups of patients. The first group included subjects suffering from sepsis with mean Sequential Organ Failure Assessment score of severity of illness (SOFA) ≤ 10 ($n = 25$) and the second group included patients with septic shock (SOFA > 10, $n = 20$). This study found in the group of patients with SOFA > 10, statistically significant inverse correlations between CRP and LF/HF ratio ($r = -0.61$), (**Figure 1A**) and positive correlations with HF ($r = 0.80$). At the same time, IL-10 proved to be significantly correlated with HF and SOFA score in a positive way and with LF, LF/HF and SDNN in a negative way. Finally, the total variability of heart rate signals (SDNN) was found to be negatively correlated with both CRP ($r = -0.79$) and SOFA score ($r = -0.84$) (**Figure 1B**). IL-6 was not significantly correlated with any HRV parameter. It is possible that, the pleuripotency of this cytokine

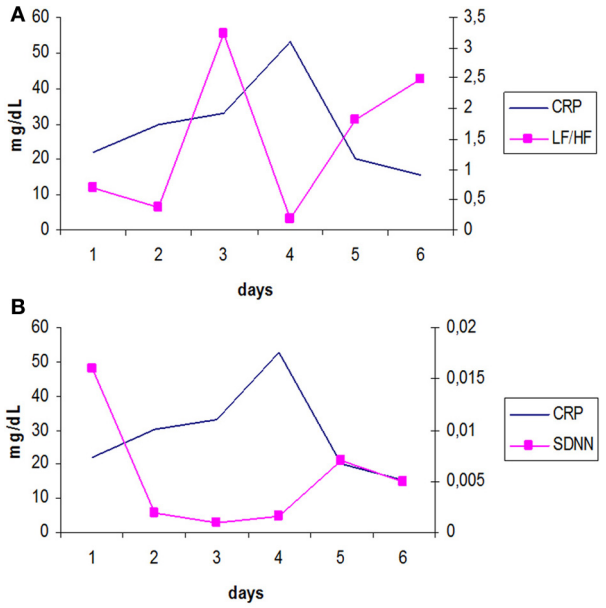

FIGURE 1 | (A) Longitudinal trends over time of mean values of CRP and LF/HF ratio, reflecting sympathovagal balance, for patients with SOFA > 10, during the 6 days of study period. [log transformed data, adapted from Papaioannou et al. (2009)]. It appears that LF/HF changes inversely with CRP. **(B)** Longitudinal trends over time of mean values of CRP and SDNN (secs), for patients with SOFA > 10, during the 6 days of study period. [log transformed data, adapted from Papaioannou et al. (2009)]. There is a progressive increase in SOFA score from day 1 until day 4 (development of septic shock) and a subsequent downward shift in its values. At the same time, the variability of heart rate signals estimated with SDNN seems to be significantly reduced during the development of septic shock.

could be responsible for our results since IL-6 can behave as both a pro-inflammatory activator (induces the production of CRP) and inhibitor (limits Tumor necrosis factor α and IL-1β secretion), at the same time (Chrousos, 1995).

These findings suggest that reduction in HRV and LF/HF is related with an augmented pro- and anti-inflammatory response during sepsis, especially in more severely ill patients. Furthermore, severity of illness is positively associated with HF and IL-10 serum concentrations and changes inversely with variability of heart rate signals. In this respect, elevated levels of IL-10 have also been found in trauma patients who developed sepsis and multiorgan failure (Sherry et al., 1996).

In conclusion, it seems that critical illness and high cytokine levels are associated with reduced HRV, however, existing literature does not elucidate whether loss of HRV is related to an endotoxin effect at the level of ANS output, baroreflex sensitivity or the pacemaker cell itself. However, results from a prospective study in a group of 40 healthy adults who received a single intravenous bolus of 2 ng/kg LPS, suggested that there is no relationship between basal cardiac ANS activity, and the inflammatory response (Kox et al., 2011). Thus, no association was found between frequency components of HRV that were determined hourly and until 8 h after LPS administration and different pro- and anti-inflammatory cytokines, measured at various time points. According to the authors, vagus nerve innervation of the heart does not reflect outflow to other organs, such as the spleen, one of the major cytokine-producing organs (Tracey, 2002, 2007). As basal vagal input to the spleen may be different from vagal input to the heart, HRV might not be an appropriate method to assess activation of the cholinergic anti-inflammatory pathway (Kox et al., 2011). These findings strengthen the notion that autonomic outflow cannot be regarded as a general response, but appears to be organ-specific. In this respect, results from different studies discussed so far lack generalization and robustness due to different study populations and design, interspecies differences and potential impact of severity of disease, sedation or mechanical ventilation upon HRV (Goldstein and Buchman, 1998). However, it appears that an inverse association between inflammation and total variability of heart rate signals could be found in the most severe cases.

POTENTIAL THERAPEUTIC IMPLICATIONS

Different clinical trials have shown that fatty acids from fish oil can be considered as powerful disease-modifying nutrients in patients with acute lung injury, sepsis and cardiovascular diseases (Christensen et al., 1999; Abuissa et al., 2005; Pontes-Arruda et al., 2006; Singer et al., 2006). Particularly, feeding with the very-long chain, ω-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA) has been found to inhibit the activity of the pro-inflammatory transcription factor nuclear factor kB (NF-kB) and subsequently, to attenuate the production of different cytokines, chemokines and other effectors of innate immune response (Singer et al., 2008). In the cardiovascular literature, it has been shown that oral supplementation of ω-3 PUFAs increase instantaneous HRV, reduce LF/HF ratio and confer protection against ischemia-induced ventricular tachycardia and sudden cardiac death (Abuissa et al., 2005). Moreover, Christensen (Christensen et al., 1999) demonstrated that fish oil feeding can induce an incorporation of DHA into the membranes of granulocytes, which is associated with a dose-response increase in HRV (SDNN), something that may protect against serious ventricular arrhythmias. Such effects of fish oil reflect an enhanced efferent vagal activity via a central-acting mechanism, due to a possible suppression of pro-inflammatory cytokines that have been found to inhibit central vagal neurons (Singer et al., 2008).

Never-the-less, different interventional studies on ω-3 PUFAs and HRV in patients with heart disease have found inconsistent results, with only 8 out of the 20 trials published so far, supporting a beneficial effect on HRV (Christensen, 2011). Indeed, Mozaffarian et al. (2008) reported that individuals with the highest fish consumption (≥5 meals/week) only exhibited 1.5 ms greater HRV compared to those with the lowest fish consumption and further that this modest reduction in HRV was associated with only a 1.1% reduction in the relative risk for sudden cardiac death. Reasons for such inconsistency might include heterogeneous populations, limited sample sizes or different study protocols with variable administered doses of ω-3 PUFA and length of intervention. Furthermore, different methods of measurement of HRV with variable time of recordings could be an additional confounder. Finally, an animal study with administration of ω-3 PUFAs in rabbits showed that a reduction in pacemaker funny

current rather than an alteration in autonomic neural regulation was responsible for heart rate reduction and increase in HRV (Verkerk et al., 2009). However, such experiments were performed in denervated hearts, excluding a potential impact of autonomic tone on HRV. More recently, the HRV response to physiological challenges was not altered by dietary ω-3 fatty acids in conscious intact preparations; data that further suggest that these lipids elicited alterations in pacemaker rate rather than cardiac autonomic regulation (Billman and Harris, 2011; Billman, 2012).

Recent evidence suggests that HMG-CoA reductase inhibitors (statins) have pleiotrophic mechanisms in patients with heart failure, such as ANS output modulation (Lefer, 2002). Experiments with animal models of heart failure have found decreased sympathetic activation and autonomic balance restoration with statins, using HRV analysis (Pliquett et al., 2002). In a cross-over study of HRV in 30 patients with hyperlipidemia (Welzig et al., 2003), pravastatin administration induced a significant increase in HF power of ECG signals, whereas others (Vrtovec et al., 2005) found that administration of 10 mg of atorvastatin for 3 months, was associated with significant increase in SDNN and RMSSD, in 80 patients with CHF and hyperlipidemia. Moreover, cholesterol lowering was not correlated with HRV changes, suggesting another mechanism than that of lipid-lowering of statins. In this context, Gao et al. (2005) showed that in experimental heart failure states there is intense free radical production and up-regulation of angiotensin receptors, in autonomic areas of the brain. Moreover, simvastatin therapy was proven to inhibit angiotensin II and superoxide pathways in the RVLM of pacing-induced heart failure rabbits, leading to an abolished renal sympathetic nerve activity (Gao et al., 2005).

Different experimental studies have shown that catecholamines, except from increasing cardiac contractility and heart rate via interaction with beta adreno-receptors, may induce myocardial damage by calcium overload and subsequent cell necrosis, upon excessive β-adrenoreceptor stimulation (Opie et al., 1985; Mann et al., 1992). Although, toxic cardiac effects of catecholamines have been recognized since 1907, Rona et al. (1959) was the first who observed that isoproterenol injection into rats produced "infarct-like" myocardial necrosis, in the absence of coronary artery lesions. He proposed the theory of "relative hypoxia" as a pathophysiological mechanism, suggesting a possible imbalance between oxygen demand and blood flow, after excessive adrenergic stimulation. Fleckenstein (1971) thought that calcium overload was the result of catecholamine-mediated cell injury, due to extensive activation of Ca-dependent ATPases and subsequent high energy phosphate deficiency, leading to mitochondrial impairment. On the contrary, Opie and co-workers (1985) were the first who demonstrated that catecholamine cell injury was due to calcium overload, mediated by the β-adrenoreceptor. More recent studies have confirmed previous results and have also demonstrated that other mechanisms could be responsible as well, for catecholamine-induced cell injury, such as increased fibrosis of the left ventricle with associated hypertrophy (Briest et al., 2001) or myocardial cellular apoptosis (Commural et al., 1998).

Since heart failure is associated with a sympathetic up-regulation and parasympathetic withdrawal, β blockers have been used to modify the effects of augmented sympathetic tone and restore autonomic imbalance. In this respect, Goldsmith et al. (1997) showed that administration of carvedilol for 4 months was associated with a significant increase in HF power, in patients with CHF under digoxin and angiotensin-converting enzyme (ACE) inhibitors. Similar results were found in post-MI subjects by Lampert et al. (2003), after treatment with propranolol for 6 weeks. An elevation in HF, which reflects restoration of sympatho-vagal balance, was found to increase final outcome (LVEF, exercise capacity, death and CHF development) in both studies.

Recent evidence suggests that a primary site of attenuated vagal control on the heart occurs at the level of the parasympathetic gaglion (Bibevski and Dunlap, 2011). Thus, cervical vagus nerve stimulation (VNS) has been recently assessed as an "add-on" therapy to optimal medical management of CHF. Li and colleagues (2004) were the first who found that VNS performed for 10 s every minute, in rats developed HF after anterior myocardial infarction, improved significantly left ventricular function and decreased mortality from 50 to 14%, in comparison with sham treated animals. Zhang et al. (2009) recently demonstrated in a canine model with high rate ventricular pacing induced HF, that VNS over 12 weeks was able to reduce left ventricular end systolic and end-diastolic volumes and increase LVEF significantly. In addition, HRV was significantly improved in VNS dogs whereas plasma norepinephrine and CRP levels were markedly attenuated with VNS treatment. Finally, vagal stimulation has also been found to limit infarct size and inflammatory response to myocardial ischemia and reperfusion in male rats that underwent myocardial ischemia for 30 min and reperfusion for 24 h (Calvillo et al., 2011). According to the authors, the anti-inflammatory and anti-apoptotic properties of the nicotinic pathway were the primary underlying mechanism in the VNS-treated animals.

Based on the results of VNS in animal models of HF, Schwartz et al. (2008) and De Ferrari et al. (2011) were the first who assessed feasibility and safety and tested possible efficacy of chronic VNS in HF patients with New York Heart Association (NYHA) class II-IV symptoms. In a two-staged study, (8-patients feasibility phase plus 24-patients safety and tolerability phase) they used CardioFit, a right cervical VNS implantable system delivering pulses synchronous with heart beats through a multiple contact bipolar cuff electrode. VNS was started 2–4 weeks after implant and patients were followed 1, 3, and 6 months thereafter. VNS was well tolerated whereas, there was a significant improvement in NYHA class and left ventricular end-systolic volume. Moreover, a significant increase in HRV, estimated with pNN50 and slightly but significantly reduced heart rate were found 6 months after VNS onset (Schwartz et al., 2008; De Ferrari et al., 2011).

As a consequence of such preliminary results, a pivotal multicenter international clinical trial, the INOVATE-HF study, has been recently designed to assess safety and efficacy of VNS in 650 CHF patients from 80 sites, with NYHA class III symptoms, sinus rhythm and QRS width less than 120 ms, using the CardioFit system (Hauptman et al., 2012). Thus, in case of

significant decrease in mortality, vagal stimulation will add significant value to current medical therapy in a narrow spectrum of patients with heart failure, through restoration of sympatho-vagal balance. Never-the-less, a lot of questions remain to be addressed, such as optimal stimulation mode (i.e., right vs. left vagus nerve stimulation, continuous vs. pulse-synchronous stimulation etc) or effective and tolerable VNS dose, before adopting this new non-pharmacologic treatment to our therapeutic armamentarium.

CONCLUSIONS AND FUTURE SUGGESTIONS

Different experimental studies have established an inter-relation between ANS output and inflammatory regulation, whereas the discovery of the "cholinergic anti-inflammatory pathway" has expanded our understanding of how the nervous system modulates the inflammatory response through an immunoreflex. Furthermore, clinical data from large epidemiological studies involving patients with CAD, heart failure and healthy subjects with increased risk factors for heart diseases suggest that there is a rather weak or moderate association between inflammation and ANS activity, estimated through HRV analysis. Finally, investigation of HRV alterations during critical illness, such as sepsis and MODS, has demonstrated loss of variability of heart rate signals that is inversely correlated with immune response, particularly in most severe cases.

Early and more accurate monitoring of cardiovascular patients, particularly in the early stages of life-threatening illnesses through continuous automated detection of abnormal variability of heart rate signals, could alert clinicians to impending clinical deterioration and allow earlier intervention. In this respect, the combination of structural indices, such as the left ventricular ejection fraction, with autonomic function indices derived from heart rate variability analysis has been proposed as the state-of-the-art method for risk assessment among patients with acute myocardial infarction or severe congestive heart failure (Priori et al., 2001). However, in-consistent findings from different studies assessing the relationship between HRV frequency components and inflammation limit adoption of HRV analysis as an indirect estimator of inflammatory response, since there is a marked heterogeneity in study protocols, time and methods of HRV measurement and patients' characteristics. Moreover, HRV analysis does not simply reflect sympathetic/parasympathetic balance, since HRV data can be influenced by artifacts related to differences in breathing characteristics, genetic factors or basal autonomic tone. Thus, any change in LF/HF ratio may correspond to central, baroreflex or cellular membrane effects of different stimuli during severe stress. Furthermore, sympathetic outflow can either induce or inhibit inflammatory activity, whereas HF component might fail to reflect vagal inputs upon different organs, such as the spleen, which are major cytokine producers. In addition, the explanatory power of HRV analysis is affected by circadian variability as well as by the estimation of inflammatory activity through biomarkers' measurements from a single blood sample (Haensel et al., 2008). Finally, lack of signals' stationarity (stable statistical properties) during measurements and the strong association between HRV data and inflammation that has been demonstrated among the most severely ill patients could reflect a non-linear relationship between ANS and inflammatory response. Thus, it has been suggested that newer methods derived from chaos theory should be implemented to assess ANS output, such as fractal and detrended fluctuation analysis (DFA) of heart rate signals (Goldberger, 1996). In addition, standardization of experimental protocols and methods used for the estimation of HRV is urgently needed, in order to allow comparisons among different studies.

In conclusion, the enormous complexity of neuro-immunological interactions cannot be captured by simple measurements, such as HRV analysis. A rather multi-parameter monitoring of ANS, through different estimators of heart rate variability and complexity has been suggested for assessment of ANS output (Goldberger, 1996). In any case and since HRV metrics are not enough for differentiating between pathophysiological states (poor specificity) or between patients (poor sensitivity), longitu-dinal alterations over time of both HRV and inflammatory markers on an intra-individual basis must be tested for establishing a potential added value of HRV analysis, as an indirect estimator of inflammatory response (Papaioannou et al., 2009).

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Heart rate variability in normal and pathological sleep

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Sleep is a physiological process involving different biological systems, from molecular to organ level; its integrity is essential for maintaining health and homeostasis in human beings. Although in the past sleep has been considered a state of quiet, experimental and clinical evidences suggest a noteworthy activation of different biological systems during sleep. A key role is played by the autonomic nervous system (ANS), whose modulation regulates cardiovascular functions during sleep onset and different sleep stages. Therefore, an interest on the evaluation of autonomic cardiovascular control in health and disease is growing by means of linear and non-linear heart rate variability (HRV) analyses. The application of classical tools for ANS analysis, such as HRV during physiological sleep, showed that the rapid eye movement (REM) stage is characterized by a likely sympathetic predominance associated with a vagal withdrawal, while the opposite trend is observed during non-REM sleep. More recently, the use of non-linear tools, such as entropy-derived indices, have provided new insight on the cardiac autonomic regulation, revealing for instance changes in the cardiovascular complexity during REM sleep, supporting the hypothesis of a reduced capability of the cardiovascular system to deal with stress challenges. Interestingly, different HRV tools have been applied to characterize autonomic cardiac control in different pathological conditions, from neurological sleep disorders to sleep disordered breathing (SDB). In summary, linear and non-linear analysis of HRV are reliable approaches to assess changes of autonomic cardiac modulation during sleep both in health and diseases. The use of these tools could provide important information of clinical and prognostic relevance.

Keywords: autonomic nervous system, heart rate variability, sleep, non-linear analysis, obstructive sleep apnea, insomnia, SUDEP

"But what interests me here is the specific mystery of sleep partaken of or itself alone, the inevitable plunge risked each night by the naked man, solitary and unarmed, into an ocean where everything changes, the colors, the densities, and even the rhythm of breathing, and where we meet the dead. What reassures us about sleep is that we do come out of it, and come out of it unchanged, since some mysterious ban keeps us from bringing back with us in their true form even the remnants of our dreams"

Memories of Adrian, Marguerite Yourcenar, 1951

INTRODUCTION

The simple observation that human beings, mammals and other animal species spend about one third or more of their lifetime sleeping strongly suggest how fundamental the physiological process of sleeping is (Chou et al., 2003; Saper et al., 2005).

Despite the fact that a large amount of data has been published on the biological meaning and function of sleep, several key points still need to be clarified.

The sleep process is characterized by the activation of a number of cortical, subcortical and medullar neural circuits, which cooperate in order to control sleep according to hormonal changes (i.e., melatonin and orexin), local factors such as adenosine accumulation, circadian variations (i.e., dark-light cycles) and other unknown factors (Saper et al., 2005). A key role in the physiology of sleep is played by the autonomic nervous system (ANS), whose regulation modulates cardiovascular functions during sleep onset and the transition to different sleep stages.

The analysis of heart rate variability (HRV) has been widely used as a non-invasive and reliable tool to evaluate cardiovascular autonomic control in health and disease. The application

Abbreviations: AHI, apnea/hypopnea index; ANS, autonomic nervous system; BP, blood pressure; BRS, baroreflex sensitivity; CAP, cyclic alternating pattern; CCE, corrected conditional entropy; CE, conditional entropy; CPAP, continuous positive airway pressure; EEG, electroencephalogram; HF, high frequency; HR, heart rate; HRV, heart rate variability; LF, low frequency; MSNA, muscle sympathetic nerve activity; NCAP, non-CAP; NREM, non-REM; NU, normalized units; OSA, obstructive sleep apnea; PI, primary insomnia; REM, rapid eye movement; Ro, regularity index; SDB, sleep disordered-breathing; SE, Shannon entropy; SUDEP, sudden unexpected death in epilepsy; SWS, slow wave sleep; VLF, very low frequency; VLPO, ventrolateral preoptic nucleus.

of different tools, such as linear and non-linear analysis of HRV during different sleep stages provided fundamental insight on the physiological autonomic changes that characterize wake-to-sleep transition, sleep onset, and different sleep stages [rapid eye movement (REM) and non-REM sleep (NREM)]. In addition, different HRV tools revealed important modifications of autonomic cardiac control in different pathological conditions, such as insomnia, primary neurological sleep disorders, and sleep disordered breathing (SDB), a group of diseases associated with an alteration in the normal breathing during sleep (Parish and Somers, 2004; Nobili et al., 2011).

The present review will focus on the fundamental principles of sleep structure, the most used linear and non-linear analyses of HRV and the application of these tools to assess the autonomic cardiac control in normal and pathological sleep.

WAKEFULNESS-SLEEP TRANSITIONS

Transition from wake to sleep is relatively rapid, considering that sleep onset is a process which lasts no more than one minute in human beings (Takahashi et al., 2010).

Interestingly, the switch from wake to sleep and among the different sleep stages is not monodirectional (i.e., from NREM, to REM), but, on the contrary, it oscillates from NREM to REM and vice versa, together with fast changes in electroencephalographic (EEG) cerebral waves and the occurrence of arousals (Saper et al., 2010).

The neural regulation of this process is very complex, counting several neural networks of neurons located both in the cortex and in the medulla. A key regulatory mechanism in the wake to sleep shift is the relation between hypothalamic and monoaminergic neurons. In fact, it has been shown that monoaminergic neurons in the pons project to noradrenergic neurons in the locus coeruleus and to dopaminergic and serotoninergic relay stations in the raphe (Saper et al., 2010). Wake period is characterized by firing activity of these monoaminergic neurons, which inhibit ventrolateral preoptic nucleus (VLPO) and neurons which regulate the transition to REM sleep; this mechanism is capable of limiting a direct transition from wake to REM sleep. On the contrary, sleep is characterized by an increased activity of VLPO neurons, which, in turn, inhibits monoaminergic neurons, and, consequentially, triggers the sleep onset. This regulatory mechanism is only one of the most important neural network involved in the wakefulness—sleep switch, and it is called the flip-flop switch model (Saper et al., 2010).

The full description of the neural networks regulating the shift from NREM to REM sleep and vice versa is out of the scope of this review.

SLEEP STRUCTURE

Sleep macrostructure is characterized by two separate physiological stages, REM sleep and NREM sleep.

REM sleep, identified in 1953, is a physiological state that includes REM and low muscle tone. On the opposite, NREM sleep is usually divided into two states, light sleep (NREM 1 and NREM2, or N1 and N2) and deep sleep (NREM3, or N3, also called Slow Wave Sleep, SWS); during NREM sleep rapid eyes movements are absent while muscle tone is usually considerable. REM and NREM sleep are characterized by typical EEG features.

In fact, during wakefulness with closed eyes, on the EEG a typical rhythm, the alpha rhythm, becomes evident. Alpha rhythm is characterized by low voltage and high frequency waves, with a frequency band bounded between 8 and 12 Hz.

During doziness and sleep onset, cortical rhythm slows, alpha rhythm disappears and theta rhythm appears alongside the alpha; lower frequency (3.5–7.5 Hz) and higher voltage waves create theta rhythm; alpha disappears as sleep becomes deeper.

During N2, alpha rhythm completely disappears and theta rhythm is the dominant EEG oscillation. In addition, two peculiar EEG elements become evident at this time: spindles and K complexes. Spindles derive from bursts of brain activity and are characterized by high frequency waveform (12–14 Hz) lasting more than 0.5 s, while waves with a first negative-high voltage peak followed by slow positive complex and a second negative peak are called K complexes. Their rate of occurrence is roughly every 1–2 min.

The transition from N2 to N3 is associated with the appearance of delta waves, which have high voltage (greater than 75 μV) and low frequency (bounded between 0.5 and 3 Hz). N3 is the sleep stage of highest synchronization of neural activity in the brain, with low muscle activity and no REMs. From N3 to REM, delta waves disappear and high frequency and low voltage waves become predominant; complete muscle atonia and REMs are observed.

HEART RATE VARIABILITY AS A WINDOW OVER AUTONOMIC CARDIOVASCULAR CONTROL

The two branches of the ANS, sympathetic and parasympathetic nervous system, regulate visceral functions in order to maintain the homeostatic milieu of the body and to render the body able to react and to adapt to external and internal stressor stimuli (Malliani et al., 1991; Montano et al., 2009) (see **Figure 1**).

A very composite and interconnected regulating mechanisms operate at different levels, both central and peripheral, in order to coordinate ANS functions (Montano et al., 2009).

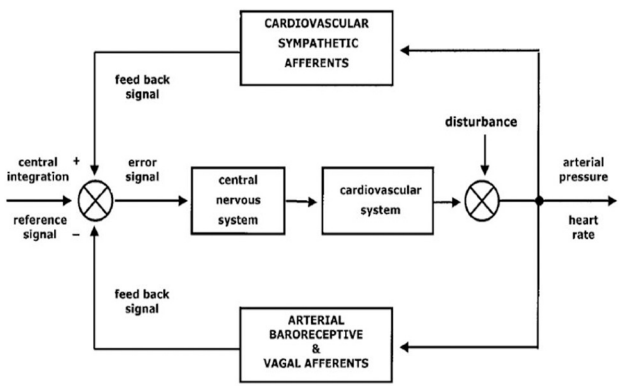

FIGURE 1 | A schematic representation of autonomic nervous system functions and its integration with internal and external stressor stimuli (from Montano et al., 2009; with permission).

Within physiological conditions, the regulation of several fundamental visceral functions, such as cardiovascular, respiratory, and gastrointestinal systems, is based on the reciprocal activation of the two autonomic subsystems, the so-called "sympatho-vagal balance": the activation of one branch, i.e., sympathetic outflow, is associated with a withdrawal of the other, i.e., parasympathetic drive, and vice versa (Malliani et al., 1998). This mechanism has been considered a key stone paradigm of the ANS function for many years; however, it has been suggested that the coactivation of both sympathetic and parasympathetic systems is not only physiologically possible, but it is also a rule in peculiar situations such as chemoreceptor reflexes (i.e., during apneas), exercise, and cold face immersion (Koizumi et al., 1982; Malliani and Montano, 2002; Paton et al., 2005).

These brief observations suggest the high degree of complexity of the ANS regulation, mainly due to the strong interconnection with several biological systems (central and peripheral nervous systems, immunity, inflammation, metabolism, hormones etc.) and to its multifaceted mechanisms of action of sympathetic and parasympathetic limbs (i.e., as antagonists or agonists).

For many years, several techniques have been developed for the assessment of ANS: (a) dosage of plasmatic and urinary catecholamines (Goldstein et al., 1983), which is nowadays not considered as a highly reliable index of sympathetic activity (Esler, 1993; Montano et al., 2009), (b) muscle sympathetic nerve activity (MSNA), a direct but invasive recording of sympathetic activity using a microneurography technique (Wallin and Charkoudian, 2007), (c) analysis of HRV, a non-invasive tool able to provide reliable information on sympathetic and parasympathetic oscillations of the heart period and arterial pressure time series, (d) more recent non-linear approaches based on entropy-derived measures and symbolic analysis of heart period time series (Porta et al., 2007a,b; Tobaldini et al., 2009).

LINEAR ANALYSIS OF HRV

A pioneering study by Lee and Hon first described significant changes in beat-to-beat intervals during fetal distress before evident changes in heart rate (HR) (Lee and Hon, 1965). During the next years, several evidences supported the hypothesis that rhythmical oscillations of both HR and blood pressure (BP) are indirect measures of sympathetic and parasympathetic modulation (Pagani et al., 1986; Malliani and Montano, 2002).

Therefore, HRV has been considered a non-invasive and reliable tool able to provide information on the sympathetic and parasympathetic modulation both in physiological and pathological conditions (Malliani et al., 1998; Montano et al., 2009). It is worth noting that HRV has been widely accepted not only as a tool to assess physiological autonomic functions, but also as a method able to provide important clinical information. Pioneering studies showed that, in post-myocardial infarction patients, total HRV was an independent predictor of mortality (Kleiger, 1987; Malik, 1989; Fei et al., 1996).

As stated above, HRV is based on the assessment of rhythmical oscillations embedded in heart period and blood pressure time series, which represent the sympathetic and parasympathetic modulations of cardiovascular function. Several computational methods have been validated, classically divided into non parametric (based on a simple algorithm, usually a fast Fourier transform) and parametric tools, such as autoregressive algorithm, in which spectral components are identified independently of preselected frequencies (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

On the heart period and blood pressure time series, three main components can be recognized: a very low frequency component (VLF), frequency band below 0.04 Hz; a low frequency component (LF), bounded between 0.04–0.15 Hz, and a high frequency component (HF, bounded between 0.15 and 0.4 Hz), synchronous with respiration. The VLF component is considered to be a marker of humoral and hormonal fluctuations; the LF component is discussed to represent a marker of both sympathetic and parasympathetic modulation while the HF component is considered a marker of vagal modulation.

In addition to the frequency band, each oscillation can be described in terms of amplitude, which can be expressed both in absolute (ms^2 or $mmHg^2$ for heart period and blood pressure time series, respectively) and in normalized units (nu). LFnu and HFnu represent the relative value of LF or HF with respect to the total variability, minus the VLF component (Malliani et al., 1991, 1998; Montano et al., 2009; Malliani and Montano, 2002).

It is worth noting that while HF oscillation is commonly accepted as a marker of parasympathetic modulation, the physiological meaning of the LF band is still debated. In fact, some authors have suggested that LF can be the result of sympathetic and parasympathetic modulation (Berntson et al., 1997; Eckberg, 1997; Billman, 2011, 2013), while, on the contrary, other experimental and clinical data support the hypothesis that LF is a marker of sympathetic modulation (Malliani et al., 1991, 1998; Montano et al., 2009).

NON-LINEAR ANALYSIS OF HRV

Classic power spectral analysis of HRV is based on the assumption that heart period time series contain only linear and stationary dynamics; however, in the last years, increasing interest has been paid to non-linear dynamics that characterize autonomic cardiovascular control (Goldberger et al., 1988; Kaplan et al., 1991; Voss et al., 1995). Interestingly, it has been demonstrated that some non-linear parameters are better predictors of morbidity and mortality than standard linear spectral parameters in cardiac patients (Mäkikallio et al., 2001). Although several non-linear methods have been developed, in the present review we will briefly present entropy-derived measures, which have been recently applied for the assessment of autonomic cardiovascular complexity in physiological and pathological sleep.

Entropy-derived measures

Physiologically, biological variables are controlled by the interaction of several systems, which actively interact with each other as agonists or antagonists at different time scales. In this perspective, beat-to-beat regulation is under the influence of sympatho-vagal balance, central oscillators, reflexes circuits such as baroreflex and chemoreflex control, sympatho-sympathetic reflexes, molecular, and hormonal regulation. All these mechanisms are responsible for HRV complexity (Goldberger et al., 1988; Kaplan et al., 1991).

During aging and pathological situations, one of these mechanisms may become predominant, with a decrease or an inhibition of the others, thus leading to a decrease of complexity of HRV and to a simplification of cardiovascular regulation. If this is the case, plasticity of cardiovascular control is strongly impaired and the ability of the system to respond to internal and external stressor stimuli is significantly damaged. Interestingly, some complexity indices are powerful predictors of mortality in high-risk patients (Voss et al., 1996; Huikuri et al., 2003; Clariá et al., 2008). Complexity is measured by evaluating the amount of information carried by a biological series, based on entropy-derived non-linear indices: the larger is the information, the greater is the complexity (Porta et al., 2001).

Entropy-derived indices, such as approximate entropy, sample entropy, corrected conditional entropy (CCE) and Shannon entropy (SE) have been proposed (Porta et al., 2001). Although a detailed description of the mathematical basis of these measures is beyond the scope of this review, we will briefly describe the key aspects of SE, Conditional Entropy (CE), and CCE, which have been applied to provide information on cardiac complexity in normal and pathological sleep.

Shannon entropy (SE)

SE evaluates the complexity of patterns distribution of length L, $RR_L = \{RR_L(i) = (RR(i), RR(i-1), \ldots, RR(i\text{-}L+1)), i = 1, \ldots, N\text{-}L+1)\}$, by describing the shape of this distribution [SE(L) describes the shape of the distribution of RR_L]. When SE(L) is calculated with $L = 1$, it depends on the shape of the distribution of the heart period time series. When SE(L) is large, the pattern distribution is flat, meaning that all the patterns are equally distributed and the amount of information carried by the series is highest. On the opposite, when SE(L) is small, the pattern distribution is not flat but characterized by specific shapes (i.e., Gaussian or skewed distribution), suggesting that some patterns are more present while others are less present or absent (Porta et al., 2001).

Conditional entropy (CE) and corrected conditional entropy (CCE)

CE measures the quantity of information carried by the current RR sample when the previous samples are known. In other words, CE corresponds to the difficulty in predicting future values of RR intervals based on past values of the same series. CE is 0 when future values of RR are completely predictable given RR past values and it is equal to SE(1) when the knowledge of past values of RR is not helpful to reduce the uncertainty of future RR values. However, because the estimation of CE is biased (CE became unreliable as a function of L, decreasing very fast toward 0 with L independently of the ability of past values of RR to predict future RR samples), CCE was designed to overcome this mathematical limitation. CCE decreased to 0 when new sample is completely predictable, remained to the maximum value [i.e., SE(1)] when the new sample is fully unpredictable and showed a minimum when the knowledge of past values was helpful to reduce the uncertainty associated to future values (Porta et al., 2007a).

From CCE, it is possible to derive an index of regularity, Ro (obtained by dividing CCE by the Shannon entropy), which is bounded between 1 (maximum regularity, lowest complexity) to 0 (lowest regularity, maximum complexity).

HEART RATE VARIABILITY IN WAKE/SLEEP STATES

The interaction between ANS and sleep is complex, bidirectional and regulated by several different factors. In fact, changes in ANS regulation can profoundly affect sleep onset and sleep homeostasis and, on the opposite, modifications of physiological sleep can impinge upon autonomic cardiovascular regulation. It is well known that sleep is a complex phenomenon in which autonomic cardiac control fluctuates between sympathetic and parasympathetic predominance, mainly according to the transition to different sleep stages (wakefulness, NREM and REM). Somers and colleagues showed that the cardiovascular system is strongly affected by the sleep stage: in fact, from N1 to N3, the stage of highest neural synchronization, a gradual decrease is observed in HR, BP and MSNA, with minimum values reached during N3, also called "quiet sleep" (Somers et al., 1993). REM sleep, however, is characterized by an opposite behavior, with a sort of "activation" of cardiovascular system to levels sometimes higher than wakefulness. Thus, the transition from NREM to REM is accompanied by a significant increase of HR, BP, and MSNA, and, more interestingly, not stable but with continuous fluctuations of the cardiovascular system, suggesting that cardiovascular control is very complex and influenced by several factors during this sleep stage.

Interestingly, animal studies showed that during REM sleep, sympathetic outflows from different sources (i.e., renal and lumbar sympathetic outflows) may change independently from each other, supporting the hypothesis that a differentiate control of sympathetic outflows could be important for arterial pressure regulation (Yoshimoto et al., 2011).

So far, several studies investigating the change of autonomic cardiovascular control by means of HRV during wake and different sleep stages provided fundamental information regarding the relation between autonomic fluctuations and the shift to different sleep stages (Vanoli et al., 1995; Vaughn et al., 1995; Elsenbruch et al., 1999; Crasset et al., 2001; Trinder et al., 2001). According to the changes of HR, BP and MSNA, transition from wake to NREM sleep is associated with a gradual increase in parasympathetic modulation, expressed by an increased HF component and a decreased of LF component of HRV (Cajochen et al., 1994; Busek et al., 2005). Although the meaning of LF oscillation is someway still debated, the decrease of LF rhythm together with the changes in MSNA and BRS seem to suggest a global decrease of sympathetic modulation from wake to NREM sleep.

On the opposite, from NREM to REM sleep a significant reduction in total HRV together with a shift of sympatho-vagal balance toward a vagal withdrawal and a possible sympathetic predominance has been reported (Berlad et al., 1993; Baharav et al., 1995; Versace et al., 2003).

Beyond these general considerations, it is worth noting that several factors can influence autonomic control during sleep stages, such as the stage preceding the change and the sleep cycle time point. In fact, it has been suggested that the degree of increased sympathetic modulation during REM sleep depends on the previous sleep stage, with significantly higher values of LF/HF ratio during N2 preceding REM sleep compared to N2

preceding N3, supporting the hypothesis that autonomic control varies not only according to the sleep stage but also in relation to the preceding and following stages (Busek et al., 2005).

As to the sleep cycle, it has been reported that REM sleep at the end of the night is characterized by an increased sympathetic modulation compared to REM sleep that occurs during the first part of the night. From a clinical point of view, this fact can be a potential link between increased sympathetic drive, REM sleep and the incidence of cardiovascular events in the early morning (Muller et al., 1995; Scholz et al., 1997; Verrier and Josephson, 2009).

The evaluation of spontaneous baroreflex sensitivity (BRS) revealed important information on the cardiac and vascular interaction through baroreflex circuit during sleep. In fact, it has been observed that during REM sleep, BRS is higher compared to nocturnal wakefulness (Monti et al., 2002). During the first sleep cycle, BRS is higher during NREM compared to wakefulness and REM, while, during the last one, BRS is increased during REM compared to NREM, thus suggesting that the efficacy of baroreflex control in blunting REM sympathetic drive is higher during the late sleep cycles (Legramante et al., 2003). Interestingly, this phenomenon is even more evident in hypertensive patients, in which the blunted BRS and the increase of sympathetic modulation are greater at the end of the night, possibly implicating the occurrence of cardiovascular events (Drager et al., 2009).

In addition to classic HRV analysis, the application of non-linear entropy-derived measures revealed important changes of autonomic cardiovascular complexity peculiar for each sleep stage. However, due to the large amount of non-linear methods and experimental protocol differences, conclusive results are still lacking.

For instance, previous data showed that NREM sleep is characterized by an increased Sample Entropy while during REM sleep controversial results have been reported, from an increase of Approximate Entropy to a decrease of Sample Entropy (Virtanen et al., 2007; Vigo et al., 2010). A recent study by Viola and colleagues showed that CCE and SE were significantly lower during REM sleep compared to wake and NREM sleep (Viola et al., 2011), and this reduction was more evident in aged people. These data suggested the hypothesis that in aged people, REM sleep more than NREM sleep could be considered a stage of reduced complexity of cardiovascular control, thus, a stage of potential increased cardiovascular risk (see **Figure 2**).

However, it is worth noting that NREM sleep is not a stable phenomenon; indeed it is punctuated by the occurrence of arousals, which represent transient episodes of cortical and autonomic activation. Moreover, besides the conventional arousals, characterized by fast EEG frequencies (within the alpha, 8–12 Hz, and beta, >16 Hz range), other EEG phasic patterns, such as K-complexes and bursts of slow waves are associated with an activation of vegetative and somatomotor functions (Ferini-Strambi et al., 2000; Ferri et al., 2000; De Carli et al., 2004; Halász et al., 2004). Therefore, both fast and slow EEG arousal components represent a certain degree of cerebral activation reverberating upon the ongoing autonomic activity (Terzano et al., 1985; Parrino et al., 2012). During NREM sleep, arousal fluctuations appear with a pseudo-rhythmic modality, recurring about every

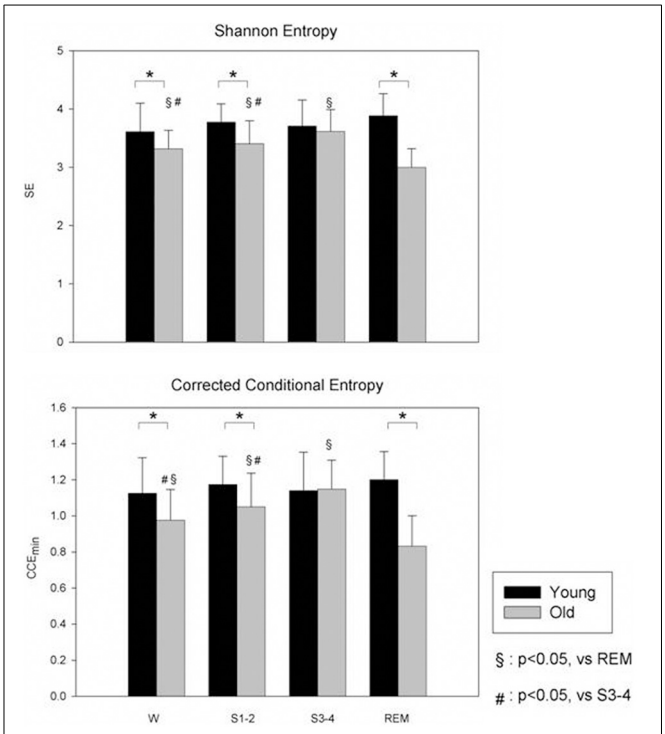

FIGURE 2 | Non-linear analysis of HRV during different sleep stages in young (black bars) and old (gray bars) subjects during wake (W) and different sleep stages. Compared to young, the old group have a lower SE. CCE is significantly reduced in old group compared to young and this reduction was more evident during REM sleep (from Viola et al., 2011; with permission). SE, Shannon Entropy; CCE, Corrected Conditional Entropy. $^{*}p < 0.05$, Young vs. Old.

20–40 s. This arousal rhythm, defined under the term of cyclic alternating pattern (CAP) (Terzano et al., 1985, 1988), represents a condition of sustained arousal instability.

CAP alternates with phases of stable sleep (non-CAP, NCAP), characterized by rare and randomly distributed arousal-related phasic events. Different studies have shown that CAP and NCAP are accompanied by significant changes of HRV parameters both in adults and children (Ferini-Strambi and Smirne, 1997; Ferini-Strambi et al., 2000; Ferri et al., 2000). In particular, during a CAP phase an increase in LF and LF/HF ratio has been observed, suggesting that during arousal instability the sympatho-vagal balance is shifted toward a sympathetic predominance (Ferini-Strambi and Smirne, 1997; Ferri et al., 2000). Moreover, the CAP-related increase in sympathetic modulation was higher than the mean value expressed by the corresponding sleep stage as a whole (Ferri et al., 2000). Interestingly, it has been demonstrated that BRS was higher during CAP than NCAP sleep, and it was similar during CAP and REM sleep (Iellamo et al., 2004). The analysis of the relationships between autonomic functions and CAP/NCAP phases could represent a more sensitive tool for the investigation of the effect of sleep disorders on HRV.

HRV AND SLEEP DISORDERS

In the last decades, a growing interest has been focused on sleep disorders, mainly due to their epidemiological and clinical

relevance and to their possible association with cardiovascular diseases. The International Classification of Sleep Disorders classified sleep disorders into several categories, such as (1) insomnias, (2) SDB, (3) hypersomnias, (4) parasomnias, and (5) sleep related movement disorders.

It is worth noting that most sleep disorders are characterized by important modifications of physiological sleep, and often by signs and symptoms of sleep loss, which have been demonstrated to be independent risk factors for cardiovascular morbidity and mortality (Wingard et al., 1982; Gallicchio and Kalesan, 2009; Cappuccio et al., 2010; Redline and Foody, 2011). It has been suggested that autonomic cardiovascular control could be importantly implicated as a potential physiopathological link between sleep disorders and their physiological consequences.

This section will briefly explore HRV changes in three pathological conditions, i.e., SDB, Insomnia, and Sudden Unexpected Death in Epilepsy, which are characterized by noteworthy alterations of cardiovascular autonomic regulation and increased cardiovascular risk.

HRV IN SLEEP DISORDERED BREATHING

SDB is a group of diseases characterized by significant alterations of breathing during sleep, which cause fragmentation of physiological sleep, symptoms of sleep deprivation and altered gas exchanges during the night. The most common SDB is Obstructive Sleep Apnea (OSA), which has a prevalence of 2–4% in middle age population.

OSA is associated with repetitive episodes of apneas during sleep, with partial or complete collapse of the upper airway during inspiration; thus, the increased resistance in the upper airways, caused by the collapse of the pharynx and hypopharynx muscles, lead to paradoxical thoracic and abdominal movements in order to overcome the airway obstruction. The frequent episodes of apneas have three main effects: hypoxia and hypercapnia due to gas exchange alterations, sleep fragmentation with repetitive arousal and, finally, irritability, daytime sleepiness and altered cognitive performance.

The clinical relevance of OSA is related to its strong association with obesity, hypertension, and increased cardiovascular risk (Kales et al., 1984; Bliwise et al., 1988; Hung et al., 1990; Somers et al., 1995; Narkiewicz and Somers, 2001). Although the pathophysiological factors linking OSA and cardiovascular risk are not completely understood, several evidences support the hypothesis that sleep fragmentation and intermittent hypoxia cause a chronic hyperactivation of the sympathetic nervous system, a key component of the progression to cardiovascular disease. In addition, endothelial dysfunction and activation of inflammatory cascade have been described in OSA patients, possibly mediated by the activation of the SNS.

OSA must be suspected in patients with snoring, obesity, resistant hypertension and in patients with signs and/or symptoms of sleep loss (fatigue, hypersomnolence, daytime sleepiness, cognitive impairment etc.). The diagnosis of OSA is confirmed in the presence of apneas (defined as a stop in airflow of at least 10 s) and hypopneas (reduction of 50% of the flow, with an oxygen desaturation of >4% and lasting at least 10 s). The number of apneic events is calculated as the apnea/hypopnea index (AHI), i.e., the total amount of apneas and hypopneas per hour of sleep. An AHI lower than five identifies a normal subjects, an AHI between 5 and 15 identifies mild OSA, an AHI between 15 and 30 a moderate OSA and an AHI greater than 30 a severe OSA (Somers et al., 1995).

The repetitive episodes of apneas have important hemodynamic and cardiovascular consequences, which result evident both during nighttime and daytime. Subjects with OSA usually have higher resting HR and BP (Vanninen et al., 1996). The autonomic consequences of OSA mainly involve chemoreflex and baroreflex regulation, with a global shift of the sympatho-vagal balance toward a sympathetic predominance and a blunted parasympathetic control (Narkiewicz et al., 1998), evident either during wake, and during night. Namely, during apneic events, the physiological inhibition of sympathetic activity by lung inflation is lacking, causing a significant sympathetic activation which is responsible for increased in BP and changes in HR. Sympathetic activity is highest at the end of an apnea, when hypoxia and hypercapnia reach their maximum levels; after the upper airways re-opening, it is possible to observe a large raise in blood pressure, which activates baroreflex control and induces a temporary withdrawal of sympathetic overactivity (Narkiewicz and Somers, 2001). The analysis of HRV revealed that during daytime, moderate to severe OSA patients were characterized by increased cardiac sympathetic modulation compared to mild-OSA and controls (Narkiewicz et al., 1998). Compared to controls, OSA subjects are characterized by a lower total variability and a possible shift of the sympatho-vagal balance toward a sympathetic predominance and a vagal withdrawal, as shown by the increase of LF component and LF/HF and the decrease of HF component (Narkiewicz and Somers, 2003; Smietanowski et al., 2006; Kesek et al., 2009) either during wakefulness and during nighttime (Shiomi et al., 1996; Vanninen et al., 1996).

It is important to underline a limitation of HRV application to sleep studies in OSA at this point. Indeed, the analysis of HRV during sleep is limited just by the presence of repetitive apneas, leg movements, or arousals, which artificially modify HRV analysis, introducing a "rhythmic" biological noise able to alter autonomic cardiovascular oscillations. This problem was highlighted by few studies that found that a higher AHI was associated with a higher vagal modulation during NREM sleep (da Silva et al., 2009) and, in contrast to the general expectations, in severe OSA patients, a decreased sympathetic regulation during REM sleep was observed (Gula et al., 2003). In fact, severe OSA induced an important modification in breathing pattern, which could have *per se* been a relevant confounding factor able to impinge upon HRV rhythmical oscillations. For this reason, cardiovascular autonomic assessment in severe OSA patients can importantly be affected by non-neural oscillations related to the continuous episodes of apnea which could thereby alter HRV analysis (Wang et al., 2008); this factor must be taken into account when analyzing PSG data and interpreting the results. To this regard, in a recent paper assessing HRV during sleep in patients with Brugada syndrome diagnosed with or without SDB, ECG recordings derived from polysomnographic studies were analyzed, carefully avoiding periods with apneas/hypopneas and considering only ECG segments associated with stable and regular

breathing (Tobaldini et al., 2013). This approach allowed the observation that Brugada syndrome, a rare but life- threatening disease characterized by ventricular arrhythmias and sudden cardiac death, more frequent during nighttime, was associated with an impaired autonomic cardiovascular control in the presence of comorbid SDB (Tobaldini et al., 2013).

The gold standard therapy for OSA is the application of continuous positive airway pressure (CPAP), a device able to maintain open the upper airways during sleep by inflating a positive pressure airflow, preventing the repetitive airway collapses as previously mentioned (Patel et al., 2003). CPAP therapy is able to improve cardiovascular outcome (Marin et al., 2005), with a significant reduction of arterial pressure (Faccenda et al., 2001), inflammatory markers, insulin resistance (Brooks et al., 1994), and coagulation factors (Phillips et al., 2012). As to autonomic effects of CPAP, a pioneer study by Somers and colleagues showed that CPAP is able to acutely affect ANS, with a significant decrease of MSNA during wake and sleep (Narkiewicz et al., 1999). Interestingly, even one night of CPAP treatment was able to affect HRV with a reduction of sympathetic modulation and an improvement of baroreflex control (Bonsignore et al., 2006; Kufoy et al., 2012). Longer CPAP treatments revealed positive effects on hemodynamic and metabolic variables, such as an improvement of arterial stiffness, a reduction of inflammatory response (Arias et al., 2008; Dorkova et al., 2008) and a decrease of platelet aggregation (Shimizu et al., 2002). However, the effects of longer CPAP treatments showed contrasting results and conclusive results on its consequences on HRV are still lacking. For instance, a reduction of the LF component and the LF/HF ratio, have been described, likely to be related to an improvement of chemoreflex and baroreflex responses (Roche et al., 1999; Khoo et al., 2001). Very recently, it has been described that long term CPAP (2 years treatment) is able to improve the coupling between parasympathetic modulation and delta wave sleep (Jurysta et al., 2013), suggesting a positive effect of this therapy on central and peripheral oscillations.

HRV IN INSOMNIA

Insomnia is a sleep disorder characterized by an important inability to fall asleep, to stay asleep or to wake up too early, causing daytime sleepiness, fatigue, mood alterations, and memory impairment. Insomnia is one of the most common sleep disorders and it can be classified into (1) comorbid insomnia, i.e., associated with other diseases, either physical and mental, (2) primary insomnia (PI), i.e., unrelated to any other disease, and (3) chronic (or long-standing) insomnia.

Apart from the relevant effects on day-life activity, the importance of insomnia as a potential modifiable risk factor for the development of cardiovascular diseases has been recently underlined (Spiegelhalder et al., 2010; Redline and Foody, 2011).

The assessment of ANS control in insomniac patients revealed interesting results. A pioneer study by Bonnet and colleagues showed a significant increase of the LF component and a decrease of the HF component of HRV in insomniacs compared to healthy subjects during sleep; these data suggested for the first time that insomnia is characterized by a predominant sympathetic modulation not only during wake but also across sleep stages (Bonnet and Arand, 1998).

These results have been confirmed by successive studies, which showed that PI patients exhibit a constant sympathetic overactivity during night (de Zambotti et al., 2013) and a marked reduction of vagal modulation, indicated by the decrease of the HF component of HRV during the night (Spiegelhalder et al., 2011; Yang et al., 2011).

These data support the hypothesis that in PI patients, sympatho-vagal balance is shifted toward a sympathetic predominance. Furthermore, the analysis of complexity indexes using entropy derived measures showed a considerable decrease in complexity during nighttime compared to healthy subjects, thus suggesting a possible link with cardiovascular diseases (Yang et al., 2011).

It is worth noting that PI patients do not only have an impaired autonomic cardiac modulation, but also an altered coupling between HRV and delta sleep. In fact, analyzing the relationship between the HF component and EEG delta power, PI patients showed a decreased HF-delta EEG coherence with respect to controls, thus suggesting a significant change in the interaction between central and peripheral drives (Jurysta et al., 2009).

In summary, insomnia is a very common sleep disorder, relevant for its prevalence over general population and for its clinical consequences. An altered autonomic cardiovascular control has been described in insomniac patients, who show a shift of the sympatho-vagal balance toward a predominance of sympathetic modulation both during wake and night; this alteration could be responsible for increased risk of cardiovascular diseases.

HRV, SLEEP, AND SUDDEN UNEXPECTED DEATH IN EPILEPSY (SUDEP)

Epilepsy is a brain disorder characterized by an enduring predisposition to generate seizures. Seizures are paroxysmal transient disturbances of brain functions that may be manifested as episodic impairment or loss of consciousness, abnormal motor phenomena and psychic or sensory disturbances. Moreover, seizures can induce a perturbation of the ANS; indeed, neurovegetative symptoms such as cardiovascular and respiratory changes, gastro-intestinal, cutaneous, and genito-urinary manifestations, frequently occur during epileptic seizures (Baumgartner et al., 2001; Leutmezer et al., 2003). Finally, it has been shown that also sub-clinical epileptic discharges may be associated with autonomic instability (Brotherstone and McLellan, 2012).

In epileptic patients, especially in those with pharmacoresistant epilepsy, the risk of sudden unexpected death (SUDEP) is 24–40 times higher with respect to the general population (Ficker et al., 1998; Mohanraj et al., 2006). SUDEP is considered to be the result of a peri-ictal concurrence of a number of predisposing and precipitating factors (Nobili et al., 2011); nevertheless SUDEP is primarily a sleep related phenomenon and sleep related seizures seem to be an independent risk factor for SUDEP (Lamberts et al., 2012). Previous studies have shown decreased HRV in chronic epileptic patients suggesting that this might play a role in the pathophysiology of SUDEP (Tomson et al., 1998; Ansakorpi et al., 2000; Ronkainen et al., 2005). There are many reports suggesting that the autonomic changes observed in epileptic patients are mainly evident during nocturnal sleep. Indeed, patients (both

adult and children) with focal epilepsy exhibit a higher reduction of HRV during night-time with respect to control subjects, indicating that the sleep period in epileptic patients might be more at risk of developing alterations of autonomic heart control (Ferri et al., 2002; Ronkainen et al., 2005; Persson et al., 2007). In a recent study, conducted in a population of drug resistant epileptic children with different clinical syndromes, authors found a striking reduction in vagal modulation during slow-wave sleep and a small autonomic modulation capacity (Jansen et al., 2011).

There is no definitive interpretation whether the observed autonomic changes in epileptic patients are due to the recurrence of seizures, the interictal epileptic discharges, and/or to the drug treatment. Seizures and periodic epileptic discharges, increasing arousal fluctuations during NREM sleep, might lead to a chronic stimulation of the ANS which are reflected by changes in HRV parameters. The relevant role of seizures and epileptic discharges on HRV change seems to be confirmed by the observation that, in drug resistant patients, HRV improves after epilepsy surgery especially in those with a positive outcome (Hilz et al., 2002; Dütsch et al., 2004; Persson et al., 2005). Also antiepileptic drugs could influence the autonomic state; in particular carbamazepine and polytherapy seem to reduce HRV (Persson et al., 2003; Yildiz et al., 2011). A recent systematic review of case-control studies, enrolling patients with different epilepsy syndromes and from infancy to adulthood, confirmed that epileptic patients present lower HF values, indicating impaired vagal control associated with increased cardiovascular risk and arrhythmias (Lotufo et al., 2012). Moreover a trend for lower LF ratios was identified in epileptic patients using pharmacotherapy.

In conclusion, the reduction of HRV that has been found in patients with epilepsy seems to be more pronounced during the night, thus affecting the circadian HRV. Further studies are needed to assess the possible association between altered HRV and risk of sudden death during sleep in epileptic patients.

CONCLUSIONS

In summary, sleep is a complex biological phenomenon regulated by different biological pathways. Cardiovascular autonomic control plays a key role, varying among the transition to different sleep stages. In addition, the sleep-autonomic link has to be considered bidirectional: in fact, autonomic changes can importantly alter sleep regulation and, on the other side, sleep disturbances can profoundly alter the physiological cardiac autonomic modulation. Nowadays, an increasing prevalence of sleep disorders such as SDB and neurological sleep related disturbances have been described. The assessment of autonomic cardiovascular control using classical linear and more recent non-linear analysis of HRV have been widely used as non-invasive tools to provide important information on autonomic changes in physiological and pathological sleep.

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Do physiological and pathological stresses produce different changes in heart rate variability?

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Although physiological (e.g., exercise) and pathological (e.g., infection) stress affecting the cardiovascular system have both been documented to be associated with a reduction in overall heart rate variability (HRV), it remains unclear if loss of HRV is ubiquitously similar across different domains of variability analysis or if distinct patterns of altered HRV exist depending on the stressor. Using Continuous Individualized Multiorgan Variability Analysis (CIMVA™) software, heart rate (HR) and four selected measures of variability were measured over time (windowed analysis) from two datasets, a set ($n = 13$) of patients who developed systemic infection (i.e., sepsis) after bone marrow transplant (BMT), and a matched set of healthy subjects undergoing physical exercise under controlled conditions. HR and the four HRV measures showed similar trends in both sepsis and exercise. The comparison through Wilcoxon sign-rank test of the levels of variability at baseline and during the stress (i.e., exercise or after days of sepsis development) showed similar changes, except for LF/HF, ratio of power at low (LF) and high (HF) frequencies (associated with sympathovagal modulation), which was affected by exercise but did not show any change during sepsis. Furthermore, HRV measures during sepsis showed a lower level of correlation with each other, as compared to HRV during exercise. In conclusion, this exploratory study highlights similar responses during both exercise and infection, with differences in terms of correlation and inter-subject fluctuations, whose physiologic significance merits further investigation.

Keywords: dimensions of variability, domains of variability, exercise, physical activity, disease, sepsis

INTRODUCTION

Several studies have shown that heart rate variability (HRV) can be used to characterize physiological (e.g., physical exercise, heat and cold stress), as well as pathological stress affecting the cardiovascular system. The utility of HRV in identifying illness states has been discussed in multiple reviews, and specific applications include the classification of heart failure, the prediction of mortality after myocardial infarction, and the estimation of autonomic modulation (Task Force, 1996; Seely and Macklem, 2004; Huikuri et al., 2009; Bravi et al., 2011). Similarly for physiological stress, measurement of HRV during physical exercise has been shown to estimate autonomic modulation, assess the level of fitness, characterize the beneficial effects of physical exercise, and many other applications (Perini and Veicsteinas, 2003; Lewis and Short, 2010; Routledge et al., 2010).

One of the major findings extracted from this collection of results is that when the cardiovascular system is under stress, either physiological or pathological, there is a decrease in variability. When pathological stresses are considered, a widespread view is to interpret this loss as a decomplexification of the cardiac system due to illness (Goldberger, 1996; Varela et al., 2010). In the domain of physiological exercise, however, a clear explanation for this phenomenon is lacking (Lewis and Short, 2010), nor is there a theory interpreting these two phenomena together. Developing a theoretical framework in this area of investigation might generate critical knowledge to understand the boundaries between health and disease, and allow knowledge translation from one domain to the other.

The first step to develop this framework consists of an extended comparison of how examples of pathological and physiological stresses affect HRV. With this in mind, the overall aim of this pilot investigation was to initiate, for the first time, a focused comparison of the patterns of change in HRV from a physiologic (i.e., exercise) and a pathologic (i.e., sepsis) stressor. The underlying hypothesis was that physiological and pathological stresses produce different effects on the body, and therefore their effects on HRV should differ. To test this hypothesis, we studied the changes in mean heart rate (HR), as well as four measures of variability belonging to different domains (Bravi et al., 2011), and associated to distinct physiological dimensions (Barrera-Ramirez

et al., 2013). Each domain of variability highlights the mathematical nature of a measure of variability, while the physiological dimensions are supposed to provide a physiological rationale to the changes in variability. Therefore, by investigating those four measures, we can shed light on the values of the two classification systems. In particular, we evaluated the trends (variation over time) of those measures from two datasets, one describing healthy subjects undergoing physical exercise under dry/warm conditions, the other ambulatory patients who developed sepsis after bone marrow transplant (BMT). Through analysis of the changes between baseline variability and variability during stress (i.e., either exercise or sepsis), as well as the analysis of the correlation between the measures, we highlighted similarities and differences between the two types of stressors.

MATERIALS AND METHODS

SUBJECTS

This study focuses on two datasets: (1) R-R interval (time between two successive R peaks during normal sinus rhythm) monitoring of 13 patients who developed systemic infection (i.e., sepsis) after BMT, and (2) R-R interval monitoring of 13 matched (by gender, age, weight, and height) healthy subjects undergoing controlled physical exercise under heat exposure, as described in more detail below. **Table 1** summarizes the subject characteristics.

SEPSIS

The first dataset (SEPSIS) consisted of patients undergoing BMT for hematological malignancy or other disorders. Inclusion criteria were treatment with myeloablative chemoradiotherapy followed by an allogeneic or autologous BMT. Exclusion criteria were pre-existing cardiopulmonary disease, taking beta-blockers or calcium-channel blockers, pre-existing arrhythmia (e.g., atrial fibrillation, atrial bigeminy), and contraindication to electrocardiogram adhesives (e.g., allergy, severe psoriasis). Sepsis was defined as the systemic inflammatory response syndrome along with a clinically suspected infection requiring treatment.

Table 1 | Demographic information.

	Dataset		*p*-value*
	EXERCISE (*n* = 13)	**SEPSIS (*n* = 13)**	
Gender			
• Male, *n* (%)	9 (69%)	9 (69%)	1
• Female, *n* (%)	4 (31%)	4 (31%)	
Age [years] – Median (95% CI)	50 (29 – 62)	49 (34 – 60)	0.83
Height [cm] – Median (95% CI)	169 (166 – 178)	173 (162 – 178)	0.97
Weight [kg] – Median (95% CI)	75 (62 – 90)	76 (63 – 105)	0.79
VO_2max [ml/kg/min] – Median (95% CI)	38.2 (31.5 – 43.2)	Not available	–

*CI, Confidence Interval; * Wilcoxon rank-sum test was used to compare the medians, χ^2 test was used to compare the proportions.*

Over 50% of the patients were diagnosed with sepsis based on the presence of fever, defined a priori as one recording greater than 38.5°C or two recordings greater than 38.0°C within 12 h. The remaining diagnoses were based on clinical suspicion, bacteremia, productive cough, and mucositis. For further details refer to (Ahmad et al., 2009; Bravi et al., 2012). The dataset consisted of 17 subjects: 3 who did not develop sepsis and showed an increase in HRV, 13 who developed sepsis and showed a reduction in HRV, and one insulin dependent diabetic subject who developed sepsis but showed an increase in HRV during the development of sepsis. To enable the comparison with the reduction in HRV during exercise, in this study we focused only on the 13 subjects who developed sepsis and showed a reduction in HRV. The average length of a recording was 12 days. A Zymed DigiTrak-Plus (Philips Healthcare, Canada) Holter system was used to record the R-R interval time series of each subject. Written informed consent was obtained from all participants, and the Ottawa Hospital Research Ethics Board authorized the study.

EXERCISE

The second dataset (EXERCISE) included healthy normally active (i.e., untrained but not sedentary) volunteers performing intermittent exercise in warm/dry conditions. We selected 13 subjects from a pool of 73, to match the septic patients. The matching was done prior to any data analysis. The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board. Prior to the experimental session, participants were asked to complete a Physical Activity Readiness Questionnaire (PAR-Q) to assess their eligibility to do physical activity. Maximal oxygen uptake (VO_{2max}) was subsequently measured during a progressive cycle ergometer protocol which consisted of a 2-min warm-up at 40 W followed by 20 W increments every minute until the participant could no longer maintain a pedaling cadence of at least 60 rpm.

The experiments were performed at the same time of day. Participants were asked to arrive at the laboratory after eating a small breakfast and to refrain from consuming alcohol and caffeine for 24 h prior to experimentation and to avoid major thermal stimuli on their way to the laboratory. Participants were also encouraged to arrive well-hydrated as no fluid replacements were provided during the experiment. After 30 min of rest on a chair, participants performed four bouts of 15-min cycling (Corival recumbent cycle ergometer, Lode, Netherlands) at a constant rate of heat production (400 W) in an environmental chamber regulated at 35°C and 20% relative humidity. Each exercise bout was separated by 15-min of rest on the recumbent cycle, with a final 60 min recovery (not reported in the following analyses). Therefore, the length of each recording was of 150 min (60 of exercise, 90 of rest).

Electrocardiographic waveforms for each subject were recorded through a Holter DigiTrak XT (Philips Medical Systems, USA) physiological monitor. For simplicity we will refer to this dataset as EXERCISE.

VARIABILITY COMPUTATION

For both datasets, only the beats characterized as normal sinus rhythm were included, while all premature beats were excluded.

The classification was automatically performed by the Holter monitors and through delta detector on both datasets (Clifford et al., 2002). Using Continuous Individualized Multiorgan Variability Analysis (CIMVA™) software (Bravi, 2013), HR and four measures of variability were extracted from the R-R interval time series of each subject through a windowed analysis, namely by using 5-min windows for both EXERCISE and SEPSIS. This means that five values were computed using 5 min of data, and successive values of those measures were computed by repeatedly shifting the 5-min window by 30 s for EXERCISE and 2.5 min for SEPSIS; these window steps were different because of the shorter length of the EXERCISE recordings compared to the SEPSIS recordings. This procedure created five variability time series per subject. Together with mean HR, the measures we investigated are standard deviation (statistical domain), the ratio between the power at low (LF) and high (HF) frequencies (LF/HF ratio—computed through the Lomb–Scargle periodogram, energetic domain), sample entropy (informational domain), and the Hurst exponent (computed through the Scaled windowed variance method, invariant domain). For details on the domains of variability, refer to Bravi et al. (2011).

STATISTICAL ANALYSIS

Before any analysis, each of the time series for the patients in the SEPSIS dataset were aligned with respect to the time of administration of antibiotics, and the time series for the subjects in the EXERCISE dataset were aligned with respect to the start of the exercise routine. Following the alignment, three analyses were performed. (1) Qualitative inspection of the population trends of HR and the four HRV measures (reported as median and 95% confidence intervals around the median). The population trends were computed by taking at each time instant the median value across the different subjects, as show in **Figure 1**. This led to five population trends for each dataset. (2) Statistical evaluation of the change between variability at baseline, and variability during stress. First, the variability time series were segmented, as summarized in **Table 2**, and then for each subject the median variability over time was computed, creating a distribution of values representative of either baseline or stress, for both SEPSIS and EXERCISE. The Wilcoxon sign-rank test was used to evaluate the null hypothesis of zero change between baseline and stress. It is worth mentioning that what we defined as "variability at baseline" for the SEPSIS dataset is not really representative of baseline conditions, because likely some subjects were already developing sepsis 72 h before the administration of antibiotics. However, not all the subjects had more than 72 h of data prior the administration of antibiotics, therefore to include all of them we decided to use that time interval. (3) Spearman's non-linear correlation analysis across the five population trends of SEPSIS and EXERCISE during the time intervals representative of "stress," as specified in **Table 2**.

RESULTS

The alignment of the variability time series of each subject, as well as the creation of the population trends of the mean HR

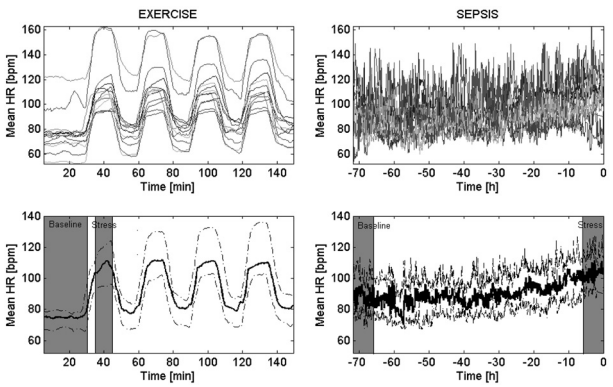

FIGURE 1 | Trends of mean heart rate in EXERCISE and SEPSIS. Top panels show how the mean heart rate (HR) changed over time for all the subjects (above), for either EXERCISE (left) or SEPSIS (right) datasets. **Bottom panels** report the median trends across each population—i.e., population trend—(bold solid line), together with the 95% confidence interval (dashed line). The EXERCISE time series start from 30 min prior the beginning of the first exercise bout, to the end of the last resting period. The SEPSIS time series start from 72 h prior the administration of antibiotics, to the time of administration of antibiotics ($t = 0$). In gray are highlighted the areas we referred to as "baseline" and "stress."

Table 2 | Segmentation of the time series.

Dataset	Baseline			Stress		
	Start	**End**	**Length**	**Start**	**End**	**Length**
EXERCISE	Thirty minutes before the beginning of the first* exercise bout	At the beginning of the first* exercise bout	30 min	Ten minutes before the end of the first exercise bout	At the end of the first exercise bout	10 min
SEPSIS	Seventy-two hours prior the administration of antibiotics	Sixty-six hours prior the administration of antibiotics	6 h	Six hours prior the administration of antibiotics	At the time of administration of antibiotics	6 h

*This table shows the time intervals that were used to compare the changes in HRV between baseline condition and stress. The variability time series were segmented as specified, and the median value in each time interval was computed. This procedure created two values (i.e., at baseline and during stress) for each subject and for each variability time series. Those intervals are reported in **Figures 1**, **2** as gray areas. *Different exercise bouts did not produce significant changes in the results.*

are reported in **Figure 1**. The figure shows that the mean HR considerably increased during exercise, and slightly increased during sepsis. Because of the low inter-subject variability, the 95% confidence intervals around the population trends are tight. Similarly, **Figure 2** shows the population trends for standard deviation, LF/HF ratio, sample entropy and Hurst exponent. Standard deviation presented a clear population trend in both sepsis and exercise, both in terms of a decrease in time as well as a tightening of the confidence intervals. The same phenomenon was not observed in either sample entropy nor Hurst exponent, even though they showed like standard deviation, a high sensitivity to non-stationarity (i.e., spikes in the transition between exercise and rest, and vice versa). Lastly, the LF/HF ratio showed a slight reduction in only the EXERCISE population trend, supported by a considerable reduction of the 95% confidence intervals around the population trends.

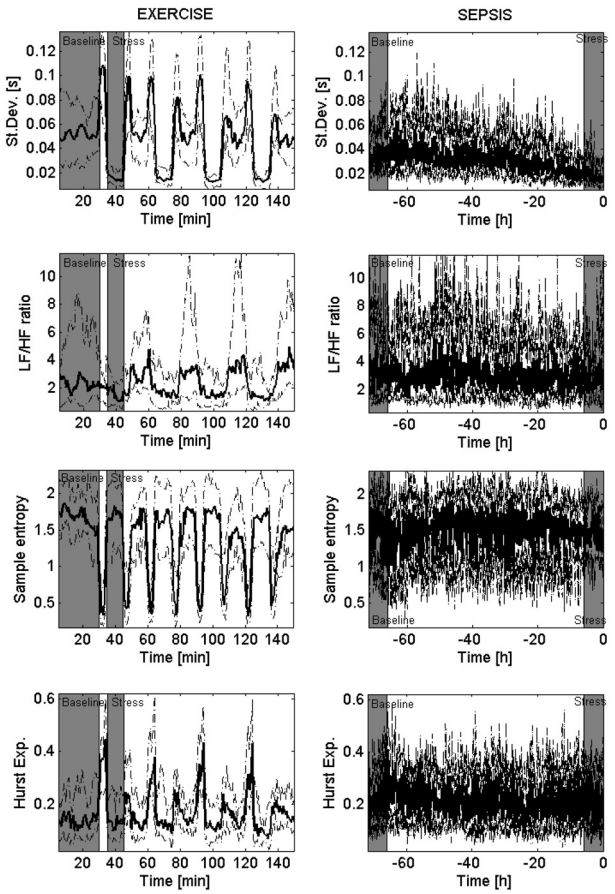

FIGURE 2 | Population trends in physiological dimensions of variability. These eight panels show the population trends of four measures of variability, hypothetically linked to separate physiological dimensions. From the top, each row presents: standard deviation, LF/HF ratio (computed through the Lomb–Scargle periodogram), sample entropy, and Hurst exponent (computed through Scaled windowed variance). The columns depict the median trend across all the subjects (bold solid line) and its 95% confidence intervals (dashed line) for the EXERCISE dataset (left) and the SEPSIS dataset (right). In gray are highlighted the areas we referred to as "baseline" and "stress."

To evaluate the changes between baseline and stress in a quantitative way, the values of the measures (median and 95% confidence intervals), together with results of the Wilcoxon sign-rank test are reported in **Table 3. Figure 3** provides a visual representation of the distributions of change from baseline to stress of **Table 3**. Mean HR and standard deviation confirmed the changes observed from a visual inspection, rejecting the null hypothesis of no change in the transition from baseline to stress for both EXERCISE ($p = 0.0002$) and SEPSIS ($p = 0.003$). The LF/HF ratio rejected the null hypothesis only in EXERCISE ($p = 0.04$). Hurst exponent and sample entropy were not able to reject the null hypothesis in neither EXERCISE nor SEPSIS.

The final analysis compared the degree of correlation between the population trends at the time of stress. The results are summarized in **Table 4**. Mean HR and standard deviation showed a good correlation (-0.68) during exercise, but lower correlation during sepsis (-0.40). Similarly, the levels of correlation between mean HR, standard deviation, LF/HF ratio and sample entropy resulted above $|0.5|$during exercise and below $|0.5|$during sepsis. The Hurst exponent showed low levels of correlation with all the measures in both sepsis and exercise, exception made for the correlation with standard deviation, which reached 0.51 during sepsis.

DISCUSSION

The main objective of this study was to explore the differences between changes in variability due to exercise vs. sepsis; the underlying hypothesis being that the two types of stress produce differential impact on HRV measures.

Before interpreting the results, it is essential to highlight at a higher level what each measure of variability represents. It is well-established that despite the differences from a mathematical point of view, many (but not all) measures of variability are highly correlated with each other (Maestri et al., 2007), and are sensitive to different types of stressors affecting the cardiovascular system; this is compatible with our findings. Therefore, we believe that each measure of variability can be interpreted as a non-specific sensor, which may be sensitive to multiple physiological mechanisms.

HRV demonstrated two main behaviors, possibly associated with two specific types of physiological phenomena: (1) sensitivity to exercise only (with "sensitivity" we intend the ability to show a change between baseline and stress—as shown in **Table 3**), or (2) to both sepsis and exercise. Although a loss of complexity might have been expected with sepsis development as opposed to exercise, this was not observed. Furthermore, we repeated the same analyses described in this article with the 11 HRV measures found to be relevant in tracking sepsis development in a previous study making use of the same SEPSIS dataset (Bravi et al., 2012), and we identified that all those measures changed significantly in both SEPSIS and EXERCISE (results not shown). This highlights that HRV responds similarly to both sepsis and exercise, although with differences, as exemplified by the particular behavior of the LF/HF ratio. This is similar to what was seen in a subsequent analysis on a set of other 17 measures of HRV, out of a pool of 96 measures (results not shown). Furthermore, when multiple measures of variability are compared through multivariate time series analysis (as done in **Table 4** through correlation analysis), further

Table 3 | Statistical results.

Measure	EXERCISE				SEPSIS			
	Baseline	**Stress**	**Change**	***p*-value**	**Baseline**	**Stress**	**Change**	***p*-value**
Mean heart rate	74 (68, 78)	110 (95, 121)	36 (31 47)	**0.0002**	82 (79, 98)	99 (92, 114)	13 (−0.2 29)	**0.003**
Standard deviation	0.050 (0.026, 0.076)	0.014 (0.010, 0.021)	−0.031 (−0.059, −0.013)	**0.0002**	0.032 (0.023, 0.044)	0.016 (0.013, 0.028)	−0.015 (−0.035, −0.002)	**0.002**
LF/HF ratio	2.41 (0.99, 5.77)	1.09 (0.81, 2.36)	−0.63 (−2.31, 0.23)	**0.04**	2.94 (1.92, 6.18)	2.58 (1.50, 4.56)	−0.82 (−2.44, 1.61)	0.30
Sample entropy	1.67 (1.53, 1.94)	1.79 (1.17, 2.13)	0.08 (−0.21, 0.27)	0.73	1.57 (1.24, 1.71)	1.43 (1.25, 1.56)	−0.14 (−0.32, 0.30)	0.63
Hurst exponent	0.15 (0.08, 0.24)	0.13 (0.07, 0.24)	−0.01 (−0.09, 0.13)	0.79	0.21 (0.16, 0.25)	0.19 (0.14, 0.25)	−0.03 (−0.06, 0.07)	0.83

*This table shows the values for each measure after the segmentation specified in **Table 2**, together with the Change (Stress minus Baseline, within each subject). Since baseline and stress were computed from repeated measurements on the same subjects, the p-values were computed through Wilcoxon sing-rank test. In bold are reported significant rejections of the null hypothesis of zero change (α-value = 0.05).*

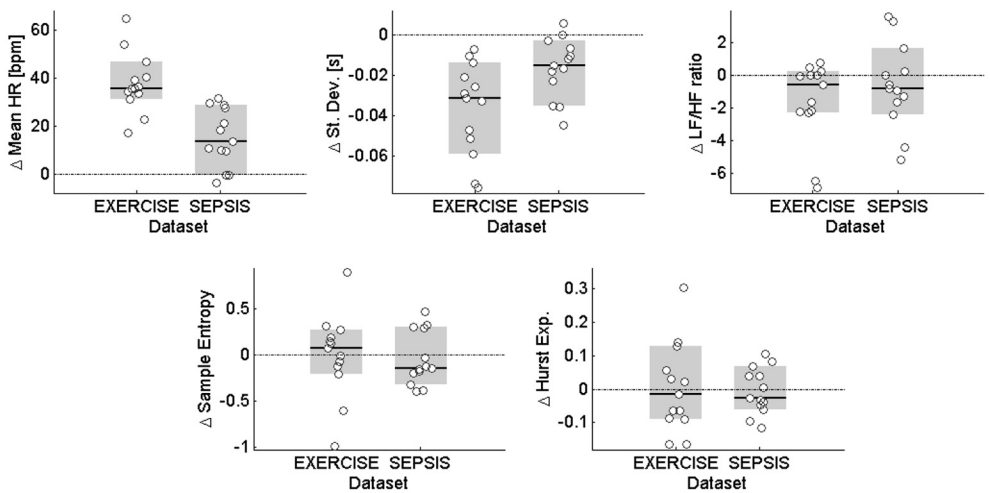

FIGURE 3 | Distributions of change from baseline to stress. The five panels show the distributions for mean HR and the four investigated measures of HRV. Each white circle represents the change in the median variability of a given subject from baseline to stress, as described in **Table 3**. The black solid line is the median of the dataset, and the gray area identifies the 95% confidence intervals for the median. The horizontal dash-dot lines highlight the lines of no change from baseline to stress.

Table 4 | Spearman correlation matrix of the population trends in EXERCISE and SEPSIS, during stress.

	Mean HR	**Standard deviation**	**LF/HF ratio**	**Sample entropy**	**Hurst exponent**
Mean HR	1.00, **1.00**				
Standard deviation	−0.68, −**0.40**	1.00, **1.00**			
LF/HF ratio	−0.76, **0.19**	0.71, −**0.17**	1.00, **1.00**		
Sample entropy	0.78, −**0.13**	−0.57, **0.05**	−0.58, −**0.14**	1.00, **1.00**	
Hurst exponent	−0.34, −**0.24**	0.22, **0.51**	−0.13, −**0.15**	−0.33, −**0.13**	1.00, **1.00**

*This table shows the correlation values between the population time series in the two datasets, making use of only those samples representative of the stress phase (as specified in **Table 2**). The coefficients for SEPSIS are reported in bold, while the others are for EXERCISE.*

differences between sepsis and exercise arise. This result supports the idea that the understanding of the physiology underlying variability may require the integration of information from multiple HRV measures.

The similarities between sepsis and exercise could be explained based on physiology. Exercise-induced immune responses and inflammatory-related immune responses share similar physiological mechanisms, such as the release of cytokines and mediators, albeit with a different intensity (Shephard, 2001; Bente Klarlund, 2005). However, elevated levels of physical stress, such as those associated with performing exercise at very high intensity (=90% of VO_2peak), produce a much lower immune response compared to the septic one (Shephard, 2001). Because we saw major changes during exercise rather than sepsis for both mean HR and standard deviation (**Table 3**), it is unlikely that immune response played a major role on the observed changes in HRV. Septic patients tend to show a normal or high cardiac output, due to an increased HR, a reduced afterload (due to the reduced vascular tone), and either increased (due to catecholamines) or reduced contractility (myocardial depression mediated by inflammatory cytokines) (Vincent, 2008). These similarities in the cardiovascular response could justify the similar behavior observed during sepsis and exercise, highlighting that major changes in HRV are likely driven by pure cardiovascular regulation.

On the other hand, the particular behavior of the LF/HF ratio is more difficult to explain, especially given the fact that this measure was documented to be sensitive to sepsis in multiple pilot studies, as specified in a recent review (Buchan et al., 2012). The key difference with the literature is that we did not compare septic patients with controls, but rather evaluated the change of LF/HF ratio during sepsis development. For instance, a study on 81 patients (Chen and Kuo, 2007) showed that septic patients ($n = 21$) who subsequently developed shock had lower LF/HF ratio respect to patients who did not develop sepsis ($n = 60$). In our study the LF/HF ratio showed a reduction during sepsis development (**Table 3**) which was not statistically significant. However, the three patients which we excluded from the analysis because did not develop sepsis showed instead an average LF/HF ratio of 3.3 (CI: 3.0, 3.6) during stress, showing therefore a larger average LF/HF ratio (see **Table 3**), consistent with the literature. Although the lack of control of posture imperils our ability to further interpret the LF/HF ratio, it is of interest that this measure exhibited different behavior during sepsis and exercise. For future reference, **Figure A1** in Appendix shows the details of LF and HF power (in normalized units) for both EXERCISE and SEPSIS, highlighting the major contribution of LF power in the LF/HF ratio population shown for EXERCISE (**Figure 2**).

An outcome of our analyses is that HRV measures taken from different domains of variability are likely to bring "unique" pieces of information, as shown by their low level of correlation (**Table 4**). The physiological interpretation of each measure in lights of the physiological dimensions of (Barrera-Ramirez et al., 2013) is instead more challenging. Standard deviation decreased during both sepsis and exercise, possibly confirming the hypothesis that it represents the level of cardiopulmonary reserve of the body (i.e., the lowest the measure, the more the body is toward its maximal capacity). LF/HF ratio, measure of sympathovagal modulation, was reduced during exercise, rather than increasing as shown in Barrera-Ramirez et al. (2013). Furthermore, despite the increase in HR, no significant change of LF/HF was detected during sepsis. Sample entropy, being a measure of complexity, was supposed to be primarily affected by a state of illness; despite the expectations, no change was observed during sepsis development. Lastly, the Hurst exponent has been linked to the capacity of the cardiorespiratory system to deliver oxygen and clear carbon dioxide. Given the increased demand of oxygen during exercise, we would have expected to see a decrease in the measure ($H = 0.5$ indicates no fractality), which however did not appear.

LIMITATIONS

While a wider analysis using additional measures of variability is possible, we are cognizant of the small size of the patient populations, and wish not to over-analyze nor over-interpret the results. With a larger dataset in the future, it may be possible to better relate changes in HRV to the current theories about the nature of variability in physiological systems (Seely and Macklem, 2012; Barrera-Ramirez et al., 2013). It is worth noticing also the need to characterize the sensitivity of each measure of variability to non-stationarity. As shown in **Figure 2**, standard deviation, sample entropy and Hurst exponent showed significant sensitivity to the transitions between rest and exercise (and vice versa). This was not a problem for the data analysis, given we knew the acquisition protocol; however, if we imagine the monitoring of ambulatory patients, the necessity of distinguishing non-stationarity from clinically relevant changes in HRV becomes clear.

An inherent limitation to comparing these two cohorts is the lack of standardization of the severity of the stressor, particularly true for the subjects developing sepsis. Indeed, most of the subjects in that group where diagnosed based on fever and clinical impression, possibly introducing a confounding factor in the analysis. Further work on the definition of what "stress" is and how to quantify it would enable a better comparison of different physiologic and pathologic forms of stress. On the same line, the choice of the specific type of physiologic and pathologic stress is a limiting factor of the analyses, and the investigation of alternative protocols or pathologies would be of interest to validate the results. Another limitation is that, the patients in the two populations were not matched by fitness level (e.g., VO_2max), because that information was unavailable for the septic population. Given the similar changes seen in univariate HRV in both populations, it is likely that the fitness level did not produce a bias in the analysis. A similar argument could be applied to other systematic differences between the two datasets, e.g., different definition of baseline state or degree of intensity of the stressor. Also, our results are based on a set of arbitrary choices determined *a priori*, e.g., chosen window size, measures of variability, their computational parameters, time segments for the definition of baseline and stress (i.e., **Table 2**), and use of the median to create the population trend. A larger dataset would enable a sensitivity analysis respect to those parameters. Despite these limitations, this work represents an initial step toward the definition of the differences between physiological and pathological stressors, and the understanding of the physiological mechanisms underlying HRV.

CONCLUSIONS

Qualitative and quantitative comparisons of the changes in HRV during physiological and pathological stress were performed on two datasets, one from patients who developed sepsis after a BMT (pathological stress), the other from a matched group of healthy subjects performing physical exercise (physiological stress). The comparison showed that HRV measures behave similarly during both exercise and sepsis development, although with subtle differences, whose explanation remains fertile ground for further investigation.

AUTHOR CONTRIBUTIONS

Andrea Bravi, Andrew J. E. Seely, and André Longtin conception and design of research; Andrea Bravi analyzed data; Andrea Bravi, Andrew J. E. Seely, André Longtin, Christophe Herry, Geoffrey Green, Glen P. Kenny interpreted results; Andrea Bravi, Andrew J. E. Seely, André Longtin, Christophe Herry, Geoffrey Green, Glen P. Kenny, Heather E. Wright edited and revised manuscript; Andrea Bravi, Andrew J. E. Seely, André Longtin, Christophe Herry, Geoffrey Green, Glen P. Kenny, Heather E. Wright approved final version of manuscript.

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Conflict of Interest Statement: Andrew J. E. Seely is founder and Chief Science Officer of Therapeutic Monitoring Systems, Inc. (TMS), created to commercialize patented Continuous Individualized Multi-organ Variability Analysis (CIMVA) technology, with the objective of delivering variability-directed clinical decision support to improve quality and efficiency of care. Geoffrey Green is currently employed by TMS in the position of Product Manager. The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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APPENDIX

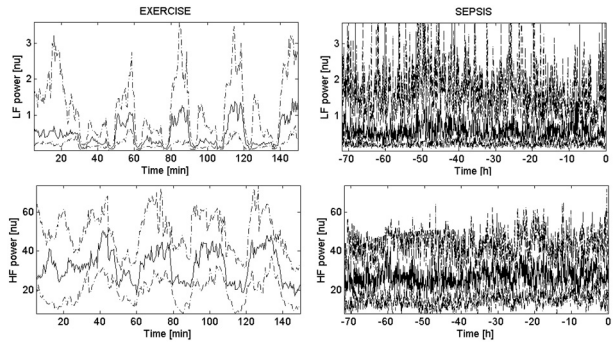

FIGURE A1 | Population trends of LF and HF power. Body: These four panels show the population trends of LF (top) and HF (bottom) power computed through the Lomb–Scargle periodogram. Each panel depicts the median trend across all the subjects (bold solid line) and its 95% confidence intervals (dashed line) for the EXERCISE dataset (left) and the SEPSIS dataset (right).

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Cardiac rehabilitation outcomes following a 6-week program of PCI and CABG Patients

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Coronary artery events requiring intervention are associated with depressed cardiac autonomic function. Whether a 6-week cardiac rehabilitation (CR) differs in effectiveness in improving exercise capacity (6MWT), cardiorespiratory function (peakVO_2), and autonomic function (HRV) following either cardiac bypass surgery (CABG) or percutaneous coronary revascularization (PCI) is unknown. The current study therefore compared the change in 6MWT and peak VO2 to HRV variables following a 6-week CR program and with patients having either PCI or CABG. Thirty-eight patients, (PCI, $n = 22$ and CABG, $n = 16$) participated in the CR program and results for pre and post 6 min walk test (6MWT), peakVO_2, and heart rate variability (HRV) were obtained. Our study has shown that a 6 weeks program following either PCI or CABG improves function. However, the effect on post-CABG differs to that of post-PCI patients. The change in distance walked (6MWT, metres) was higher in the CABG (Δ6MWT: 61, $p < 0.001$) compared to the PCI group (Δ6MWT: 41, $p < 0.001$). Maximum exercise capacity (peak VO_2, ml/kg.min) also changed significantly with a greater change in the CABG group (ΔPCI: 0.7, $P < 0.001$; ΔCABG: 1.0, $P < 0.001$) but did not reach normal population values. Although an improvement in HRV parameters was noted for the PCI group, a statistically significant improvement in HRV was observed only in the CABG group for the following; SDNN (ms) (baseline vs. post-rehabilitation (median $\pm$ IQR): 31.2 ± 25.6 vs. 51.8 ± 23.1, $p < 0.01$), RMSSD (19.32 ± 19.9 vs. 42.1 ± 34.2, $p < 0.01$); LF (ms^2) (191 ± 216 vs. 631 ± 693, $p < 0.01$) and HF (107 ± 201 vs. 449 ± 795.0, $p < 0.05$). A significant interaction in the PCI group but not in the CABG group was observed using correlation analysis between the 6MWT and peak VO_2 with HRV parameters indicating that being healthier that is, a better 6MWT and peak VO_2 led to better HRV results but no significant effect of CR in the PCI group. When the results were investigated for baseline 6MWT and peak VO_2 effect using a covariate analysis, a significant influence of CR on HRV parameters was retained in the CABG group ($p = 0.0072$). Our study indicates that a 6-weeks CR program benefits both patient groups in terms of exercise capacity, cardiorespiratory function and autonomic nervous system modulation of heart rate, with CABG patients showing the most improvement. HRV can be a useful additional variable to gauge cardiac function following CR.

Keywords: cardiac rehabilitation, exercise, percutaneous coronary angioplasty, coronary artery bypass drafting, heart rate variability

INTRODUCTION

Percutaneous coronary angioplasty intervention (PCI) and coronary artery bypass grafting (CABG) are effective and established treatments for clinically significant coronary artery disease (CAD) (Eagle et al., 2004; Bravata et al., 2007). The long-term survival benefits of cardiac rehabilitation (CR) among patients with CAD have been documented following several large-scale trials and meta-analyses with no direct comparisons between cardiac intervention (CABG vs. PCI) or current measures, including the 6-min walk test and peak oxygen volume (6MWT, peak VO_2) (Giannuzzi et al., 2003; Taylor et al., 2004; Leon et al., 2005; Suaya et al., 2009). In recent papers there has been some speculation this may be due to an improvement in cardiac autonomic function (Lucini et al., 2002; Routledge et al., 2010).

The 6MWT is now used routinely to demonstrate the physical and physiological benefits of CR following coronary intervention with peak VO_2 still used in major metropolitan hospitals (Fiorina et al., 2007). Recently Soumagne reported improvement in functional capacity following CR but did not report direct comparisons between PCI and CABG (Soumagne, 2012).

Heart rate variability (HRV) is a valid marker of cardiac autonomic activity and complexity that reflects sympathetic and parasympathetic balance and overall tone (Mäkikallio et al., 2006; Jelinek et al., 2010; McLachlan et al., 2010). HRV can be measured in both time and frequency domains, where global activity in the time domain is indicated by the standard deviation of the RR intervals (SDNN) and parasympathetic function by the root mean square of the standard deviation of the RR intervals (RMSSD) (TFESC, 1996). Sympathetic output in the frequency domain is loosely correlated with low frequency (LF) power, although a parasympathetic component has been noted, while parasympathetic/vagal output is in part correlated with high frequency (HF) power of the HRV spectrum (Grassi et al., 2009). The LF/HF ratio is a derived value from the calculated HF and LF spectral component (Reed et al., 2005; Billman, 2013). Reduced HRV may be associated with abnormal adaptability of the cardiac autonomic nervous system to changes in cardiac pathology and may increase risk of adverse or fatal cardiac events (Kleiger et al., 1987; Quintana et al., 1997; Weber et al., 1999; Jelinek et al., 2010).

Parasympathetic indices are reduced in patients within 24 h after PCI (Tseng et al., 1996; Osterhues et al., 1998; Wennerblom et al., 2000; Kanadasi et al., 2002). However, this reduction in parasympathetic function appears to be a transient phenomenon (Osterhues et al., 1998; Kanadasi et al., 2002; Janowska-Kulińska et al., 2009). In patients with greater than one target-vessel affected and/or with other comorbidities, HRV remains lower due to several factors (Tseng et al., 1996; Birand et al., 1998; Wennerblom et al., 2000; Kanadasi et al., 2002). In advanced heart failure with low left ventricular ejection fraction autonomic dysfunction may be due to a decrease in muscarinic receptor density, or changes in neuro-hormonal output leading to a decrease in parasympathetic output and an increase in sympathetic activity (Eckberg et al., 1971). In further studies, HRV has been shown to be independent of ejection fraction, extent of CAD and other variables, where a decreased HRV is a potent independent predictor of mortality in the 12 months following elective coronary angiography in patients without recent myocardial infarction (Rich et al., 1988). A number of studies have found that CR can improve both HRV and exercise capacity in patients following PCI (Chien et al., 2006; Munk et al., 2009; Baumert et al., 2011).

In general, outcomes for post-CABG reported in earlier studies differ to PCI in that impairment of cardiac autonomic regulation assessed by HRV remains suboptimal for several months or years post CABG and an increased risk of atrial fibrillation remains present (Demirel et al., 2002; Bauernschmitt et al., 2004; Cygankiewicz et al., 2004; Wu et al., 2005; Laitio et al., 2006; Kalisnik et al., 2007). Accordingly, strategies resulting in favorable recovery of cardiac autonomic tone as soon as possible after CABG may be clinically important in these patients. Long-term outpatient exercise-based CR has been reported to positively improve exercise capacity and cardiac autonomic function post-CABG hospital discharge in programs of 2 months duration or longer (Hirschhorn et al., 2008; Baumert et al., 2011). However, data evaluating the impact of CR on HRV in outpatients after CABG are limited (Iellamo et al., 2000; Baumert et al., 2011). It is uncertain whether short-term CR applied post-CABG during an outpatient CR program has a substantial beneficial impact on HRV and how this compares to current measures of exercise capacity (6MWT) and cardiorespiratory function (peak VO_2). No study has directly compared the physiological parameters measured as 6MWT and peak VO_2 and cardiac autonomic function improvements for CABG and PCI patients following a 6-week CR program.

The current study prospectively compared the impact of a 6 weeks CR program on baseline HRV parameters and exercise capacity outcomes with patients recovering from both PCI and CABG in a standardized program.

METHODS

PARTICIPANTS

The project was conducted in accordance with ethics guidelines and approval by the Sydney Adventist Hospital and Charles Sturt University Human Ethics Research Committee. All participants were provided with an information sheet and gave informed consent.

The participants who agreed to participate in this study were prospectively and consecutively enrolled between July and December 2010 and part of the CR program at the Sydney Adventist Hospital, Wahroonga, NSW, Australia. All patients had revascularization procedures (either PCI or CABG) within 1 month of enrolment. Patients enrolled into CR were excluded from the study if the principal diagnosis was of cardiac failure, valvular surgery, or patients who had a documented myocardial infarction within 6 months prior to study enrolment. Participants were also excluded from the analysis if they were unable to complete the 6-week CR program or could not participate in the designated exercise program.

REHABILITATION PROGRAM

Exercise sessions were performed three times per week for 6 weeks at 55–70% of peak VO_2, measured during the first exercise session at commencement of the CR program combined with a patient perceived exertion rating of 11–13 (fairly light to somewhat hard) on the Borg scale (Borg, 1982). The program included 16 periods of exercise training and six education sessions on cardiovascular risk factors, life style modification measures and the pathophysiology of atherosclerosis. The exercise component of the program was conducted according to the National Heart Foundation of Australia & Australian Cardiac Rehabilitation Association (NHFA and ACRA, 2004) guidelines. Each participant was given an individualized exercise program consisting of aerobic exercise (cycle ergometry, treadmill walking, and rowing) that was devised by an exercise physiologist to ensure the participants could exercise continually throughout the session at the prescribed level of intensity. Each session consisted of stretching and warm-up exercise (5–10 min), endurance training (15–20 min), resistance training (10–15 min), or strength training (10–15 min) and cool-down/relaxation exercise (5–10 min). Participants were also advised to complete a home walking program, as recommended by the National Heart Foundation to achieve 30 min of moderate intensity physical activity on most or all days of the week.

DATA COLLECTION

The 6-min walk test (6MWT) is a standard measure of functional walking capacity and as such provides insight into the likely effect of participation on patients' ability to carry out activities of daily living (Fiorina et al., 2007). This test consists of walking up and down an 18 m indoor track as many times as possible within a 6-min period (NHFA and ACRA, 2004; West et al., 2012). In accordance with the American Thoracic Society guidelines this test was conducted twice prior to commencing CR, with the better of the two tests recorded as baseline (Argyropoulos and Harper, 2002). Patients were allowed to rest if required, and the assessment was ceased if there was angina pectoris or undue shortness of breath, which would normally limit activity and/or necessitate the use of coronary vasodilator therapy. Patients were advised of elapsed time at each minute of the assessment. No other encouragement was given. The peak VO_2 was collected throughout the 6MWT and calculated using the American College of Sports Medicine formula (ACSM, 2006).

A 20-min, 3-lead ECG recording (PowerLab 4/30, LabChart Version 7, Castle Hill, NSW, Australia) was obtained prior and post CR. Time series variables were analyzed over the 20-min recording range, whereas frequency domain measures were analyzed using the accepted 5-min successive interval method to avoid the influence of non-stationarity (Task Force, 1996). The time domain variables considered in this study were the mean RR interval and its standard deviation of the N-N intervals (SDNN), representing the overall HRV and its root mean square successive difference (rMSSD), representing the vagal tone. The frequency-domain variables measured were LF and HF and the ratio LF/HF, which provide information on vagal and sympathetic input modulating HRV (Carney et al., 2005).

Other information obtained from patients attending the CR included medications, blood pressure, smoking, and diabetes status. Height and weight were measured, and the body mass index (BMI) calculated. Waist circumference was measured within an accuracy of 0.1 cm. Blood pressure was measured with a clinical zero sphygmomanometer. The average of two measurements was used to determine blood pressure. A cardiac history questionnaire was given to all participants to ascertain any other factors that may impact on their exercise capacity and HRV.

DATA ANALYSIS

All the data were analyzed by LabChart version 7 (ADInstruments, Castle Hill, Sydney, Australia). In accordance with the American Thoracic Society guidelines (ATS, 2002), 6MWT results were expressed as an absolute value. Exercise capacity was expressed as peak VO_2 in ml/kg.min.

Statistical analysis was performed using SPSS Version 20 (Copyright IBM Inc.). The Wilcoxon Signed-Ranks Test was used to compare HRV parameters for the CABG and PCI groups before and following CR. A student *t*-test was used to compare the 6MWT and peak VO_2 results before and following CR. To assess the correlation on a nominal scale the Pearson's correlation test was used. Covariate analysis to model the influence of 6MWT and peak VO_2 on effectiveness of CR was determined using Friedman's test. Differences were considered significant when $p < 0.05$. Values were expressed as means and standard deviation for normally distributed data and medians and interquartile range (IQR) if the data was not normally distributed.

RESULTS

BASELINE VALUES OF PATIENTS

Forty-two patients were consecutively enrolled after successful cardiac intervention procedures if they agree to the research and provided informed consent. The PCI group consisted of 25 patients and the CABG group of 17 patients. One patient was unable to complete the CR program, two missed the follow-up appointment and one patient was hospitalized during the rehabilitation program. No patients experienced angina during the exercise component of the rehabilitation program. Data from 22 patients in the PCI group and 16 patients in the CABG group were used for the final analysis.

There were no significant differences in risk profile including age, blood pressure, smoking, and diabetes, and clinical presentation between the two groups (**Table 1**). Medication use was similar between the two groups apart from a significant difference in the PCI group used angiotensin converting enzyme inhibitors (ACEI). One patient in the PCI group and two patients in the CABG group discontinued β-blocker therapy and were excluded from the analysis.

Table 1 | Patient demographics and clinical history.

	PCI (%)	CABG (%)	P
BASELINE CHARACTERISTICS			
Male/female (%)	18 (81.8)/4 (18.2)	13 (81.3)/3 (18.7)	NS
Age (years)[#]	62.5 ± 9.9	64.9 ± 8.8	NS
BMI (kg/m^2)	27.5 ± 3.9	27.3 ± 4.6	NS
Waist circumference (cm)	98.1 ± 10.3	97.5 ± 12.0	NS
HISTORY			
Past cardiac surgery (%)	1 (4.5)	3 (18.8)	NS
Myocardial infarction (%)	11 (50.0)	5 (31.3)	NS
Hypertension (treated) (%)	8 (36.4)	10 (62.5)	NS
Valvular disease (%)	0 (0.0)	3 (18.8)	NS
Arrhythmia (%) AF**	1 (4.5)	3 (18.8)	NS
Diabetes (%)	5 (22.7)	1 (6.3)	NS
Hyperlipidaemia (%)	17 (77.2)	14 (87.5)	NS
Stroke (%)	2 (9.1)	1 (6.3)	NS
Kidney problem (%)	1 (4.5)	1 (6.3)	NS
Family history (%)	14 (63.6)	15 (93.8)	NS
Smoker/ex smokers (%)	1 (4.5)/8 (36.4)	0 (0.0)/3 (18.8)	NS
Depression (%)	6 (27.3)	4 (25.0)	NS
MEDICATION			
β-blockers/Stopped (%)	11 (50.0)/1 (4.5)	8 (50.0)/2 (12.5)	NS
Calcium antagonists (%)	0 (0.0)	1 (6.3)	NS
Diuretics (%)	2 (9.1)	2 (12.5)	NS
Angiotensin converting enzyme inhibitors	14 (63.6)	4 (25.0)	0.025*
Antiplatelet treatment/ bleeding	22 (100.0)/1 (4.5)	16 (100.0)/0 (0.0)	NS

[#] *Mean and standard deviation; *PCI vs. CABG $p < 0.05$; **Atrial fibrillation.*

CLINICAL VARIABLES

BMI and waist circumference were measured before and after CR. No significant differences were observed after the 6-week CR programme (**Table 2**).

HEART RATE VARIABILITY

Significant increases were seen for SDNN, RMSSD, LF, and HF in the CABG group following CR compared to baseline (**Table 3**).

There was no significant change in HRV post CR in the PCI group for all HRV measures. Significant differences were seen for the majority of HRV measures for the CABG group when comparing post CR to baseline levels (**Table 3**).

Analysis of the difference in the extent of change (Δ) between the CABG and PCI groups for the time and frequency domain parameters following CR revealed no significant differences in the HRV parameters. However, CABG patients showed a greater improvement in all HRV measures.

A significant interaction in the PCI group but not in the CABG group was observed using covariant analysis between the 6MWT and peak VO_2 with HRV parameters, indicating that being healthier that is, better 6MWT and peak VO_2, led to better HRV results but no significant effect of CR in the PCI group. When the results were investigated for baseline 6MWT and peak VO_2 in the CABG group a significant influence of CR on HRV parameters was retained in the CABG group ($p = 0.0072$).

EXERCISE CAPACITY

Clinically significant improvements in exercise capacity were observed in both PCI and CABG groups following CR as judged by the American Thoracic Society guidelines (2002), which stipulate that an increase of 54 m in the 6MWT is the minimum distance required (**Table 4**).

Peak VO_2 increased significantly in both groups. However, it remained below international and Australian population norms that are set between 20 and 35 ml/kg.min (see **Table 4**). CABG patients improved by 8% and PCI by 1% compared to baseline following the CR program.

Table 2 | BMI and waist circumference in the CABG and PCI groups.

Variables	Baseline (mean ± SD)	6 weeks (mean ± SD)	*P*-value
PCI_BMI (kg/m^2)	27.5 ± 4	27.1 ± 4	0.17
PCI_Waist** (cm)	98.1 ± 10	96.6 ± 9	0.09
CABG_BMI (kg/m^2)	27.3 ± 4	27.3 ± 4	0.89
CABG_Waist (cm)	97.5 ± 12	96.5 ± 12	0.12

***waist circumference.*

Table 3 | Changes of the HRV indices in PCI and CABG group.

Variables	Baseline (median ± IQR)	6 weeks (median ± IQR)	*P*-value
PCI			
SDNN (ms)	49.1 ± 45.3	53.42 ± 35.36	NS
rMSSD	30.17 ± 46.1	35.68 ± 38.5	NS
LF (ms^2)	438 ± 1414	781 ± 1389	NS
HF (ms^2)	291 ± 1309	376 ± 821	NS
LF/HF	1.47 ± 0.9	1.87 ± 1.7	NS
CABG			
SDNN (ms)	31.19 ± 25.6	51.8 ± 23.1	0.005
rMSSD	19.32 ± 34	61.6 ± 54	0.02
LF (ms^2)	191 ± 216	631 ± 639	0.001
HF (ms^2)	106 ± 201	449 ± 795	0.02
LF/HF	1.09 ± 1.14	1.06 ± 2.01	NS

DISCUSSION

Our study is the first to investigate whether there is a difference in CR outcomes following a 6 week, moderate-intensity exercise program for patients that have undergone either CABG or PCI with differing levels of parasympathetic suppression at baseline (within the first month following intervention but immediately prior to rehabilitation). Effect of CR on HRV was compared to the traditional measures of 6MWT and peak VO_2. CABG patients clearly entered the program with lower HRV parameters, particularly for LF power activity and total HRV, which may be due to more extensive myocardial or cardiovascular disease and the more invasive intervention (Santangeli et al., 2008; Lakusic et al., 2009). CABG intervention has been shown to decrease HRV post-CABG intervention with HRV parameters not improving past preoperative levels even after 6 months (Kuo et al., 1999). There were two principal findings: (1) CR significantly improved both HF power and LF power in the CABG group, which suggests significant changes to the extent or functionality of the autonomic nervous system, (2) CR had a significant impact on the exercise capacity in both groups. Our study also supports that a short 6-week CR program as recommended by the Australian guidelines is sufficient to improve exercise capacity, cardiorespiratory function regardless of intervention (PCI or CABG) and cardiac autonomic function in CABG patients (NHFA and ACRA, 2004). Our study agrees with previous reports that despite patients with diabetes having a higher risk of adverse cardiac events, adherence to a CR program can improve functional capacity (Hindman et al., 2005).

Time after intervention is an important component that influences CR outcomes. We recruited patients between 2 and 4 weeks after intervention and observed that the baseline values for HRV in the CABG group were much lower than for the PCI group in agreement with previous studies (Demirel et al., 2002; Cygankiewicz et al., 2004; Laitio et al., 2006). While there was a correlation between HRV parameters and 6MWT as well as peak VO_2, this did not explain all the variance, hence indicating HRV is an independent physiological measure that provides information

Table 4 | 6MWT distance and metabolic variables in both groups at baseline and after 6 weeks.

Variables	Baseline	6 weeks	*P*-value
PCI_6MWT Distance (m)	548.1 ± 62.0*	589.0 ± 78.1	0.001
PCI_Peak VO_2 (ml/kg.min)	12.6 ± 1.0	13.3 ± 1.3	0.001
CABG_6MWT Distance(m)	504.3 ± 93.5	565.8 ± 98.8	0.001
CABG_Peak VO_2 (ml/kg.min)	11.9 ± 1.6	12.9 ± 1.6	0.001

**Mean and standard deviation.*

on the outcome of CR. HRV may be a more sensitive marker for effectiveness of improvement in cardiac function. HRV can be determined from 2-, 5-min, or longer lead II ECG recordings (Buchheit et al., 2007). 6MWT did show an improvement in both groups with only the CABG group meeting the recommended 56 meters cut-off compared to the PCI group. Peak VO_2, demonstrated significant improvement as well for both groups, but results remained well below published population norms for our study and age range. This lower than previously reported outcome in peak VO_2 may be due to higher baseline values on entry to the program especially in the PCI group or possibly due to patient motivation, home-based compliance, age, gender, and fitness of the patients. However, even modest improvements are correlated with better long-term outcomes in cardiorespiratory function (Swank et al., 2012).

CR has been demonstrated to decrease mortality following PCI (Goel et al., 2011) and either improved survival or morbidity compared to no CR following CABG in longitudinal studies (Hedbäck et al., 2001). However, comparative studies addressing CR effectiveness with respect to the type of cardiac intervention (PCI vs. CABG) and improvement in HRV compared to the traditional measures of 6MWT and peak VO_2 has not been investigated in the one study design. Our study indicates that CR following CABG may improve long-term outcome by reducing the risk of future sudden cardiac death by increasing parasympathetic tone and therefore HRV (Peng et al., 1995).

The effectiveness of CR is influenced by type of surgery and length of rehabilitation program (Eagle et al., 2004). CABG patients usually have more than one target vessel involved, postoperative pain, morbidity, and hence their recovery period is often longer. Patients undergoing PCI, on the other hand, can begin CR 24–48 h after PCI if there is no evidence of hematoma at the catheterization site (West et al., 2012). Length of CR may be a factor in its effectiveness, with many studies reporting an 8-week period (Austin et al., 2004; Freyssin et al., 2012).

Our study shows in a single center cohort that CR consistently improved HRV in patients following CABG even when pre CR values of 6MWT and peak VO_2 are considered.

CONCLUSION

This study indicates that the effect of CR is of benefit to patients with reduced parasympathetic tone prior to the start of CR and that CR has a greater effect in post-CABG compared to post-PCI. In addition HRV is independent of 6MWT and peakVO2, suggesting that HRV is a useful additional measure to employ for CR.

AUTHOR CONTRIBUTIONS

Herbert F. Jelinek: Lead investigator, organized study protocol, data interpretation, statistical analysis, and writing; Zhaoqi Q. Huang: Cardiologist, performing all experimental work, data analysis, and writing; Hosen Kiat: Cardiologist in charge, responsible for developing exercise protocol for participants, study protocol, interpretation, and writing of article; Dennis Chang: Data interpretation and writing of article; Ahsan H. Khandoker: ECG analysis, data interpretation, and writing of article.

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Heart rate variability during simulated hemorrhage with lower body negative pressure in high and low tolerant subjects

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Heart rate variability (HRV) decreases during hemorrhage, and has been proposed as a new vital sign to assess cardiovascular stability in trauma patients. The purpose of this study was to determine if any of the HRV metrics could accurately distinguish between individuals with different tolerance to simulated hemorrhage. Specifically, we hypothesized that (1) HRV would be similar in low tolerant (LT) and high tolerant (HT) subjects at presyncope when both groups are on the verge of hemodynamic collapse; and (2) HRV could distinguish LT subjects at presyncope from hemodynamically stable HT subjects (i.e., at a submaximal level of hypovolemia). Lower body negative pressure (LBNP) was used as a model of hemorrhage in healthy human subjects, eliciting central hypovolemia to the point of presyncopal symptoms (onset of hemodynamic collapse). Subjects were classified as LT if presyncopal symptoms occurred during the −15 to −60 mmHg levels of LBNP, and HT if symptoms occurred after LBNP of −60 mmHg. A total of 20 HRV metrics were derived from R–R interval measurements at the time of presyncope, and at one level prior to presyncope (submax) in LT and HT groups. Only four HRV metrics (Long-range Detrended Fluctuation Analysis, Forbidden Words, Poincaré Plot Descriptor Ratio, and Fractal Dimensions by Curve Length) supported both hypotheses. These four HRV metrics were evaluated further for their ability to identify *individual* LT subjects at presyncope when compared to HT subjects at submax. Variability in individual LT and HT responses was so high that LT responses overlapped with HT responses by 85–97%. The sensitivity of these HRV metrics to distinguish between individual LT from HT subjects was 6–33%, and positive predictive values were 40–73%. These results indicate that while a small number of HRV metrics can accurately distinguish between LT and HT subjects using group mean data, individual HRV values are poor indicators of tolerance to hypovolemia.

Keywords: lower body negative pressure, hypovolemia, hemorrhage, heart rate variability, heart period variability

INTRODUCTION

In trauma patients, the primary cause of death within the first hour of injury is hemorrhage (Bellamy, 1984; Sauaia et al., 1995; Champion et al., 2003). Early detection of hypovolemia in trauma patients is therefore critical to improving triage and providing life saving interventions (LSIs). Unfortunately, the severity of hemorrhage or central hypovolemia is difficult to detect by first responders or other emergency medical personnel, as the traditional vital signs currently available to them, such as arterial pressure, heart rate, respiration rate, and pulse character do not consistently change until hemorrhage has progressed to the point of decompensation (i.e., hemodynamic collapse; Heckbert et al., 1998; Parks et al., 2006; Convertino et al., 2008). Assessing the severity of hemorrhage in trauma patients is further complicated by the reality that there are individuals who have a low tolerance to hemorrhage such that, for the same level of blood loss, low tolerant (LT) individuals reach the point of cardiovascular collapse in less time than those who are high tolerant (HT; Shoemaker et al., 1973, 2006; Klemcke et al., 2011). Thus, there is an urgent need to identify physiological parameters that can accurately track hypovolemia in trauma patients, and have the sensitivity to provide early identification of a patient that has a low tolerance to this physiological insult. New vital signs are needed which can detect the onset, progression, and severity of hypovolemia in individual patients.

The measurement of the variation in R–R intervals, i.e., heart rate variability (HRV; Malik, 1996), can provide information regarding overall cardiovascular status. For instance, healthy individuals with normal autonomic function have high HRV, while individuals with a stressed cardiovascular system (via activity, disease, or dysfunction) can be identified by reduced HRV (Thayer and Sternberg, 2006). HRV has been extensively studied in hospital settings such as intensive care units to assess cardiovascular status and predict mortality in trauma patients (Winchell and Hoyt, 1996, 1997; Grogan et al., 2005; Norris et al., 2005, 2008; Morris et al., 2006). Considering that monitoring electrocardiogram

(ECG) signals is non-invasive, can be accomplished in all echelons of care (including the pre-hospital environment), and can potentially provide information regarding cardiovascular status, the utility of HRV as a pre-hospital triage assist tool is the focus of many investigations (Cooke et al., 2006a,b; Batchinsky et al., 2007a; Cancio et al., 2008; Ryan et al., 2011).

One criterion for the development of accurate and timely assessment of the clinical status of patients with hemorrhage is the capability to investigate the continuous physiological response to progressive reductions in central blood volume. This capability has become available with the emergence of lower body negative pressure (LBNP) as a human experimental model for hemorrhage (Cooke et al., 2004). Cardiovascular responses during LBNP are reproducible, and are consistent with those observed during hemorrhage (Convertino and Sather, 2000; Convertino, 2001; Cooke et al., 2004). In addition, LBNP allows for the assessment of cardiovascular responses which are specific to hypovolemia as confounding factors associated with trauma such as pain and inflammation are absent. Thus, the controlled experimental environment of LBNP allows for optimal conditions in which to study HRV. Using the LBNP model to induce a clinical endpoint (i.e., presyncope), human subjects can be identified as having HT or LT to central hypovolemia (Sather et al., 1986; Convertino and Sather, 2000; Rickards et al., 2011). If HRV metrics reflect the status of cardiovascular stability during progressive central hypovolemia, then LT subjects would be expected to display greater reductions in HRV compared to HT subjects. We therefore used LBNP tolerance as a model to test the hypotheses that: (1) HRV at presyncope is similar in LT and HT subjects when both groups are on the verge of cardiovascular collapse, and (2) LT subjects have lower HRV at presyncope compared to HT subjects who remain hemodynamically stable.

MATERIALS AND METHODS

SUBJECTS

This study was conducted under a protocol reviewed and approved by the Brooke Army Medical Center and US Army Medical Research and Materiel Command Institutional Review Boards and in accordance with the approved protocol. All studies were conducted at the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX, USA. Normotensive, non-smoking healthy human volunteers ($N = 120$; male = 75, female = 45, age 28 ± 1 years; height 174 ± 1 cm; weight 76 ± 1 kg) participated in this study after evaluation of their medical history and physical examination by a physician to ensure the absence of previous and current medical conditions that would exempt them as participants. All female subjects were confirmed as not pregnant by a urine pregnancy test conducted within an hour before the experiment. For 24 h prior to the study, participants were instructed to maintain their normal sleep patterns, refrain from exercise, and abstain from alcohol and autonomic stimulants including prescription and non-prescription drugs such as caffeine, alcohol, and decongestants. The potential effect of caffeine withdrawal on baseline hemodynamics was not assessed in this study. Subjects were given a written description of the experimental protocol and the risks associated with the study. On the day of the study, the subjects were made familiar with the laboratory, given a verbal briefing on the protocol and procedures, and encouraged to ask questions of the investigators. All subjects signed an IRB approved informed consent form.

LBNP PROTOCOL

Subjects were instrumented for standard lead II ECG to record R–R intervals (RRI), and a finger cuff to record beat-by-beat finger arterial pressure by photoplethysmography (Finometer Blood Pressure Monitor, TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). To simulate hemorrhage in conscious humans, central hypovolemia was induced by application of LBNP. Previous studies have shown that the LBNP protocol can closely simulate the hemodynamic challenges associated with pre-shock hemorrhage (Cooke et al., 2004; Ward et al., 2010). Subjects were positioned supine within an airtight chamber that was sealed at the level of the iliac crest by a neoprene skirt. The LBNP protocol consisted of a 5-min control period (baseline) followed by 5 min of chamber decompression at −15, −30, −45, and −60 mmHg, and then additional increments of −10 mmHg every 5 min until the onset of cardiovascular collapse followed by a 10-min recovery period. The application of LBNP was terminated based on three criteria.: (a) sudden onset of relative bradycardia, (b) progressive fall of systolic pressure below 80 mmHg; and, (c) voluntary subject termination due to the onset of presyncopal symptoms such as sweating, nausea, dizziness, vision alterations, or general discomfort. During the LBNP protocol, arterial pressure waveform (Finometer) and ECG signals were sampled continuously at 500 Hz and digitally recorded with WinDaq data acquisition software (DATAQ Instruments, Akron, OH, USA).

HIGH TOLERANCE VS. LOW TOLERANCE

Subjects were categorized as LT or HT based on the level of LBNP at which they experienced the onset of cardiovascular collapse (Sather et al., 1986; Rickards et al., 2011). LT subjects experienced cardiovascular collapse before or during −60 mmHg of LBNP, and HT subjects experienced cardiovascular collapse after −60 mmHg of LBNP.

DATA ANALYSIS

Arterial pressure waveform and ECG signals were analyzed during the last 3 min of each LBNP level using a commercially available software program (WinCPRS, Absolute Aliens, Turku, Finland). Beat-to-beat stroke volume was derived from the arterial pressure waveform using the pulse contour method (Jansen et al., 1990).

Electrocardiogram signals were visually inspected and confirmed to be free of noise, persistent ectopic beats, or arrhythmias. When ectopic beats were identified, the presence of a single event within the 3-min analysis window resulted in the correction of the beat by linear interpolation prior to analysis. If more than one ectopic beat occurred during an LBNP level, the ECG data for that level were not analyzed. Acceptable ECG signals were used to calculate RRI and heart rate. The RRI signal was used to derive 20 indices of HRV described in detail in previous publications from this laboratory (Rickards et al., 2010a; Ryan et al., 2010). Linear methods were used to calculate HRV metrics in both the time domain [$n = 9$; RRI standard deviation (RRISD), RRI root mean squared standard deviation (RMSSD), percentage adjacent RRIs varying by at least 50 ms (pNN50), Poincaré

plot descriptors standard deviation 1 and standard deviation 2 (SD1 and SD2), SD1/SD2 ratio, SD2/SD1 ratio, complex demodulation high frequency (CDM HF), complex demodulation-low frequency (CDM LF)]; and frequency domain [$n = 2$; RRI low frequency power (RRI LF), RRI high frequency power (RRI HF)]. For frequency domain analysis, the RRI signals were plotted using linear interpolation, sampled at 5 Hz, and then passed through a low-pass impulse response filter with a cutoff frequency of 0.5 Hz. Data sets were submitted to a Fourier transform with a Hanning window. Non-linear methods were used to calculate heart rate complexity metrics from the RRI signal ($n = 9$; sample entropy (SampEn), Lempel-Ziv entropy (LZEn), fractal dimensions by curve length (FD-L), fractal dimensions by dispersion analysis (FD-DA), symbol dynamics entropy (SymDyn), normalized symbol dynamics entropy (DisnEn), long-range detrended fluctuation analysis (DFA long), short-range detrended fluctuation analysis (DFA short), and Forbidden Words (FW).

Heart rate variability metrics were compared in LT and HT subjects at the level of LBNP at which cardiovascular collapse was imminent (presyncope). In HT subjects, HRV metrics were also calculated one level of LBNP prior to the presyncopal level (submax), and compared with LT presyncopal values. HRV metrics which measured equal variability in LT and HT at presyncope and also less variability in LT at presyncope compared to HT at submax were further evaluated for their ability to distinguish between individual LT and HT subjects.

SENSITIVITY AND POSITIVE PREDICTIVE VALUE

The effectiveness of HRV metrics to accurately determine group membership (HT or LT) was evaluated by calculating sensitivity and positive predictive value. Sensitivity assesses the ability of a HRV metric to correctly identify a LT subject from all the subjects that were actually LT (i.e., true positives/true positives + false negatives). Positive predictive value is a measure of the ability of a HRV metric to identify a subject as LT from all the subjects that "appear" to be LT (i.e., true positives/true positives + false positives). A logistic regression model was used to evaluate the validity of HRV metrics as predictors of low tolerance to hemorrhage. Using this model, cutoff values were determined for each parameter at each level of LBNP based on receiver-operator characteristic analysis with specificity no less than 0.95. Sensitivity and positive predictive values were then calculated based on this criterion (Pregibon, 1981).

STATISTICAL ANALYSIS

Data are expressed as mean ± SE. Statistical analysis was conducted with commercially available software (SigmaStat; Systat Software, Richmond, CA, USA). Unpaired t-test, Chi-square, and z-test analyses were used to compare subject demographics and conditions at LBNP termination between tolerance groups. For hemodynamic responses, a two way analysis of variance with repeated measures on one variable was used to determine significant variable (tolerance and LBNP level) main effects ($p \leq 0.05$). Subsequent multiple comparison tests (Holm–Sidak) determined significant differences between HT and LT groups ($p \leq 0.05$). HRV measurements at presyncope and submax in LT and HT subjects were compared by unpaired t-test.

RESULTS

SUBJECTS

The LBNP procedure was conducted on 120 subjects. LBNP was terminated in 2 subjects during −30 mmHg, in 5 subjects during −45 mmHg, in 26 subjects during −60 mmHg, in 43 subjects during −70 mmHg, in 29 subjects during −80 mmHg, in 13 subjects during −90 mmHg, and in 2 subjects during −100 mmHg. As a result, 33 subjects were identified as LT (27.5%) and 87 subjects were identified as HT (72.5%). The demographics and baseline mean arterial pressure and heart rates of these two groups of subjects were not statistically different (**Table 1**). However, baseline RRI was significantly lower in LT subjects compared to HT subjects. The presyncopal conditions observed at LBNP termination are shown in **Table 2**. Cardiovascular conditions include systolic pressure below 80 mmHg and/or sudden onset of relative bradycardia, and presyncopal symptoms represent discomfort reported by the subject. LT and HT subjects experienced similar presyncopal conditions at LBNP termination.

RRI TRACKS HYPOVOLEMIA

Stroke volume and RRI at baseline (LBNP = 0 mmHg) and during LBNP in LT and HT subjects are shown in **Figures 1A,B**. LT subjects experienced presyncopal conditions and/or symptoms before or during LBNP level of −60 mmHg. HT subjects experienced presyncopal conditions and/or symptoms after −60 mmHg.

Table 1 | Demographics and baseline hemodynamic variables for low tolerant and high tolerant subjects.

	Low tolerant	High tolerant	*P* value
N	33	87	<0.001
Male/female	18/15	57/30	0.37
Age (years)	27 ± 1	29 ± 1	0.32
Height (cm)	173 ± 2	174 ± 1	0.60
Weight (kg)	76 ± 3	76 ± 1	0.98
Mean arterial pressure (mmHg)	99 ± 2	97 ± 1	0.23
Heart rate (beats/min)	69 ± 2	64 ± 1	0.06
R–R interval (ms)	896 ± 22	973 ± 17	0.01

Data are expressed as mean ± SE.

Table 2 | Prevalence of presyncopal conditions at LBNP termination in low tolerant and high tolerant subjects.

Conditions at LBNP termination	Low tolerant ($n = 33$)	High tolerant ($n = 87$)	*P* value
Cardiovascular + symptoms	17 (52%)	51 (59%)	0.622
Cardiovascular only	5 (15%)	12 (14%)	0.917
Symptoms only	11 (33%)	24 (27%)	0.694

Cardiovascular conditions include systolic blood pressure below 80 mmHg and/or sudden bradycardia. Symptoms represent presyncopal symptoms such as sweating, nausea, dizziness, vision alterations, or general discomfort as reported by the subject. Data are expressed as number of occurrences; group percentages are shown in parentheses.

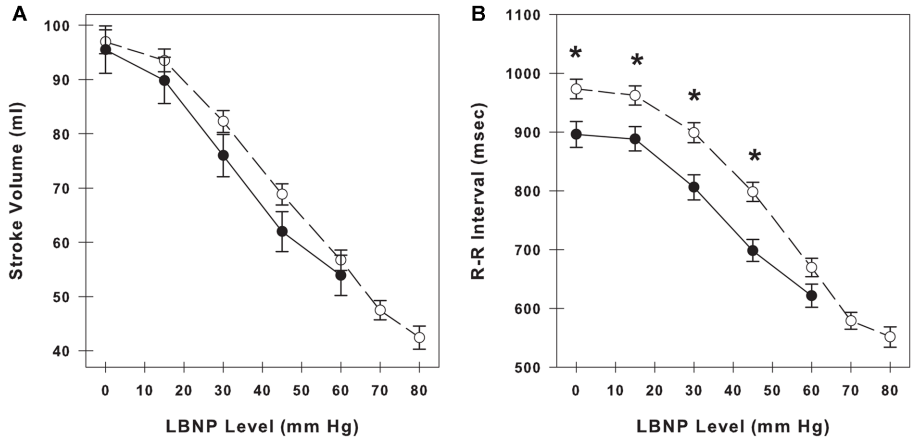

FIGURE 1 | Stroke volume (A) and R–R interval (B) during progressive lower body negative pressure (LBNP) in low tolerant subjects (closed circles, solid line) and high tolerant subjects (open circles, dashed line). At baseline, there are 33 low tolerant subjects and 87 high tolerant subjects. The number of subjects at the subsequent time points during LBNP decline as subjects experience presyncopal symptoms at various levels of LBNP. Data are shown as mean ± SE. * Denotes group differences at the same LBNP level ($p \leq 0.05$).

Data for HT subjects are shown to LBNP level of −80 mmHg. Both stroke volume and RRI decreased in a similar incremental fashion during the progression of LBNP, and average RRI measurements reflect and are correlated with the hypovolemia induced by LBNP (amalgamated $r^2 = 0.988$ between stroke volume and RRI). Stroke volume was not different between LT and HT groups, but RRI was significantly lower in LT subjects than HT subjects at rest and during −15, −30, and −45 mmHg LBNP ($p \leq 0.01$).

HRV MEASUREMENTS AT PRESYNCOPE AND SUBMAX LEVELS OF LBNP

Figure 2 demonstrates the variation in RRI in a single LT subject and a single HT. These sample tracings of RRI were obtained during LBNP level of −60 mmHg, and are 3 min in duration. The variation in RRI is low in the LT subject who is on the verge of cardiovascular collapse (at presyncope). In contrast, the variability in RRI is high in the HT subject who is hemodynamically stable (at submax).

Heart rate variability was quantified at presyncope in all LT and HT subjects, and at submax in HT subjects by 20 HRV metrics. These results are shown in **Table 3**. The first two columns in **Table 3** indicate the parameter or HRV metric and its proportionality to variability. All HRV metrics are quantitatively proportional (P) to HRV except SD2/SD1, DFA long, DFA short, and FW, which are inversely proportional (I) to HRV. The values of proportional metrics decrease as variability decreases, while the values of inversely proportional metrics increase as variability decreases. Columns 3, 4, and 5 in **Table 3** display the data for three groups: LT at presyncope, HT at presyncope, and HT at submax. The comparisons of variability between LT at presyncope verses HT at presyncope, and LT at presyncope verses HT at submax are also shown in **Table 3**, in columns 6 and 7, respectively. The hypotheses of our study were (1) that at presyncope HRV would be equal in LT and HT subjects (column 6) because both groups are on the verge of cardiovascular collapse; and (2)

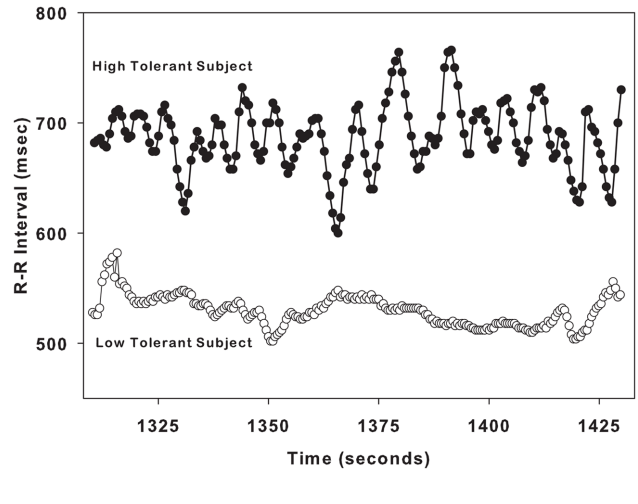

FIGURE 2 | Sample R–R interval tracings from one low tolerant subject (lower tracing, open circles) and one high tolerant subject (upper tracing, closed circles) are shown for a 2-min time span during LBNP level of −60 mmHg.

that LT subjects would have less HRV at presyncope compared to HT subjects who remain hemodynamically stable at submax (column 7). These hypotheses are stated in **Table 3** above the comparisons of HRV metrics (i.e., "LT = HT," "LT < HT"). When comparing HRV between groups it is important to keep in mind that the numerical values of *proportional* metrics are consistent with variability (i.e., high value indicates high variability), but numerical values of metrics which are *inversely* proportional to variability are opposite to variability (i.e., high value indicates low variability). At presyncope, HRV was equivalent between LT and HT groups as measured by 7 HRV metrics (bold type in column 6 of **Table 3**). When HRV was compared between LT subjects at presyncope and HT subjects at submax, 8 metrics were less

Table 3 | HR, RRI, and HRV metrics in LT at presyncope, HT at presyncope, and HT at submax.

Metric	Proportional (P), Inversely proportional (I)	LT at presyncope	HT at presyncope	HT at submax	LT @ Presyncope vs. HT@ Presyncope	LT @ Presyncope vs. HT @ Submax
ECG SIGNAL						
HR		97.3 ± 3.0	117.9 ± 2.3	106.3 ± 2.1	LT < HT	LT < HT
RRI		643.6 ± 22.0	530.6 ± 11.6	587.1 ± 113.4	LT > HT	LT > HT
HRV metrics					Hypothesis	Hypothesis
					LT = HT	LT < HT
TIME DOMAIN						
RRISD	P	64.1 ± 6.0	38.9 ± 2.3	36.7 ± 1.9	LT > HT	LT > HT
RMSSD	P	23.9 ± 5.8	9.6 ± 0.9	14.8 ± 1.2	LT > HT	LT > HT
pNN50	P	3.2 ± 1.1	0.9 ± 0.2	2.5 ± 0.6	LT > HT	LT = HT
SD1	P	13.2 ± 1.9	6.8 ± 0.6	10.5 ± 0.9	LT > HT	LT = HT
SD2	P	88.2 ± 8.1	54.0 ± 3.2	50.1 ± 2.6	LT > HT	LT > HT
SD1/SD2*	P	0.146 ± 0.010	0.129 ± 0.010	0.201 ± 0.011	**LT = HT**	**LT < HT**
SD2/SD1	I	7.80 ± 0.50	10.17 ± 0.53	5.85 ± 0.24	LT > HT	**LT < HT**
CDM HF	P	12.8 ± 1.7	6.9 ± 0.6	11.7 ± 1.1	LT > HT	LT = HT
CDM LF	P	27.1 ± 2.3	20.1 ± 1.7	27.9 ± 1.9	LT > HT	LT = HT
FREQUENCY DOMAIN						
RRI HF	P	216.1 ± 88.7	55.6 ± 10.6	188.9 ± 38.1	LT > HT	LT = HT
RRI LF	P	432.7 ± 91.4	302.4 ± 62.3	742.7 ± 111.4	**LT = HT**	LT = HT
COMPLEXITY DOMAIN						
SampEn	P	0.707 ± 0.048	0.57 ± 0.029	0.821 ± 0.031	LT > HT	LT = HT
LZEn	P	0.504 ± 0.026	0.420 ± 0.017	0.566 ± 0.016	LT > HT	**LT < HT**
FD-L*	P	1.572 ± 0.026	1.529 ± 0.016	1.701 ± 0.011	**LT = HT**	**LT < HT**
FD-DA	P	1.152 ± 0.020	1.128 ± 0.012	1.186 ± 0.011	**LT = HT**	LT = HT
SymDyn	P	0.521 ± 0.018	0.474 ± 0.011	0.580 ± 0.010	LT > HT	**LT < HT**
DisnEn	P	3.142 ± 0.111	2.846 ± 0.067	3.482 ± 0.059	LT > HT	**LT < HT**
DFA long*	I	1.054 ± 0.031	1.100 ± 0.025	0.887 ± 0.022	**LT = HT**	**LT < HT**
DFA short	I	1.564 ± 0.052	1.619 ± 0.034	1.605 ± 0.029	**LT = HT**	LT = HT
FW*	I	67.3 ± 0.9	69.1 ± 0.7	63.8 ± 0.7	**LT = HT**	**LT < HT**

Data are shown in three groups: low tolerant (LT) at presyncope; high tolerant (HT) at presyncope; and HT at submax. Values (mean ± SE) are shown for Heart Rate (HR), R–R interval (RRI), RRI standard deviation (RRISD), RRI root mean squared standard deviation (RMSSD), percentage adjacent RRIs varying by at least 50 ms (pNN50), Poincaré plot descriptors standard deviation 1 (SD1), and standard deviation 2 (SD2), SD1/SD2 ratio, SD2/SD1 ratio, complex demodulation high frequency (CDM HF), complex demodulation-low frequency (CDM LF), RRI high frequency power (HF), RRI low frequency power (LF), sample entropy (SampEn), Lempel-Ziv entropy (LZEn), fractal dimensions by curve length (FD-L), fractal dimensions by dispersion analysis (FD-DA), symbol dynamics entropy (SymDyn), normalized symbol dynamics entropy (DisnEn), long-range detrended fluctuation analysis (DFA long), short-range detrended fluctuation analysis (DFA short). P indicates HRV metrics values proportional to RRI variability; I indicates HRV metrics values inversely proportional to RRI variability. Comparisons between groups are shown as LT = HT, LT < HT, and LT > HT; <, > denote significant difference between LT and HT group means ($p \leq 0.05$); bold type comparisons support the related hypothesis. ∗ Denotes the metrics with responses to support both hypotheses.

variable in LT at presyncope (bold type in column 7 of **Table 3**). The responses of DFA long, FW, SD1/SD2, and FD-L were the only metrics to support both hypotheses, and are indicated by an asterisk (*) in **Table 3**. These four HRV metrics were further evaluated for their ability to distinguish individual LT and HT subjects.

DFA LONG, FW, SD1/SD2, AND FD-L IN INDIVIDUAL LT AND HT SUBJECTS

DFA long, FW, SD1/SD2, and FD-L in individual LT subjects at presyncope and individual HT subjects at submax are shown as box and whisker plots in **Figures 3A–D**. Statistical analysis of group averages (**Table 3**) reveal significant differences in the four HRV measurements between LT and HT groups. However, when the measurements of DFA long, FW, SD1/SD2, and FD-L from individual subjects are graphically displayed (**Figures 3A–D**), it is evident that individual responses from LT subjects at presyncope are predominantly within the range of responses observed in HT subjects at submax despite the fact that LT subjects are on the verge of cardiovascular collapse and HT subjects are hemodynamically stable. The percentage of LT responses that were coincident with HT responses were 97% for DFA long and FW, 94% for SD1/SD2, and 85% for FD-L. The sensitivities of DFA long, FW, SD1/SD2, and FD-L to identify individual LT subjects were low (12, 6, 24, 33%), and positive predictive values were 50, 40, 67, and 73%.

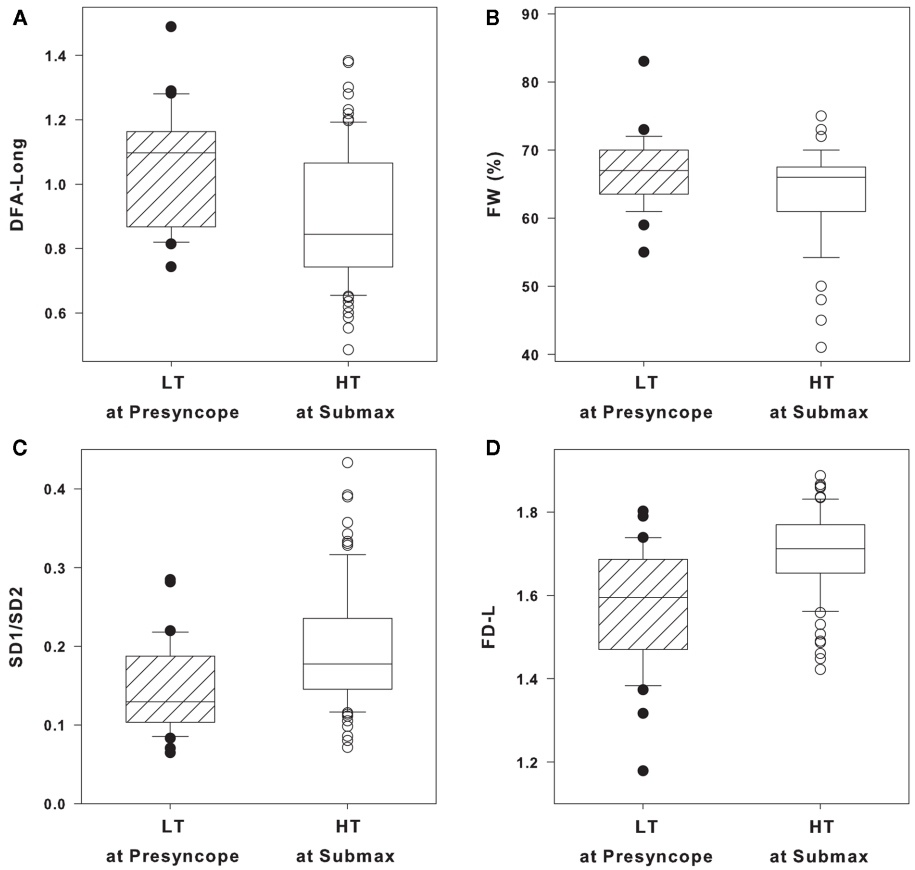

FIGURE 3 | (A) Long-range detrended fluctuation analysis (DFA long), **(B)** forbidden words (FW), **(C)** Poincare plot standard deviations ratio (SD1/SD2), and **(D)** fractal dimensions by curve length (FD-L) in low tolerant (LT) at presyncope (solid circles, $n = 33$) and high tolerant (HT) at submax (open circles, $n = 87$) level of lower body negative pressure (LBNP). Data are shown as box (25th/75th percentiles) and whisker (90th/10th percentiles) plots with median value (black line).

DISCUSSION

To investigate the application of HRV monitoring for assessment of hemodynamic stability during hemorrhage, we used an experimental model to compare HRV metrics in LT and HT subjects during LBNP. In order for any specific HRV metric to be considered as a valid candidate for the assessment of cardiovascular stability in the context of central blood volume loss, two conditions must hold true using this model: (1) LT and HT subjects should display similar HRV at presyncope; and (2) HRV in LT subjects should be less (i.e., LT subjects would be less stable) than HRV in HT subjects at the point in time when LT subjects experienced presyncope. These conditions are based on the premise that hemodynamic instability reflects a specific physiological condition defined by the inability of cardiovascular mechanisms to adequately compensate for reduced central blood volume in all subjects independent of their tolerance, and that LT subjects are hemodynamically unstable at presyncope when HT subjects remain stable. Our results indicate that of the 20 HRV metrics evaluated, only four metrics (DFA Long, FW, SD1/SD2, and FD-L; three of which were calculated by non-linear methods to assess RRI irregularity or complexity) supported both hypotheses.

We propose that a metric which can predict low tolerance in an *individual* subject prior to cardiovascular collapse would be optimal for use as a triage assist tool. Identification of LT patients is particularly important as the first responder will have less time to initiate effective treatment in this patient population compared with HT patients. Upon identifying the metrics which supported our initial hypotheses, we further evaluated DFA Long, FW, SD1/SD2, and FD-L on their utility as triage assist tools in individual subjects. First, we compared the individual responses from LT subjects at presyncope and HT subjects at submax to determine the overlap in responses between the two groups. Second, we assessed the accuracy of these HRV metrics in detecting LT subjects at presyncope from the total subject pool (sensitivity and positive predictive value).

While the group mean values of DFA Long, FW, SD1/SD2, and FD-L (**Table 3**) distinguished between LT at presyncope (unstable) and HT at submax (stable), the responses in individual LT and HT subjects varied extensively such that 85–97% of the LT responses overlapped with those of HT subjects (**Figures 3A–D**). In addition, the sensitivities of DFA Long, FW, SD1/SD2, and FD-L were 12, 6, 24, and 33%; while positive predictive values were 50, 40, 67, and

73%. Considering that identifying LT subjects by chance alone has 50% sensitivity and 50% positive predictive value, the accuracy of DFA Long and FW to identify LT subjects at presyncope was not much better than flipping a coin, or worse. Essentially, DFA Long and FW did not adequately identify a LT individual even when the subject was on the verge of cardiovascular collapse.

According to our results, FD-L had the highest positive predictive value (73%), and thus had the highest potential for accurately assessing hypovolemia of all 20 HRV metrics evaluated. However, the utility of FD-L to accurately monitor *individual* trauma patients may be limited by potentially high variability in individual responses. In the present study, under very controlled laboratory conditions, the sensitivity of FD-L to identify a LT subject was only 33%, and the overlap of FD-L measurements from LT subjects and HT subjects was 85% (**Figure 3D**). Overall, these results indicate that the utility of FD-L as an accurate triage assisting tool for first responders is also limited.

Heart rate variability has been studied extensively during hemorrhage and trauma. HRV decreases with hypovolemia in human LBNP studies (Cooke and Convertino, 2005; Cooke et al., 2008) and in animal hemorrhage experiments (Batchinsky et al., 2007b, 2010). HRV has also been studied in pre-hospital environments during transport of trauma patients. By retrospective analysis of ECG data from actual trauma patients in transport to hospital care, a number of investigators (Cooke et al., 2006a,b; Batchinsky et al., 2007a; Cancio et al., 2008; Ong et al., 2008; King et al., 2009) have identified several HRV metrics that are associated with mortality or the need for a LSI. Importantly, however, all of these analyses were based on evaluation of group mean data only, with no consideration of the appropriateness of application to individual patients.

Despite the observation of depressed HRV in subsets of trauma patients, the use of HRV as a prognostic tool to assess the severity of hemorrhage or trauma is controversial for a number of important reasons. Ryan et al. (2010) evaluated the ability of numerous HRV metrics to track hypovolemia in individual subjects undergoing simulated hemorrhage with LBNP. They observed that when group means were evaluated several metrics correlated very well with reductions in stroke volume ($r \geq 0.87$), but none of the HRV metrics consistently correlated with changes in stroke volume in individual subjects ($r \leq 0.49$).

To further investigate the prognostic relevance of HRV, Rickards et al. (2010b) evaluated a wide range of HRV metrics and their association with the administration of LSIs in actual trauma patients who all had normal vital signs during transport. These patients would benefit most from identification of an early predictor of cardiovascular collapse, as their physiological status could not be accurately determined from currently available standard vital signs. Of the HRV metrics studied, only one metric, FD-L, was uniquely associated with the administration of a LSI. However, FD-L variance in both groups of patients was too high to accurately determine group membership (LSI vs. No-LSI) on an individual basis, and the number of false negatives identified with FD-L further limited the power of this metric as an accurate indicator of LSIs in individual trauma patients. Interestingly, FD-L also showed the highest positive predictive value for identifying LT subjects in the current study but, just as in trauma patients, the overlap between individual responses from LT and HT subjects was large (85%), and we found the sensitivity (33%) of this metric to be limited.

There are several technical factors associated with ECG monitoring which can limit the usefulness of HRV metrics in a pre-hospital setting. First, ECG signals must display normal sinus rhythm for accurate calculation of HRV, but trauma patients often develop arrhythmias such as premature atrial and ventricular contractions (Sethuraman et al., 2010). Second, ECG signals must have a low level of random noise, which usually results from motion artifact, an unavoidable consequence of patient transport in the pre-hospital setting. Third, the data from the ECG signal should be stationary, i.e., a relatively stable RRI signal without wide fluctuations from interventions or patient manipulation. Unfortunately, standard pre-hospital care typically requires extensive patient interventions and manipulation. Other factors which can also contribute to ECG non-stationarity such as disease, age, recreational drugs, medications, alcohol, smoking, and postural changes are widely present in the trauma patient population (Bilchick and Berger, 2006). Finally, the length of the ECG data set required for accurate measurements varies by HRV metric, and can range from 100 to 800 RRIs; which can be an inordinately long time in a trauma patient with rapidly changing physiological status (Acharya et al., 2006; Rickards et al., 2010a). Thus, the quality of HRV measurements are optimal when they can be calculated from extended, stable ECG recordings under standardized and very stable conditions; these conditions, however, are not typically encountered when first responders are treating trauma patients.

Historically, heart rate has been closely monitored in trauma patients because of the widely accepted notion that the magnitude of tachycardia reflects the degree of hypovolemia. However, the reliability of tachycardia in response to hypovolemia has been questioned as tachycardia can be absent in many trauma patients despite the development of hypotension associated with bleeding (Victorino et al., 2003; Brasel et al., 2007). In addition, the results of the current study indicate that tachycardia was a poor indicator of tolerance to hypovolemia because LT subjects at presyncope had lower heart rates than HT subjects at greater levels of central hypovolemia (**Table 3**). Based on the clinical doctrine that tachycardia signals a more severe state of hypovolemia and the approach of cardiovascular collapse, the HT subjects would have been erroneously identified as more hemodynamically unstable and at greater risk of developing circulatory shock than LT subjects. Although a change in cardiac rhythm may represent an adverse clinical status during hemorrhage, the results of the present study reinforce the unreliability of heart rate (and subsequently calculated metrics derived from heart rate such as HRV) for patient triage.

In summary, the results of the current study are consistent with the findings that ECG-derived metrics of HRV failed to provide reliable information about clinical status in individual subjects during progressive reductions in central blood volume similar to those experienced during hemorrhage (Ryan et al., 2010), or the need for LSIs in individual trauma patients (Rickards et al., 2010b). In the present study, heart rate and HRV metrics derived from ECG signals were found to be poor indicators of LT to hypovolemia even in the controlled experimental environment of simulated

hemorrhage. This study raises further concern that monitoring heart rate or calculated derivatives of heart rate (e.g., HRV) will not reliably identify those patients who are least tolerant to hypovolemia and therefore at highest risk for early hemodynamic collapse during hemorrhage.

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DISCLAIMER

The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Why should one normalize heart rate variability with respect to average heart rate

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Keywords: heart rate variability, heart rate, autonomic nervous system, analysis, R-R interval

Heart rate variability (HRV) is a recognized risk factor in many disease states (Bravi et al., 2011; Sacha et al., 2013a). However, HRV is significantly correlated with an average heart rate (HR), and this association is both physiologically and mathematically determined. The physiological determination comes from the autonomic nervous system activity (Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology, 1996), but the mathematical one is caused by the non-linear (inverse) relationship between R-R interval and HR (Sacha and Pluta, 2005a,b, 2008).

HRV may be estimated by using R-R interval (the most frequent method) or HR signals, yet, they both do not yield the same results since they are inversely related with each other—indeed, the analyses are mathematically biased (Sacha and Pluta, 2005a,b). If one uses R-R intervals, the same changes of HR cause much higher fluctuations of R-R intervals for the slow average HR than for the fast one (**Figure 1A**). Conversely, if one employs HR signals, the same changes of R-R intervals cause much higher fluctuations of HR for the fast than slow average HR (**Figure 1B**). Consequently, due to these mathematical reasons, HRV estimated from R-R intervals should negatively correlate with average HR (or positively with average R-R interval), but HRV estimated from HR signals should be positively correlated with average HR (or negatively with average R-R interval) (Sacha and Pluta, 2005a,b). Moreover, due to the inverse relationship between R-R interval and HR, there is a possibility that a given patient may have higher HRV than another in terms of R-R intervals and lower HRV in terms of HRs—**Figure 1C** explains such a case (Sacha and Pluta, 2005a).

Another mathematical problem concerning the association between HRV and

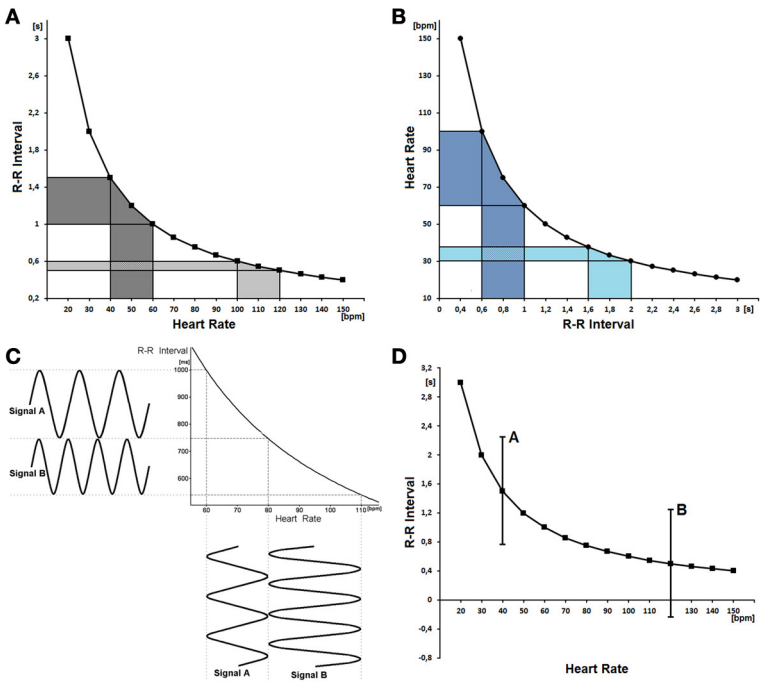

FIGURE 1 | (A) The non-linear (mathematical) relationship between R-R interval and heart rate is depicted. One can see that the oscillations of a slow average heart rate (*x*-axis, dark gray area) result in much greater oscillations of RR intervals (*y*-axis, dark gray area) than the same oscillations of a fast average heart rate (light gray area). As a consequence, the variability of R-R intervals is higher for the slow average heart rate than for the fast one, despite the fact that the variability of heart rate is the same (reprinted with modification from Sacha and Pluta, 2008). **(B)** The relationship between heart rate and R-R interval is shown—the same oscillations of R-R intervals yield much greater oscillations of HR for the fast average heart rate (dark blue area) than for the slow one (light blue area). Consequently, the variability of HR is higher for the case with fast average heart rate, despite the fact that the variability of R-R intervals is the same in both cases. **(C)** The relationship between R-R interval and heart rate is depicted along with two signals oscillating in different extents. Signal A oscillates between 60 and 80 bpm but signal B between 80 and 110 bpm. One can see that signal A is more variable (its amplitude is higher) than signal B when expressed as R-R interval signals, and conversely signal A is less variable than B if expressed as HR ones. The example clearly shows how the same signals may reveal an inverse relationship with each other depending on the way they are expressed (reprinted from Sacha and Pluta, 2005a). **(D)** The relationship between R-R interval and heart rate with two hypothetical examples of R-R interval oscillations (i.e., A and B) are presented. It is shown that the fluctuations of R-R intervals may be potentially quite high for a slow average HR (A), however, such fluctuations are not possible for a fast average HR (B) since the R-R intervals should have become negative.

HR is presented in **Figure 1D**. One can see that the fluctuations of R-R intervals may be potentially very high for slow average HR, however, the same fluctuations are not possible for fast average HR, since the R-R intervals should have become negative. The same problem can be met if one calculates HRV from HR signals, i.e., the average HR of 80 bpm may potentially fluctuate between 30 and 130 bpm (i.e., the fluctuation amplitude equals 100 bpm), however, such fluctuations are not possible for the average HR of 40 bpm, since the heart rhythm must have fluctuated between −10 and 90 bpm.

Due to the above facts, the standard HRV analysis is mathematically biased, particularly if patients differ in terms of their average HRs. The only way to overcome it is to calculate HRV with respect to the average value, i.e., to normalize the fluctuations with respect to the mean (Sacha and Pluta, 2005a,b, 2008). One can do that by division of the signal by the average R-R interval in the case of R-R interval signal, or by the average HR in the case of HR signal. Moreover, this way the same results are obtained no matter if one calculates HRV from R-R intervals or HRs (Sacha and Pluta, 2005a).

Such an approach enables to explore the HR contribution to the physiological and clinical significance of HRV (Billman, 2013; Sacha et al., 2013a). Recently, this approach has been further developed to enhance or completely remove the HR influence (even physiological one) on HRV, what turned out to provide valuable information on cardiac and non-cardiac prognosis in patients after myocardial infarction—the details have been published elsewhere (Sacha et al., 2013a,b,c).

To conclude, HRV is significantly associated with HR, which is caused by both physiological and mathematical phenomena, however, by a simple mathematical modification one may exclude mathematical bias and explore a real clinical value of HR and its variability.

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The effect of heart rate on the heart rate variability response to autonomic interventions

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Heart rate variability (HRV), the beat-to-beat variation in either heart rate (HR) or heart period (R-R interval), has become a popular clinical and investigational tool to quantify cardiac autonomic regulation. However, it is not widely appreciated that, due to the inverse curvilinear relationship between HR and R-R interval, HR *per se* can profoundly influence HRV. It is, therefore, critical to correct HRV for the prevailing HR particularly, as HR changes in response to autonomic neural activation or inhibition. The present study evaluated the effects of HR on the HRV response to autonomic interventions that either increased (submaximal exercise, $n = 25$ or baroreceptor reflex activation, $n = 20$) or reduced (pharmacological blockade: β-adrenergic receptor, muscarinic receptor antagonists alone and in combination, $n = 25$, or bilateral cervical vagotomy, $n = 9$) autonomic neural activity in a canine model. Both total (RR interval standard deviation, RRSD) and the high frequency (HF) variability (HF, 0.24–1.04 Hz) were determined before and in response to an autonomic intervention. All interventions that reduced or abolished cardiac parasympathetic regulation provoked large reductions in HRV even after HR correction [division by mean RRsec or (mean RRsec)^2 for RRSD and HF, respectively] while interventions that reduced HR yielded mixed results. β-adrenergic receptor blockade reduced HRV (RRSD but not HF) while both RRSD and HF increased in response to increases in arterial blood (baroreceptor reflex activation) even after HR correction. These data suggest that the physiological basis for HRV is revealed after correction for prevailing HR and, further, that cardiac parasympathetic activity is responsible for a major portion of the HRV in the dog.

Keywords: heart rate, heart rate variability, autonomic nervous system, cholinergic receptor antagonists, β-adrenergic receptors, exercise, baroreceptor reflex

INTRODUCTION

Heart rate variability (HRV, beat-to-beat changes in the heart period, R-R interval) is increasingly used to quantify cardiac autonomic regulation and to identify patients at an increased risk for adverse cardiovascular events (Appel et al., 1989; Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Denver et al., 2007; Thayler et al., 2010; Billman, 2011, 2013). However, it is not widely appreciated that the prevailing heart rate (HR) can influence HRV independent of changes in cardiac autonomic regulation.

As a consequence of the inverse curvilinear relationship between HR and R-R interval, identical changes in HR will elicit profoundly different changes in the R-R interval variability depending upon the baseline HR (Sacha and Pluta, 2008). For examples, as is illustrated in **Figure 1**, the same HR variability (±1.6 beats/min) is associated with a much greater R-R interval variability (RRSD) at lower (RRSD at 30 beats/min = 105.9 ms) as compared to higher (RRSD at 180 beats/min = 2.9 ms) prevailing HRs. Several studies have reported a strong inverse correlation between HR and various time domain indices of HRV (e.g., the standard deviation (SD) of normal beats, SDNN; Kleiger et al., 1987; Van Hoogenhuyze et al., 1991; Fleiss et al., 1992) such that R-R interval variability increases as average HR decreases. Frequency domain analysis of HRV is similarly affected by mean HR. Sacha and co-workers (Sacha and Pluta, 2005a,b; Sacha et al., 2013a,b) demonstrated that the high frequency (HF) component of HRV was inversely, while the low frequency (LF) component was directly, related to average baseline HR of the subject. As such, differences in average HR *per se* will influence HRV magnitude independent of cardiac autonomic nerve activity either magnifying or masking (diminishing) the autonomic component of HRV as HR changes. It is therefore essential to correct HRV for the prevailing HR in order to identify physiological (changes in cardiac autonomic regulation), as opposed to artifactual (that merely arise as a consequence of a mathematical relationship), changes in HRV.

Although Sacha and co-worker (Sacha and Pluta, 2005a,b, 2008; Sacha et al., 2013a,b) have recently examined the relationship between average HR and indices of HRV under baseline conditions and compared methods to correct HRV for HR, the effects of HR on HRV during the activation or inhibition of cardiac autonomic regulation remained to be determined. As autonomic interventions will alter the prevailing HR, it is particularly important to correct indices of HRV for HR in order to differentiate between the HRV changes that are directly related

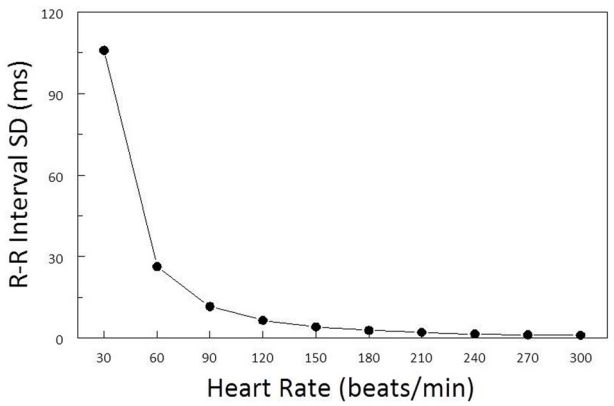

FIGURE 1 | Effect of baseline heart rate on heart rate variability. The standard deviation of R-R interval (RRSD) was calculated for a set of 5 simulated heart beats (X − 2, X − 1, X, X + 1, X + 2) over a range of mean heart rates (HR, from 30 to 300 beats/min) (solid black line). The standard deviation for HR was ±1.6 beats/min at each HR level. Note that RRSD was inversely related to HR, identical changes in HR were accompanied by much larger R-R interval variability at low as compared to high prevailing HRs.

to cardiac autonomic neural activation or inhibition from those changes that result merely as a mathematical consequence of increases or decreases in the baseline HR. It, therefore, was the purpose of the present study to evaluate the effects of well-characterized autonomic interventions on HRV after correction for average HR. Using a canine model, Cardiac autonomic neural activity was increased by submaximal exercise or the activation of the baroreceptor reflex and reduced by pharmacological (autonomic blockade: β-adrenergic receptor, muscarinic receptor antagonists alone and in combination) or by surgical (bilateral cervical vagotomy) interventions.

METHODS

All the animal procedures were approved by the Ohio State University Institutional Animal Care and Use Committee and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication N. 85-23, revised 1996).

Archived data from 74 heartworm free mixed breed dogs (1–3 y old, male $n = 32$, female $n = 42$) weighing 19.3 ± 0.4 kg (range = 11.6–26.8 kg) were used in the present study. The sole selection criterion was an ECG signal of sufficient quality to determine HRV both at baseline and in response to autonomic neural interventions (i.e., submaximal exercise, baroreceptor reflex activation, pharmacological autonomic blockade, or bilateral cervical vagotomy).

HEART RATE VARIABILITY PROTOCOLS

Body surface electrodes were placed on either side of the animal's chest and secured with surgical tape. HRV was then calculated using a Delta-Biometrics vagal tone monitor triggering off the electrocardiogram R-R interval (Urbana-Champaign, IL). This device employs the time-series signal processing techniques as developed by Porges to estimate the amplitude of respiratory sinus arrhythmia [the HF component of R-R interval variability (Porges, 1986)]. Details of this analysis have been described previously (Billman and Hoskins, 1989; Billman and Dujardin, 1990; Houle and Billman, 1999). Data were averaged over 30s intervals before and after the autonomic interventions (see below). The following indices of HRV were determined: Vagal Tone Index - the HF component of R-R interval variability (HF, 0.24–1.04 Hz), and SD of the R-R intervals (a marker of total variability) for the same 30 s time periods.

In order to remove any mathematical bias from HRV calculations, Sacha and co-workers (Sacha and Pluta, 2005a,b; Sacha et al., 2013a,b) previously demonstrated that SD of R-R and frequency data (power spectra) should be corrected by division with the corresponding mean R-R interval or mean R-R interval (in seconds) squared, respectively. These correction factors were used in all subsequent analyses.

AUTONOMIC INTERVENTIONS

Animals received the following interventions to increase or decrease cardiac autonomic regulation: pharmacological blockade ($n = 25$); baroreceptor reflex activation ($n = 20$); submaximal exercise ($n = 25$); and bilateral cervical vagotomy ($n = 9$).

AUTONOMIC BLOCKADE ($n = 25$)

First, the dogs were trained to lie quietly and unrestrained on a laboratory table. Once the animals had habituated to the laboratory environment, a catheter was percutaneously placed in a cephalic vein for the administration of a non-selective β-adrenergic receptor antagonist (propranolol HCl, 1.0 mg/kg, i.v.) followed, at least 5 min later, by a cholinergic muscarinic receptor antagonist (atropine sulfate, 50 μg/kg, i.v.). The drug doses had been previously shown to provide an effective inhibition of cardiac autonomic neural receptors (Billman and Dujardin, 1990). One week later, the study was repeated with the drugs given in the reverse order (i.e., atropine followed by propranolol). HRV was monitored continuously 5 min before and for at least 5 min after each drug injection to ensure that peak changes had been achieved.

BARORECEPTOR ACTIVATION ($n = 20$)

With the animals lying quietly on a laboratory table, a bolus injection of the α-adrenergic receptor agonist, phenylephrine (10 μg/kg, i.v.) was given to induce a 30–50 mm Hg increase in arterial pressure and thereby reflexively increase cardiac parasympathetic and decrease cardiac sympathetic neural activity (Billman et al., 1982). HRV was monitored for at least 5 min after the drug had been given to ensure that peak changes had occurred.

SUBMAXIMAL EXERCISE ($n = 25$)

Over a period of 3–5 days, the dogs learned to run on a motor driven treadmill. The cardiac response to submaximal (i.e., 60–70% of maximal HR) exercise was then evaluated as follows: Exercise lasted a total of 18 minutes with workload increasing every 3-min. The protocol began with a 3-min "warm-up" period, during which the dogs ran at 4.8 kph at 0% grade. The speed was then increased to 6.4 kph, and the grade increased every 3-min (0, 4, 8, 12, and 16%). The submaximal exercise test was repeated

three times (1/day). HRV was monitored continuously, beginning 3 min before the onset of exercise, during exercise, and for the first 3 min following the termination of exercise.

BILATERAL CERVICAL VAGOTOMY ($n = 9$)

Finally, as a terminal experiment, dogs were pre-medicated with morphine sulfate (2 mg/kg, i.m.). A catheter was percutaneously placed in a cephalic vein and used to administer the anesthesia: a mixture of α-chloralose (50 mg/kg, i.v.) and urethane (500 mg/kg, i.v). This anesthetic regimen has been shown to preserve cardiac autonomic regulation (Halliwill and Billman, 1992). The cervical vagus nerves were located via a midline incision on the ventral surface of the neck and 1 h later both vagus nerves were cut. HRV was once again monitored for at least 5 min after the nerves had been severed.

DATA ANALYSIS

All data are reported as mean SEM. The data were digitized (1 kHz) and recorded using a Biopac MP-100 data acquisition system (Biopac Systems, Inc., Goleta, CA). The HR and HRV data were averaged over 30 s intervals before and during the autonomic interventions.

All statistical analyses were performed using NCSS statistical software, (NCSS, Kaysville, UT). The relationship between baseline HR and HRV (SD of R-R interval or HF variability) with and without HR correction were evaluated by means of linear regression. The autonomic intervention data, with or without correction for HR, were compared using an ANOVA with repeated measures. Homogeneity of covariance (sphericity assumption, equal correlates between the treatments) was tested using Mauchley's test and, if appropriate, adjusted using Huynh–Feldt correction. If the F-value exceeded a critical value ($P < 0.05$), *post-hoc* comparisons of the data were then made using Tukey–Kramer Multiple-Comparison Test. The effect of anesthesia on baseline data was evaluated using a t-test.

RESULTS

RELATIONSHIP BETWEEN BASELINE HR AND HRV

The relationship between average HR and the R-R interval variability (SD of R-R interval, $n = 74$) and HF component of the R-R interval variability (cardiac vagal tone index, $n = 74$) under baseline conditions before and after correction for mean R-R interval are displayed in **Figures 2**, **3**, respectively. There were significant inverse relationships between HR and either the R-R interval SD (RRSD, Pearson's correlation coefficient $= -0.51$, $P < 0.00001$; **Figure 2A**,) or the HF variability (Pearson's correlation coefficient $= -0.51$, $P < 0.00001$; **Figure 3A**). However, HR accounted for less than 30% of this variability (R-R variability, $r^2 = 0.26$; HF variability $r^2 = 0.26$). Correction for the prevailing HR abolished the HR dependence for both RRSD (Pearson's correlation coefficient $= -0.128$, NS; **Figure 2B**) and HF variability (Pearson's correlation coefficient $= -0.221$, NS; **Figure 3B**). The portion of this variability that could be ascribed to average HR was also eliminated after correction (R-R interval variability $r^2 = 0.0165$; HF variability $r^2 = 0.0492$).

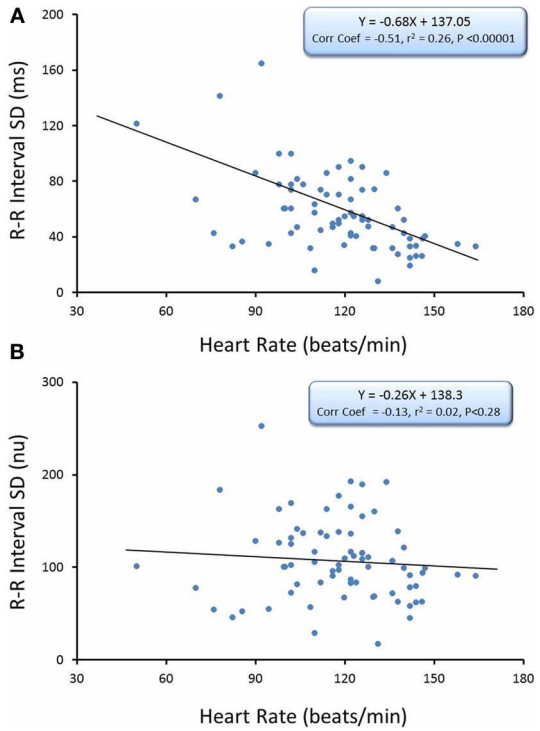

FIGURE 2 | The relationship between baseline heart rate and heart rate variability. The total heart rate (HR) variability (standard deviation of R-R interval, RRSD) was calculated for over the last 30 s before an autonomic intervention was administered and plotted against the average HR for that interval. One data point is displayed for each animal ($n = 74$). The data without and with HR correction (RRSD/mean RR) are displayed in (**A,B**), respectively. Note that HR only accounted for less than 30% ($r^2 = 0.26$) of the variability before correction for HR. nu, normalized units following HR correction.

PHARMACOLOGICAL INTERVENTIONS—AUTONOMIC NEURAL BLOCKADE

Cardiac parasympathetic regulation was inhibited using the cholinergic (muscarinic receptor) antagonist atropine sulfate. As would be expected, this drug elicited significant increases in HR (pre-atropine, 113.2 ± 4.7; post-atropine, 189.8 ± 5.2 beat/min, $P < 10^{-6}$) and decreases in R-R interval (pre-atropine, 560.9 ± 33.6; post-atropine, 321.6 ± 8.5 ms, $P < 10^{-6}$). Atropine treatment also provoked significant reductions (both $P < 10^{-6}$) in R-R interval variability (**Figure 4A**) and HF variability (**Figure 4B**). After correction for prevailing HR, corrected R-R interval (**Figure 4A**) and corrected HF variability (**Figure 4B**) were still significantly (both $P < 10^{-5}$) reduced by atropine treatment.

In contrast, inhibition of cardiac sympathetic regulation using the non-selective β-adrenergic receptor antagonist propranolol HCl elicited significant reductions in HR (pre-propranolol, 114.0 ± 5.1; post-propranolol, 96.8 ± 2.6 beat/min, $P < 0.002$) and increases in R-R interval (pre-propranolol, 524.6 ± 26.6; post-propranolol, 631.6 ± 18.6 ms, $P < 0.05$). Propranolol treatment did not alter either R-R interval ($P < 0.58$, **Figure 5A**) or HF ($P < 0.88$, **Figure 5B**) variability before HR correction. However, after correction for the propranolol induced

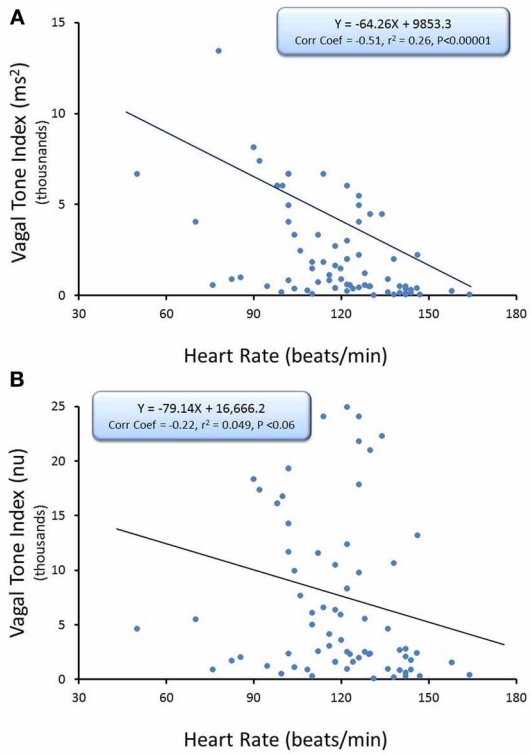

FIGURE 3 | The relationship between baseline heart rate and heart rate variability. The high frequency (HF) component of the R-R interval variability (cardiac vagal tone index, 0.24–1.04 Hz) was calculated for over the last 30 s before an autonomic intervention was administered and plotted against the average HR for that interval. One data point is displayed for each animal ($n = 74$). The data without and with HR correction [cardiac vagal tone index/(mean RRsec)^2] are displayed in **(A,B)**, respectively. Note that HR only accounted for less than 30% ($r^2 = 0.26$) of the variability before correction for HR. nu, normalized units following HR correction.

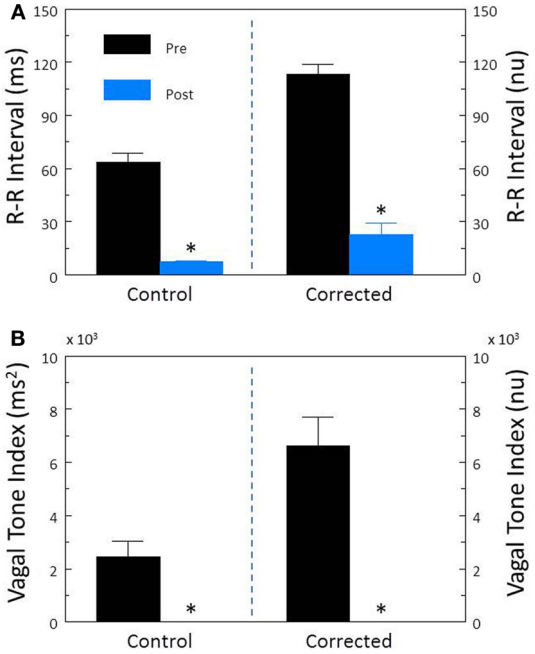

FIGURE 4 | The effect of the cholinergic receptor antagonist atropine on heart rate variability. The effect of atropine sulfate (50 μg/kg i.v.; $n = 25$) on total heart rate variability (standard deviation of R-R interval, RRSD) without and with correction (RRSD/mean RRsec) are displayed in **(A)**. The effects of this drug on the high frequency variability (cardiac vagal tone index, 0.24–1.04 Hz) without and with correction [cardiac vagal tone/(mean RRsec)^2] are shown in **(B)**. Note that despite correction for large increases in heart rate, atropine still provoked large reductions in both RRSD and the cardiac vagal tone index. Thus, cardiac parasympathetic activity is responsible for a large portion of the heart rate variability independent of changes in HR. $^*P < 0.01$ pre (black bars) vs. post (blue bars); pre = last 30 s before atropine administration, post = 30 s interval recorded 5 min after atropine treatment. nu, normalized units following HR correction.

reductions in HR, this drug provoked significant reductions in corrected R-R interval variability ($P < 0.007$, **Figure 5A**) but not in corrected HF variability ($P < 0.37$, **Figure 5B**).

Complete autonomic blockade (atropine + propranolol) provoked significant increases in HR (pre-treatment, 113.2 ± 4.7; post-treatment, 149.3 ± 5.8 beats/min, $P < 0.00007$) and reductions in R-R interval (pre-treatment, 560.9 ± 33.6; post-treatment, 415.2 ± 14.6 ms, $P < 0.0007$). Autonomic blockade also provoked significant reductions in R-R interval variability ($P < 10^{-6}$, **Figure 6A**) and HF variability ($P < 0.0003$, **Figure 6B**). After correction for HR, corrected R-R interval (**Figure 6A**) and corrected HF (**Figure 6B**) variability still significantly (both $P < 10^{-6}$) decreased following complete autonomic blockade. As the post-autonomic blockade HR was higher than baseline HR, these data indicate the presence of a tonic parasympathetic regulation of HR under basal conditions in the dog.

PHYSIOLOGICAL INTERVENTIONS—EXERCISE OR BARORECEPTOR REFLEX ACTIVATION

In agreement with previous studies (Billman and Hoskins, 1989; Billman and Dujardin, 1990; Houle and Billman, 1999; Billman, 2006, 2009) exercise elicited significant increases in HR (pre-exercise, 119.5 ± 3.8; peak-exercise, 181.7 ± 4.7 beats/min, $P < 10^{-6}$) and reductions in R-R interval (pre-exercise, 514.2 ± 16.2; peak-exercise, 336.1 ± 10.0 ms, $P < 10^{-6}$) that were accompanied by significant ($P < 10^{-6}$) reductions in both R-R interval (**Figure 7A**) and HF (**Figure 7B**) variability. After correction for the prevailing HR, exercise still provoked large reductions in both the corrected R-R variability ($P < 10^{-6}$, **Figure 7A**) and the corrected HF variability ($P < 10^{-6}$, **Figure 7B**; both).

The α-adrenergic receptor agonist, phenylephrine (PE) was used to increase arterial pressure (via vasoconstriction) and thereby reflexively augmented cardiac parasympathetic and reduced cardiac sympathetic neural activity (baroreceptor reflex activation). In agreement with previous studies (Billman et al., 1982; Billman and Dujardin, 1990), phenylephrine provoked significant decreases in HR (pre-PE, 122 ± 5.0; PE, 74.3 ± 4.0 beats/min, $P < 10^{-6}$) and increases in R-R interval (pre-PE, 507.3 ± 23.5; PE, 864.6 ± 58.1 ms, $P < 10^{-5}$) that were accompanied by significant (both $P < 10^{-6}$) increases in R-R interval (**Figure 8A**) and HF (**Figure 8B**) variability. After correction for

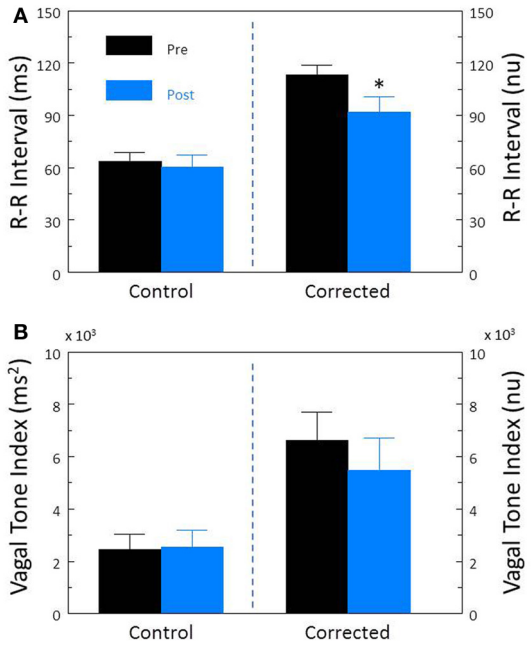

FIGURE 5 | The effect of the β-adrenergic receptor antagonist propranolol on heart rate variability. The effect of propranolol HCl (1.0 mg/kg i.v.; $n = 25$) on total heart rate variability (standard deviation of R-R interval, RRSD) without and with correction (RRSD/mean RRsec) are displayed in **(A)**. The effects of this drug on the high frequency variability (cardiac vagal tone index, 0.24–1.04 Hz) without and with correction [cardiac vagal tone/(mean RRsec)^2] are shown in **(B)**. Note that after correction for propranolol-induced reductions in baseline HR, total (RRSD) heart rate variability significantly decreased following this treatment. $^*P < 0.01$ pre (black bars) vs. post (blue bars); pre = last 30 s before propranolol administration, post = 30 s interval recorded 5 min after this drug treatment. nu, normalized units following HR correction.

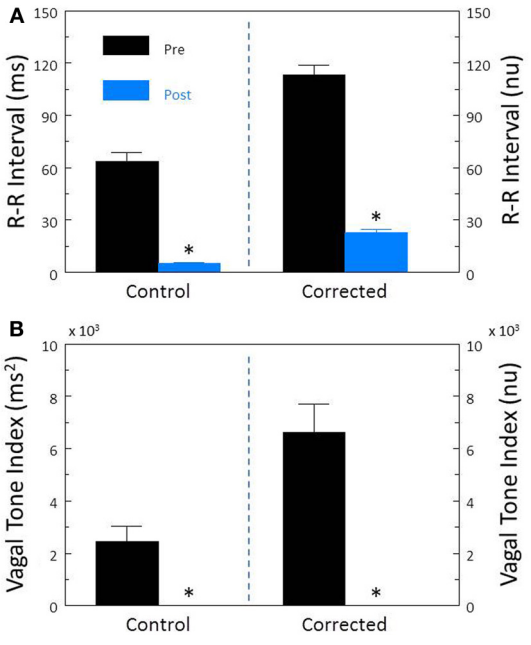

FIGURE 6 | The effect of total autonomic neural inhibition on heart rate variability. Cardiac autonomic blockade ($n = 25$) was achieved using the combination of a cholinergic receptor antagonist (atropine sulfate, 50 μg/kg i.v.) and a non-selective β-adrenergic receptor (propranolol HCl, 1.0 mg/kg i.v.). The effects of autonomic blockade on total heart rate variability (standard deviation of R-R interval, RRSD) without and with correction (RRSD/mean RRsec) are displayed in **(A)**. The effects of this treatment on the high frequency variability (cardiac vagal tone index, 0.24–1.04 Hz) without and with correction [cardiac vagal tone/(mean RRsec)^2] are shown in **(B)**. Note that despite correction for large increases in heart rate, this treatment still provoked large reductions in both RRSD and the cardiac vagal tone index. As baseline HR increased following autonomic blockade, these data indicate the presence of a tonic parasympathetic restraint of intrinsic HR under basal conditions in the dog. $^*P < 0.01$ pre (black bars) vs. post (blue bars); pre = last 30 s before atropine + propranolol administration, post = 30 s interval recorded 5 min after this drug treatment. nu, normalized units following HR correction.

the PE induced reductions in HR, baroreceptor activation produced similar increases in both corrected R-R interval ($P < 10^{-5}$, **Figure 8A**) and corrected HF ($P < 10^{-6}$, **Figure 8B**).

SURGICAL INTERVENTION—BILATERAL CERVICAL VAGOTOMY

In contrast to previous reports (Halliwill and Billman, 1992), anesthesia reduced baseline HRV. Although baseline HR was not affected by anesthesia (conscious 113.2 ± 4.7 vs. anesthesia 110.8 ± 7.4 beats/min; $P < 0.34$), both RRSD (conscious, 63.7 ± 5.0 vs. anesthesia, 34.2 ± 4.4 ms; $P < 0.001$) and HF variability (conscious, 6622.3 ± 1089.5 vs. anesthesia, 3090.6 ± 1382.8 ms^2, $P < 0.05$) were significantly lower in anesthetized ($n = 9$) as compared to conscious ($n = 33$) dogs. These differences in HRV were not altered by HR correction. Thus, anesthetic agents that were believed to have minimal effects on cardiac autonomic regulation (Halliwill and Billman, 1992) reduced HRV in the present study.

Disruption of the cardiac parasympathetic regulation by bilateral cervical vagotomy elicited significant increases in HR (pre-vagotomy, 110.2 ± 7.4; post-vagotomy, 186.0 ± 9.8 beat/min, $P < 0.00008$) and decreases in R-R interval (pre-vagotomy, 561.3 ± 37.5; post-vagotomy, 329.7 ± 17.0 ms, $P < 0.00004$) that were accompanied by significant reductions in both R-R interval ($P < 0.00009$, **Figure 9A**) and HF ($P < 0.0007$, **Figure 9B**) variability. After correction for HR, vagotomy still produced significant reductions in both corrected R-R interval ($P < 0.0002$, **Figure 9A**) and corrected HF variability ($P < 0.05$, **Figure 9B**). These results are very similar to those obtained following treatment with atropine sulfate and further demonstrate that cardiac parasympathetic activity is responsible for a major portion of the HRV, independent of changes in the prevailing HR.

DISCUSSION

The present study investigated the effects of well-characterized autonomic interventions on HRV with and without correction for the prevailing HR. The major findings of the study are as follows: (1) In agreement with previous studies (Kleiger et al., 1987; Van Hoogenhuyze et al., 1991; Fleiss et al., 1992; Sacha and Pluta, 2005a,b, 2008; Sacha et al., 2013a,b), there were significant inverse relationships between HR and both total

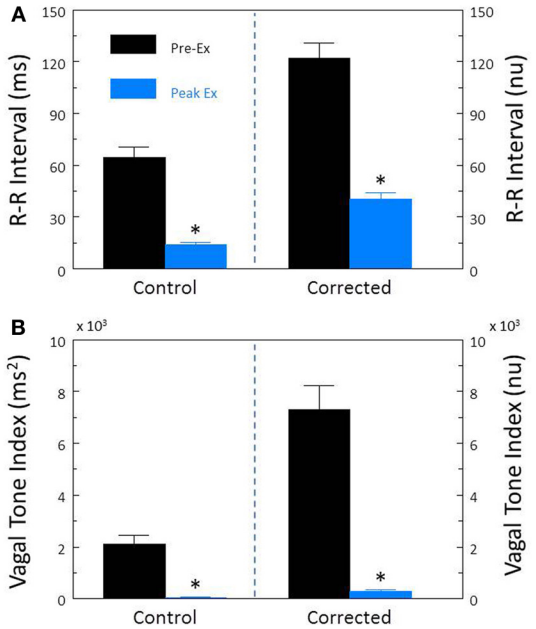

FIGURE 7 | Effect of submaximal exercise on heart rate variability. The effect of exercise ($n = 25$) on total heart rate variability (standard deviation of R-R interval, RRSD) without and with correction (RRSD/mean RRsec) is displayed in **(A)**. The effect of exercise on the high frequency variability (cardiac vagal tone index, 0.24–1.04 Hz) without and with correction [cardiac vagal tone/(mean RRsec)^2] are shown in **(B)**. Note that despite correction for large increases in heart rate, exercise provoked even large reductions in both RRSD and the cardiac vagal tone index than were noted before correction. The data were averaged over the last 30 s before exercise onset (Pre-Ex, black bars) and during the last 30 s of exercise (Peak Ex, blue bars) level. Peak exercise = 6.4 kph and 16% grade. $^*P < 0.01$ Pre-Ex vs. Peak Ex. nu, normalized units following HR correction.

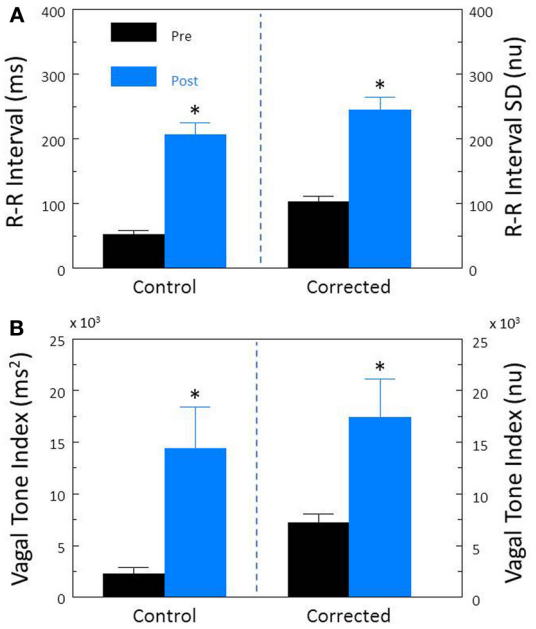

FIGURE 8 | The effect of activation of the baroreceptor reflex on heart rate variability. The α-adrenergic agonist phenylephrine HCl (10 μg/kg, i.v; $n = 20$) was used to increase arterial blood 30–50 mm Hg and thereby reflexively induce reductions in heart rate. The effects of this intervention on total heart rate variability (standard deviation of R-R interval, RRSD) without and with correction (RRSD/mean RRsec) are displayed in **(A)**. The effects of this treatment on the high frequency variability (cardiac vagal tone index, 0.24–1.04 Hz) without and with correction [cardiac vagal tone/(mean RRsec)^2] are shown in **(B)**. Note that both before and after correction for the phenylephrine-induced decreases in HR, baroreceptor reflex activation provoked significant increases in both RRSD and the cardiac vagal tone index. These data suggest that a reflexively mediated increase in cardiac parasympathetic activity is responsible for a large portion of the heart rate variability response to increases in arterial pressure independent of changes in HR. $^*P < 0.01$ pre (black bars) vs. post (blue bars); pre = last 30 s before phenylephrine administration, post = 30 s interval recorded 5 min after this physiological intervention. nu, normalized units following HR correction.

variability (R-R interval SD) and the variability within the HF band (0.24–1.04 Hz), an indirect marker of cardiac parasympathetic regulation (Appel et al., 1989; Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Denver et al., 2007; Thayler et al., 2010; Billman, 2011). However, HR accounted for less than 30% of the HRV; (2) division of the HRV indices by the mean R-R (reported in seconds) or mean R-R interval (in seconds) squared (Sacha and Pluta, 2005b) successfully removed variability due to HR (**Figures 2**, **3**) under basal conditions.; (3) Surgical (bilateral cervical vagotomy), pharmacological (cholinergic or complete autonomic blockade), and physiological (submaximal exercise) interventions that reduced or abolished cardiac parasympathetic regulation provoked large reductions in HRV even after correction for the accompanying increases in mean HR that were induced by these interventions; and (4) interventions that reduced HR yielded mixed results. β-adrenergic receptor blockade (propranolol) reduced rather than increased R-R interval variability after correction for the drug-induced HR reductions while, in contrast, increases in arterial blood pressure still provoked increases in both HF and R-R interval variability even after correction for the reflexively mediated reductions in HR. When considered together these data suggest that the physiological basis for HRV is revealed after correction for prevailing HR. These data further demonstrate that cardiac parasympathetic activity is responsible for a major portion of the HRV independent of changes in the prevailing HR and further that cardiac parasympathetic regulation provides a tonic restraint (inhibition) of the baseline pacemaker rate (i.e., the presence of a high basal vagal tone) in the dog.

As was previously noted, due to mathematical considerations, identical changes in HR can elicit profoundly different changes in R-R interval variability depending on the prevailing HR (larger changes at low, as compared to high, basal HR heart rates) independent of changes in cardiac neural activity (Sacha and Pluta, 2008). For example, a simulated set of heart beats with the same HR variability ($SD = \pm 1.6$ beats/min) at each HR level yielded markedly different values for R-R variability depending upon the prevailing HR ($HR = 30$ beats/min, RRSD = 105.9 ms vs. $HR = 300$ beats/min, RRSD = 1.1 ms; **Figure 1**). Similar, albeit

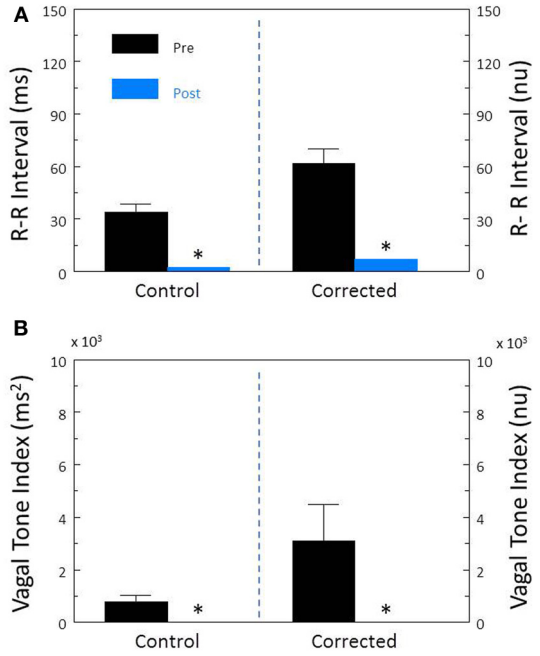

FIGURE 9 | The effect of bilateral cervical vagotomy on heart rate variability. The effect of surgical disruption of the vagus nerves ($n = 9$) on total heart rate variability (standard deviation of R-R interval, RRSD) without and with correction (RRSD/mean RRsec) are displayed in **(A)**. The effects of this interval on the high frequency variability (cardiac vagal tone index, 0.24–1.04 Hz) without and with correction [cardiac vagal tone/(mean RRsec)^2] are shown in **(B)**. Note that despite correction for large increases in heart rate, the vagotomy still provoked large reductions in both RRSD and the cardiac vagal tone index. These changes are very similar to those noted after treatment with the cholinergic receptor antagonist atropine. Thus, the vagotomy data further indicate that cardiac parasympathetic activity is responsible for a large portion of the heart rate variability independent of changes in HR $^*P < 0.01$ pre (black bars) vs. post (blue bars); pre = last 30 s before vagotomy, post = 30 s interval recorded 5 min after this treatment. nu, normalized units following HR correction.

less dramatic, results have been reported for data obtained from healthy subjects and in patients following myocardial infarction or with congestive heart disease (Kleiger et al., 1987; Van Hoogenhuyze et al., 1991; Fleiss et al., 1992). Indeed, a strong inverse correlation between HR and various time domain indices of HRV (e.g., the SD of normal beats, SDNN) was reported in these patient populations. Frequency domain analysis of HRV is similarly affected by mean HR. Sacha and co-workers (Sacha and Pluta, 2005a,b, 2008; Sacha et al., 2013a,b) demonstrated that the HF component of HRV was inversely, while the LF component was directly, related to average baseline HR of the subject. In agreement with these studies, similar results were obtained for the dog in the present study. Under basal conditions, both total variability (R-R interval SD) and the variability within the HF band (0.24–1.04 Hz) increased as HR decreased. However, only about 30% of this variability could be attributed to HR, demonstrating that other factors must also contribute to this variability. Thus, it is critical to remove the HR contribution from indices of the HRV in order to identify any physiological components to this variability. This HR correction is particularly important when cardiac autonomic neural regulation is altered, as the activation or inhibition of these cardiac nerves will produce corresponding changes in the prevailing HR, thereby making it difficult to discern the direct autonomic neural contribution to HRV under these conditions.

Recently, Sacha and co-workers (Sacha and Pluta, 2005b; Sacha et al., 2013a,b) demonstrated that division by corrections factors weakened the HR dependence of HRV. In particular, they found that the mathematical contribution to R-R interval (RRSD) and HF variability could be removed by dividing these variables by the corresponding mean R-R interval and (mean R-R)^2, respectively. These earlier observations in human subjects were confirmed for healthy dogs in the present study, as these correction factors eliminated the correlation with prevailing HR under basal conditions (**Figures 2**, **3**). Using these correction procedures, it was then possible to evaluate the effects of autonomic interventions on HRV that arise independent of changes in HR.

Interventions that reduce cardiac parasympathetic activation provoke HR increases and could, thereby, exaggerate the resulting reductions in HRV. In the present study, both pharmacological and surgical disruption of the cardiac parasympathetic nerves produced similar increases in HR and reductions in HRV. Exercise [a physiological challenge known to decrease cardiac parasympathetic and increase cardiac sympathetic activity (Billman, 2009)] also provoked large increases in HR that were accompanied by decreases in HRV. However, the HRV response to these interventions was not altered by correction for prevailing HR. These data strongly suggested that, even after correction for HR, cardiac parasympathetic regulation was responsible for a major portion of the reduction in indices of HRV provoked by these interventions. In a similar manner, the HRV reductions that resulted from complete autonomic blockade were not altered by correction for HR. As the prevailing HR increased following this treatment, these data further suggest that cardiac parasympathetic regulation provides a tonic inhibition of the basal HR in the dog.

In contrast to interventions that increased HR, autonomic interventions that reduced HR yielded mixed results. β-adrenergic receptor blockade (propranolol) did not alter HRV despite reductions in HR. However, after correction for drug-induced HR reductions, total (RRSD), but not HF, variability significantly decreased. For mathematical reasons, as previously discussed, one would expect that any intervention that decreases HR would produce increases in HRV. Thus, it is initially surprising that HF variability did not change and R-R interval variability decreased rather than increased as the result of propranolol treatment. There are at least two possible explanations for these observations: (1) Sympathetic neural activity could modulate the HF component of the R-R interval variability (Taylor et al., 2001; Cohen and Taylor, 2002). Taylor et al. (2001) found that cardioselective β-adrenergic receptor blockade increased the amplitude of respiratory sinus arrhythmia [a widely used marker of cardiac parasympathetic activity (Billman, 2011)] over a wide range of respiratory frequencies (i.e., the increases were not restricted to lower frequencies, <0.15 Hz). Thus, they concluded that "*cardiac sympathetic outflow can oppose vagally mediated R-R interval oscillations and sympathetic blockade removes this effect*"

(Cohen and Taylor, 2002). However, since β-adrenergic receptor blockade decreased rather than increased R-R interval variability and did not alter HF variability in the present study, it is unlikely that the removal of a sympathetic restraint on cardiac vagal regulation can explain this observation. Indeed, it is possible that the HRV increases reported by Taylor and associates following β-adrenergic receptor blockade resulted as a mathematical consequence of declining HR rather than from the removal of any sympathetic "restraint" of cardiac parasympathetic regulation. (2) It is much more likely that inhibition of the cardiac sympathetic activity provoked the withdrawal of cardiac parasympathetic activity in order to maintain a more constant cardiac output. If this hypothesis is correct, then would one predict that this putative parasympathetic withdrawal should become more obvious during physiological challenges that increase tissue oxygen demand (that must be matched by increased oxygen delivery). In fact, both submaximal exercise and acute myocardial ischemia provoked much larger reductions in HF variability, despite smaller increases in HR, following β-adrenergic receptor blockade (Billman and Hoskins, 1989; Collins and Billman, 1989; Billman and Dujardin, 1990; Billman, 2006). Finally, and in contrast to β-adrenergic receptor blockade, the increase in HRV (both RRSD and HF) elicited in response to the increases in arterial blood pressure (induced by phenylephrine) was not altered after correction for the reflexively mediated reductions in HR. These data further suggest that an augmentation of cardiac parasympathetic activity can increase HRV independent of reflex mediated reductions in HR.

In conclusion, the present study demonstrates that prevailing HR can dramatically affect HRV with HRV increasing as HR decreases. The HR dependence of HRV becomes particularly important as HR changes in response to the activation or inhibition of cardiac autonomic neural regulation. It is, therefore, essential to correct HRV for the average HR in order to differentiate between physiologically- and mathematically- mediated changes in HRV. For the dog, as has been previous shown for human subjects (Sacha and Pluta, 2005b), R-R (as measured by SD) and HF variability can be corrected by division by mean R-R interval (in seconds) and (mean R-R interval, in seconds)^2, respectively. HR correction did not attenuate the HRV response to interventions that inhibited cardiac parasympathetic regulation. As such, these data demonstrate that cardiac parasympathetic activity is responsible for a major portion of the HRV independent of changes in the prevailing HR. In contrast, interventions that reduced HR yielded mixed results after HR corrections. β-adrenergic receptor blockade decreased rather than increased some HRV indices after correction for HR suggesting that this treatment provoked compensatory reductions in cardiac parasympathetic activity (to maintain a more constant cardiac output in the face of changing environmental demands). In contrast, even after correction for baroreceptor reflex mediated reductions in HR, increases in arterial pressure still provoked large increases in HRV (both RRSD and HF variability). These data suggest that baroreceptor reflex mediated increases in are HRV largely result from the direct cardiac actions of parasympathetic activation. When considered together, these data are further evidence that HRV provides an indirect and largely qualitative assessment of cardiac parasympathetic regulation; an assessment that must also be corrected for prevailing HR. Since an accurate assessment of nerve activity can only be obtained from direct nerve recordings, HRV data should always be interpreted with care.

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A comparison between heart rate and heart rate variability as indicators of cardiac health and fitness

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Quantification of cardiac autonomic activity and control via heart rate (HR) and heart rate variability (HRV) is known to provide prognostic information in clinical populations. Issues with regard to standardization and interpretation of HRV data make the use of the more easily accessible HR on its own as an indicator of autonomic cardiac control very appealing. The aim of this study was to investigate the strength of associations between an important cardio vascular health metric such as VO_2max and the following: HR, HRV indicators, and HR normalized HRV indicators. A cross sectional descriptive study was done including 145 healthy volunteers aged between 18 and 22 years. HRV was quantified by time domain, frequency domain and Poincaré plot analysis. Indirect VO_2max was determined using the Multistage Coopers test. The Pearson correlation coefficient was calculated to quantify the strength of the associations. Both simple linear and multiple stepwise regressions were performed to be able to discriminate between the role of the individual indicators as well as their combined association with VO_2max. Only HR, RR interval, and pNN50 showed significant ($p < 0.01$, $p < 0.01$, and $p = 0.03$) correlations with VO_2max. Stepwise multiple regression indicated that, when combining all HRV indicators the most important predictor of cardio vascular fitness as represented by VO_2max, is HR. HR explains 17% of the variation, while the inclusion of HF (high frequency HRV indicator) added only an additional 3.1% to the coefficient of determination. Results also showed when testing the normalized indicators, HR explained of the largest percentage of the changes in VO_2max (16.5%). Thus, HR on its own is the most important predictor of changes in an important cardiac health metric such as VO_2max. These results may indicate that during investigation of exercise ability (VO_2max) phenomena, quantification of HRV may not add significant value.

Keywords: autonomic cardiac control, prognostic indicators, exercise ability

INTRODUCTION

It is known that cardiovascular disease (CVD) has reached pandemic proportions worldwide. It is identified as one of the most important causes of death in several countries including USA, Pacific and Middle Eastern countries, China and Europe (Barakat et al., 2012; Go et al., 2013). Factors, contributing to this pandemic is: smoking, physical activity/fitness, blood pressure, blood glucose, total cholesterol levels, weight, and diet. Of these factors, also called cardio vascular health metrics (Yang et al., 2012), the level of activity and fitness is a relative easy way to modify a person's CVD risk factor. However, low physical activity levels is also one of the most common problems identified across different age group contributing toward the CVD pandemic (Barakat et al., 2012). It is known that an increase in fitness/exercise capacity measured by VO_2max, results in a reduction in CVD risk (Barakat et al., 2012). Active individuals present half the coronary artery disease risk compared to a very inactive group. Exercise and increased fitness not only help to prevent disease risk factors but can also treat existing risk factors such as high insulin levels, hypertension, hyperlipidaemia and obesity.

The link between cardiac autonomic neuropathy (increased sympathetic, decreased parasympathetic activity or a combination of the two), and CVD is well established (Lahiri et al., 2008). Quantification of cardiac autonomic activity and control via heart rate (HR) and heart rate variability (HRV) is known to provide prognostic information in clinical populations. HR is used as a CV mortality risk factor indicator and HRV as a predictor of disease outcome (Kannel et al., 1987; Bravi et al., 2011; Sacha et al., 2013).

Quantification of HRV, integrating the sympathetic and parasympathetic cardiac influences, is a complex measurement of the autonomic nervous system and its responses to internal and external stimuli (Lahiri et al., 2008). Issues with regards to standardization and interpretation (Grant et al., 2011) of HRV data make the use of the more easily accessible HR on its own as an indicator of autonomic cardiac control very appealing. It is important to establish if the more complex concept of HRV determination adds prognostic value above HR measurements. In an effort to quantify the contribution of HR to the prognostic value of HRV, the aim of this study was to investigate the strength of associations between an important cardio vascular health metric

such as VO_2max and HR, as well as between VO_2max and HRV indicators including RMSSD, pNN50, LF, HF, LF/HF, SD1, and SD2 (abbreviations explained in **Table 1**). HR normalized HRV indicators were also calculated and used as regressors to determine if HR independent variability shows similar associations with VO_2max than standard HRV indicators. Regression analyses were employed to determine the relative importance of HR and HRV indicators as predictors of an important cardio vascular health metric such as VO_2max.

The primary hypothesis was that HR and RR intervals show stronger correlations with VO_2max than the HRV indicators, and are on their own stronger predictors of VO_2max than the more complex indicators of HRV (RMSSD, pNN50, LF, HF, LF/HF, SD1, and SD2). A secondary hypothesis was that, when including HR and HR normalized indicators of variability (RMSSD/RR, LF/RR^2, HF/RR^2, LF/HF, SD1/RR, SD2/RR) regression analysis will show that HR is still the most important predictor of VO_2max.

MATERIALS AND METHODS

BACKGROUND AND DETERMINATION OF THE HEALTH METRIC VO_2MAX

The gold standard in fitness testing is generally regarded as the VO_2max test for measuring aerobic fitness or cardiorespiratory endurance. VO_2max is defined as the maximum volume of oxygen that can be utilized in 1 min during maximal exercise. It is measured in millilitres of oxygen used in 1 min per kilogram of body weight, (ml/kg/min) (Wilmore and Costill, 2004). Elite endurance athletes are known to have high VO_2max values and it is thought that the more oxygen the body can use during strenuous exercise, the more energy it can produce (Wilmore and Costill, 2004).

VO_2max can be measured directly or indirectly. The direct measurement needs to be done in a laboratory and is expensive, time consuming and requires special expertise. The test subject must reach his/her maximum work capacity in order to determine VO2 max accurately. The test is done on a treadmill or a bicycle and a strict protocol is followed. The athlete is required to exercise while the speed and intensity is gradually increased. The volume and oxygen concentration of the inhaled and exhaled air of the athlete is measured to determine how much oxygen is used. The oxygen consumption usually rises in direct relationship to the increase in exercise intensity up to a certain point. At this point the oxygen consumption reaches a plateau and does not rise further even with a further rise in intensity. This plateau marks the VO_2max.

VO_2max can also be determined indirectly, as an estimate of the true VO_2max. The Cooper 12 min run test was used in the current study. This assessment uses a set period of time (12 min) and scoring according to distance (in meters). The participants were asked to run or walk for 12 min as fast as possible (Cooper, 1968). This test was performed on a 400 m tartan athletics track. A warm-up of light aerobic activities and flexibility exercises was performed for 5 min before the start of the test. Testers were placed at 100 m intervals around the track as a source of verbal motivation. A prediction of VO_{2max} from the distance covered at the end of the 12 min period was obtained by applying the distance run to the Cooper regression equation: VO_2max (ml/kg/min) = 0.0268 (distance covered in meters)—11.3 (Cooper, 1968).

The sedentary person has a much lower VO_2max generally compared to the active fit individual, with the elite endurance athlete having the highest VO_2max. Genetics play a role in an individual's exercise ability but VO_2max can be improved with physical training. The more unfit, the more the VO_2max can be improved. As exercise capacity improves, skeletal muscle strength and endurance also improve (Paterson et al., 2004; Stringer, 2010).

Table 1 | HRV indicators defined.

HRV indicator	Definition
Mean RR (s)	The mean of the intervals between successive QRS complexes
RMSSD (ms)	Root mean square of the standard deviation between RR intervals
pNN50 (%)	The percentage of successive RR interval differences over the entire measurement larger than 50ms
LF Power (ms^2)	Peak between 0.04 and 0.15Hz
HF Power (ms^2)	Peak between 0.15 and 0.5Hz
LF/HF	LF Power (ms^2) divided by HF Power (ms^2)
SD1 (ms)	Indicator of the standard deviation of the instantaneous RR variability
SD2 (ms)	Indicator of the standard deviation of the continuous or long term variability of the heart rate

STUDY DESCRIPTION

A hypotheses driven, cross sectional, descriptive study was performed. The study protocol was submitted and approved by the Ethics Committee of the University. A total of 145 healthy participants from a group of 235 volunteers were accepted to take part in the research study. Exclusion criteria included refusal to give voluntary written informed consent; a history of cardiovascular, hepatic, respiratory, or renal impairment, as well as pulmonary, metabolic, and orthopaedic diseases requiring medical attention; lung/respiratory tract infection in the previous 2 weeks; and medication that could influence cardiovascular control and psychological disorders. None of the participants were professional athletes or high-level sport participants. All participants gave written informed consent before commencement of the intervention. Participants fasted overnight and were asked not to use any caffeine, alcohol or to smoke 24 h prior to the measurements. Measurements were taken in a temperature regulated, quiet environment, in the morning before 12H00. The Polar 810E HR monitor system was used to record supine RR intervals for a 10 min period. The 5 to 10 min period of this recording was used to quantify the HRV. Computer software from the University of Kuopio, Finland calculated the HRV indicator values by time domain analysis (RR, STDRR, RMSSD, and pNN50), frequency domain analysis (LF, HF, and LF/HF), and Poincaré plot analysis (SD1 and SD2) (Mourot et al., 2004). HRV indicators determined are listed in **Table 1**; Mourot et al., 2004).

STATISTICAL ANALYSES

The Pearson's correlation coefficient (r) was calculated. Correlation strength were defined as very low if rho is smaller than 0.2; low to moderate if rho is larger than 0.2 but smaller than 0.4; and moderate if rho is larger than 0.4 but less than 0.6 (Landis and Koch, 1977). The level of significance was set at the conventional 5%, thus, when p-values were less than 0.05, the associations were identified as statistically significant.

To test the hypotheses, linear regression was used to determine the most important predictors of changes in cardio vascular fitness and exercise ability as represented by VO_2max. Indicators included were: HR, RR interval, RMSSD, pNN50, LF, HF, LF/HF, SD1, SD2. The following HR normalized HRV indicators were also included in a separate set of regression analyses: RMSSD/RR, LF/RR^2, HF/RR^2, LF/HF, SD1/RR, and SD2/RR. Both simple linear and multiple stepwise regressions were performed to be able to discriminate between the role of the individual indicators as well as their combined relationship with VO_2max (Field, 1994).

The HR, RR intervals, and HRV indicators (normalized and non-normalized) were compared to the VO_2max of participants results, to establish any significant relationships (**Table 4**).

The results shown in **Table 4** indicate that only one HRV indicator value, pNN50, correlated significantly with VO_2max. When indicators were HR normalized by dividing by RR or RR^2, no significant correlations were found. All significant correlation coefficients were between 0.19 and 0.41 indicating very low to moderate associations.

Simple linear regression analyses (**Table 5**) showed that only HR, RR, and pNN50 explained a significant ($p < 0.05$) proportion of the variation in VO_2max. Stepwise multiple regression (**Table 5**) indicated that, when including all nine indicators (HR, RR, RMSSD, pNN50, LF, HF, LF/HF, SD1, SD2), the statistical model containing a linear combination of HR and HF, explained the largest proportion of the variation, i.e., is the best predictor of variation in VO_2max 20.1%). However, HR entered the model first and by itself explains 17% of the variation, while the

RESULTS

A summary of the participants ($N = 145$) and HRV indicator values are displayed in **Tables 2**, **3**.

Table 2 | Median, mean and standard deviation (*SD*) of participant body mass index (BMI) and exercise capacity (VO_2max).

	Mean	*SD*	Median
BMI (kg/m^2)	23.07	2.36	22.62
VO_2max (ml/kg/min)	49.54	8.79	53.53
2.4 km run time (s)	733.71	179.10	661.50

Table 3 | Mean, standard deviation (*SD*), median and inter quartile range of HRV indicators and normalized HRV indicators.

	Mean	*SD*	Median	Inter quartile range(Q1;Q3)
Mean RR (ms)	894.10	137.03	884.50	186.25 (791.00;977.25)
Mean HR (beats per minute)	69.22	10.5	68.78	14.61 (61.83;76.44)
RMSSD (ms)	76.04	44.84	65.00	53.25 (43.40;96.65)
pNN50 (%)	39.08	22.84	38.30	39.85 (18.40;59.25)
LF (ms^2)	1148.19	1291.74	999.00	1336 (582.50;1918.50)
HF (ms^2)	2760.15	3465.31	1721.00	2627 (582.50;1918.50
LF/HF	0.81	0.58	0.67	0.74 (0.35;1.09)
SD1(ms)	54.06	31.83	46.10	37.80 (30.95;68.75)
SD2(ms)	90.48	37.17	83.10	51.60 (64.00;115.60)
NORMALIZED HRV INDICATORS				
RMSSD/RR	0.08	0.04	0.07	0.05 (0.05;0.10)
LF/RR^2	0.0017	0.0013	0.0012	0.001 (0.001;0.002)
HF/RR^2	0.0031	0.0034	0.0020	0.002 (0.001;0.003)
SD1/RR	0.060	0.030	0.050	0.04 (0.03;0.07)
SD2/RR	0.10	0.040	0.090	0.05 (0.07;0.12)

Table 4 | Pearson's correlation coefficients (r) and significance (p-value) for VO_2 max and HRV indicators.

HRV Indicators	Indirect VO_2max r (p-value)	Normalized HRV indicators	Indirect VO_2max r (p-value)
Mean RR	0.41(< 0.01)*	NA	NA
Mean HR	−0.41 < 0.01*	NA	NA
RMSSD	0.09 (0.29)	RMSSD/RR	0.02 (0.80)
pNN50	0.19 (0.03)*	NA	NA
LF	0.10 (0.21)	LF/RR^2	0.02 (0.95)
HF	0.001 (0.99)	HF/RR^2	−0.03 (0.72)
LF/HF	0.02 (0.82)	NA	NA
SD1	0.09 (0.29)	SD1/RR	0.02 (0.80)
SD2	0.14 (0.10)	SD2/RR	0.02 (0.79)

*NA, Not applicable; *, significant: $p < 0.05$.*

Table 5 | Simple and stepwise multiple linear regression results.

Regression type	Predictors of VO_2max included	Significant predictors ($p < 0.05$)	Coefficient of determination R^2
Simple Regression	HR	HR	0.170
Simple Regression	RR	RR	0.161
Simple Regression	pNN50	pNN50	0.035
Multiple Regression	Nine indicators HR,RR,RMSSD, pNN50,LF, HF, LF/HF, SD1, SD2	HR and HF	0.201
Multiple Regression	HR and six normalized HRV indicators: HR/RR, RMSSD/RR, LF/RR^2, HF/RR^2, LF/HF, SD1/RR, SD2/RR	HR and HF/RR^2	0.190

inclusion of HF added only an additional 3.1% to the coefficient of determination. Stepwise multiple regression also showed that, when including HR and all normalized HRV indicators (RMSSD/RR, LF/RR^2, HF/RR^2, LF/HF, SD1/RR, SD2/RR), the statistical model consisting of a linear combination of HR and HF/RR^2, will be the best predictor of variation in VO_2max, that is 19.0%. HR on its own explained 16.5% and a combination of HR and HF/RR^2 thus, explained only a further 2.5%.

DISCUSSION

The primary hypothesis was that straightforward measurements such as HR and RR intervals show stronger correlations with VO_2max than the HRV indicators, and is on their own stronger predictors of VO_2max than the more complex indicators of HRV. Study results partially confirmed the first hypotheses. Moderately strong correlations were found between HR and RR intervals vs. VO_2max. The only other correlation found (pNN50) was of very low strength ($r = 0.19$). The stepwise multiple regression analysis indicated that HR predicted 17%, and the combination of HR and HF predicted 20.1% of the variation in VO_2max, when entering the following HRV indicators: HR, RR, RMSSD, pNN50, LF, HF, LF/HF, SD1, SD2. With regards to the second hypothesis multiple *r*egression analysis showed that the combination of HR and HF/RR^2 explained only 2.5% more of the variation in VO_2max, than HR alone (16.5%), when including all normalized indicators.

Recent publications by Sacha et al. (2013) suggested that, because of physiological and mathematical relationships between HR and HRV, the dependence of HRV indicator values on HR can be modified by mathematical manipulation. This is due to the known fact that RR variation is significant higher for a slow average HR than for higher mean HR. Following suggestions by Sacha et al. (2013) to minimize the HRV indicator's dependence on HR, correlational and regression statistical analyses in this study were performed firstly with the actual HR, RR, and HRV indicator values and again after each were normalized by dividing by the mean RR or RR^2.

Correlations found between the HR, RR intervals, pNN50, and VO_2max were significant ($p < 0.05$), but of very low to medium strength ($0.19 \leq r \leq 0.41$). A significant positive correlation was found between mean RR interval and VO_2max ($p < 0.01$; $r = 0.41$) and as expected a significant negative correlation between mean HR and VO_2max ($p < 0.01$; $r = -0.41$). These associations concur with some previous study findings. Higher fitness is associated with a lower HR, higher VO_2max, and also higher vagal cardiac control (Nagai et al., 2004; Spierer et al., 2007; Gilder and Ramsbottom, 2008). The strength of the associations found in this study are lower than values reported in literature but this may be due to the fact that VO_2max was only indirectly determined and not directly with gas analyses.

The fact that only one HRV indicator (pNN50) correlated significantly with VO_2max, may indicate that correlations between HRV indicators and VO_2max exist mainly due to the relationship between HR (independent of variability) and VO_2max.

Regression analysis confirmed that, when including HR, RR, RMSSD, pNN50, LF, HF, LF/HF, SD1, and SD2, the most important predictor of cardio vascular fitness and exercise ability as represented by VO_2max, is HR. Results also showed when testing the normalized indicators, HR explained the largest percentage of the changes in VO_2max. Thus, HR on its own is the most important predictor of changes in an important cardiac health metric such as VO_2max. Thus, the more complex concept of HRV quantification unlock only a small percentage of additional information with regards to autonomic function, compared to information obtained from only HR and RR interval measurements. These results may indicate that during investigation of exercise ability (VO_2max) phenomena, quantification of HRV may not add significant more value. This is a novel idea which should be investigated further in clinical populations.

Limitations of this study include the fact that VO_2max was not directly determined with gas analyses, but only indirectly determined. Another drawback is that the tachogram used to determine HRV, was sampled only in a resting, supine position. Previous studies indicated that HRV measured during a stressor, such as standing upright, show more significant correlations with cardiopulmonary fitness indicators than supine HRV measurements (Grant et al., 2009). For future studies it is recommended to investigate participants from other age groups as well as clinical groups to answer the question whether these results are only applicable to healthy young participants and if not; how it differs in other groups.

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Interplay between heart rate and its variability: a prognostic game

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Keywords: heart rate, heart rate variability, heart rate dynamics, prediction, correlation, risk factor

In the last decades, heart rate (HR) and heart rate variability (HRV) were extensively investigated in various clinical and laboratory settings and proved to be significant risk factors for different outcomes and patients' populations (Kannel et al., 1987; Bravi et al., 2011; Antoni et al., 2012). During that time, a number of new methods permitting to explore different aspects of HR variability and dynamics have been implemented (Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology, 1996; Schmidt et al., 1999; Bauer et al., 2006). However, most of them yield parameters which are essentially associated with HR (Tsuji et al., 1996; Cygankiewicz et al., 2004; Sacha and Pluta, 2005, 2008; Lewek et al., 2009; Sacha, 2013), therefore, they actually carry information not only on the variability but also on HR itself (Sacha, 2014a,b). In fact it is hard to ascertain which of these two (i.e., HR or variability) really matters in the prognostic significance of HRV (Sacha, 2013, 2014a,b). Recently, a new approach which enables to weaken (or even eliminate) or strengthen the association between HR and its variability has been proposed (Sacha et al., 2013a). The principles of this method are simple, i.e., by division of RR interval tachograms by the corresponding average RR intervals, the variability of RR intervals of slow HR is attenuated, but that of fast HR is relatively amplified and the resulting HRV loses its correlation with HR. On the other hand, by multiplication of RR interval tachograms by their average RR intervals, the variability of slow HR is boosted but that of fast HR is relatively suppressed, and consequently, the correlation between HRV and HR is growing. By division or multiplication by higher powers of average RR intervals stronger effect on the HRV/HR relationship can be achieved (Sacha et al., 2013a). Moreover, by division by very high powers, this relationship may even be inverted, i.e., from negative to positive one (**Figure 1A**) (Sacha et al., 2013b).

Recent studies with implementation of this method have shown that HR may have different impact on the prognostic ability of HRV for different outcomes (Sacha et al., 2013c). In general, it seems that for populations and events where HR is a significant risk factor the enhancement of its impact improves the prognostic value of HRV, however, for groups and outcomes where HR is not or is a weak risk factor, the exclusion of its influence increases the HRV prediction capacity (Sacha et al., 2013c, 2014; Sacha, 2014a,b). In particular, such phenomena were observed in the study addressing HRV and HR in different genders after myocardial infarction (Sacha et al., 2014). In other words, HR was a strong risk factor of cardiac death in men and strengthening its influence on HRV boosted the HRV prediction performance for cardiac mortality, conversely, HR was a poor predictor of non-cardiac death in male subgroup and weakening its impact augmented the HRV prognostic value for non-cardiac mortality. However, HR did not predict any outcomes in females and the exclusion of its influence improved the HRV prognostic ability for every mode of death in women (**Figure 1B**) (Sacha et al., 2014).

The concept of such prognostic HRV and HR interaction has been recently confirmed in a large group of patients undergoing exercise tests (i.e., 1288 participants) (Pradhapan et al., 2014). The study showed that HR right before exercise was not a risk factor of death and elimination of its influence improved the predictive capability of the respective HRV, conversely, HR during recovery phase was a significant mortality predictor and the enhancement of its impact augmented the respective HRV prognostic performance (Pradhapan et al., 2014).

Low HRV and high HR are usually associated with worse prognosis, however, there are also situations where such a coincidence represent favorable prospect—this can be seen during exercises (Dewey et al., 2007). In the aforementioned study by Pradhapan et al., higher HRV and lower HR (i.e., below 125 bpm) during recovery phase were related with an increased risk of both cardiac and non-cardiac death (Pradhapan et al., 2014). However, if one inverts the HRV/HR relationship from negative to positive one (i.e., by division of HRV indices by high powers of their corresponding average RR intervals), higher HRV represents good prognosis—**Figure 1C** (Pradhapan et al., 2014).

Thus, the interplay between HRV and HR turns out to be quite complicated. However, the unraveling of this remarkable game, by using the method of strengthening or weakening the HRV/HR dependence (Sacha et al., 2013a), may yield valuable prognostic information (Sacha et al., 2013c, 2014; Pradhapan et al., 2014; Sacha, 2014a,b). This is particularly important in women, in whom

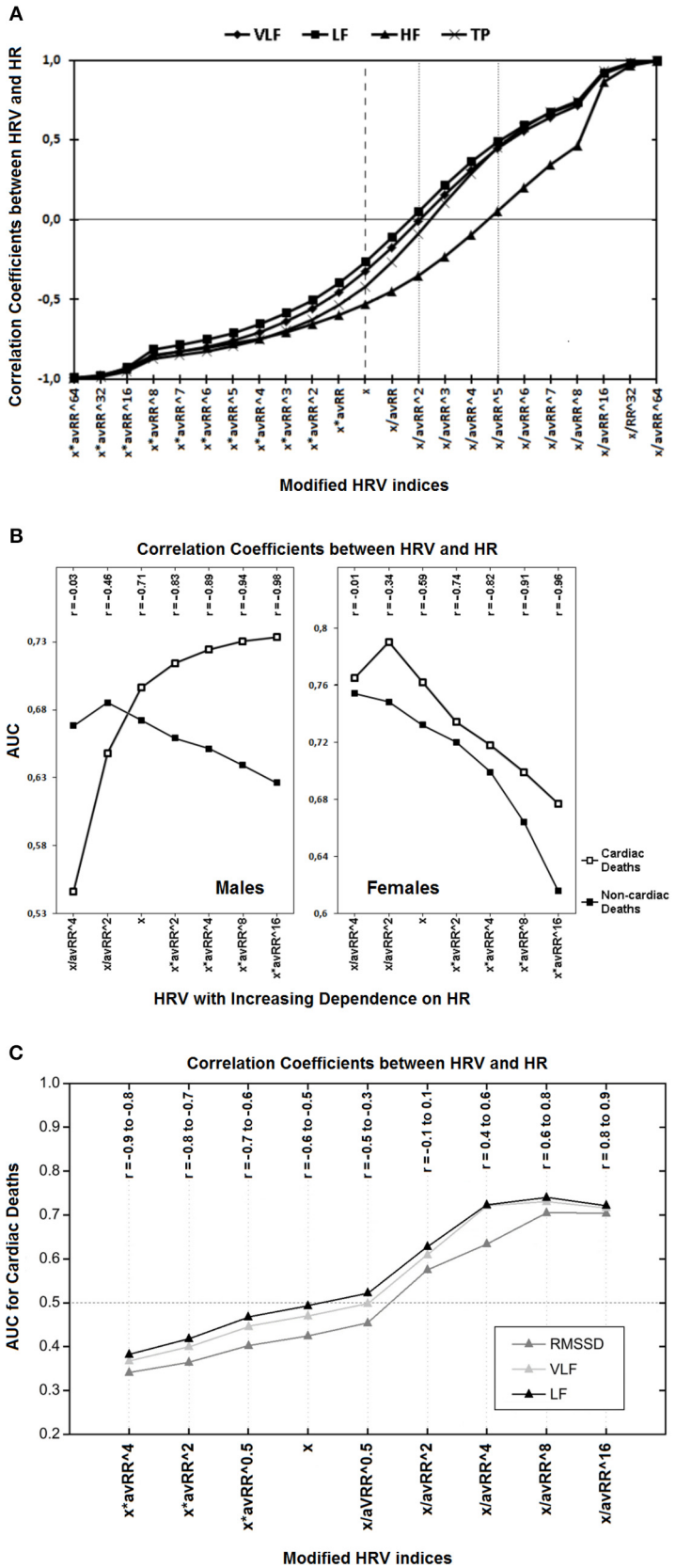

FIGURE 1 | Continued

FIGURE 1 | (A) Correlation coefficients between different modified spectral HRV indices and HR are presented. The "x" denotes standard HRV indices which all are inversely associated with HR; multiplication by different powers of the corresponding average RR interval (avRR) increases this negative relationship (the left half of the diagram); but division by avRR to different powers diminishes the HRV dependence on HR and may even inverse this relationship, i.e., from negative to positive one (the right half of the diagram). Of note, to become HR-independent (i.e., to achieve insignificant correlation coefficients) VLF, LF and TP have to be divided by avRR^2, but HF by avRR^5 (see respective markers on dotted lines)—this is due to the fact that HF is initially more dependent on HR (compare respective markers on the dashed line). HF, high frequency component; LF, low frequency component; VLF, very low frequency component; TP, total power (Reprinted from Sacha et al., 2013b). **(B)** The prediction performance (i.e., AUC, area under receiver-operator characteristic curves) for different classes of modified HRV indices (i.e., very low frequency components of HRV spectrum) and their correlation coefficients (r) with HR in males and females are depicted. The "x" denotes standard HRV indices, other indices were calculated by division or multiplication of "x" by different powers of the corresponding average RR interval (avRR). In the first two classes (i.e., x/avRR^4 and x/avR^2), the HRV dependence on HR was weakened, while it was strengthened in the last four classes (i.e., x*avRR^2, x*avRR^4, x*avRR^8 and x*avRR^16). As HRV is becoming more dependent on HR (i.e., from the first to the seventh class), its predictive ability increases in men for cardiac death but decreases for non-cardiac one, while in women, it decreases for both outcomes. It is noteworthy that HR was a strong predictor of cardiac death and a weak predictor of non-cardiac death in males, however, in females HR did not predict any mode of death (Reprinted with modification from Sacha et al., 2014). **(C)** Predictive performance for cardiac mortality (i.e., AUC, area under receiver-operator characteristics curves) for different classes of modified HRV indices and their correlation coefficients (r) with HR, during the recovery after exercise test are presented. The "x" denotes standard HRV indices, other indices were calculated by multiplication or division of "x" by different powers of the corresponding average RR interval (avRR). The AUC < 0.5 indicates that higher HRV is related with worse prognosis but AUC > 0.5 means that higher HRV is associated with better prognosis. Standard HRV indices (i.e., x) are negatively correlated with HR and after multiplication by different powers of avRR this negative correlation becomes tighter, along with the improvement in their predictive ability (i.e., AUC is getting lower and lower)—of note, higher values of these indices are related with worse prognosis. However, the division by different powers of avRR makes HRV indices either independent on HR (i.e., x/avRR^2) or positively correlated with HR (i.e., x/avRR^4, x/avRR^8 and x/avRR^16) along with the increase in their predictive power—higher values of these indices are associated with better prognosis (i.e., AUC > 0.5). LF, low frequency component; RMSSD, root mean square successive differences; VLF, very low frequency component (Reprinted with modification and permission from Pradhapan et al., 2014).

HR is a weak (or even is not) risk factor and actually can cover the prognostic value of HRV—yet, this detrimental effect may be eliminated by the removal of HRV dependence on HR (Sacha et al., 2014).

Currently, it is hard to conclude how to practically employ the aforementioned method in clinical settings. However, a concept of a separate approach to HR and its variability should enable us to determine which of the two quantities presents higher predictive performance for a given population and outcome. Probably, it will give us possibilities to increase the prognostic value of HRV by the suitable modification of its relationship with HR. It should be stressed that such a method may be employed to any other HR dynamics analysis which parameters are significantly correlated with HR (Sacha, 2014a,b). The first experiences with using this approach are encouraging, however, the concept requires further investigations in various clinical situations.

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Effect of heart rate correction on pre- and post-exercise heart rate variability to predict risk of mortality—an experimental study on the FINCAVAS cohort

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The non-linear inverse relationship between RR-intervals and heart rate (HR) contributes significantly to the heart rate variability (HRV) parameters and their performance in mortality prediction. To determine the level of influence HR exerts over HRV parameters' prognostic power, we studied the predictive performance for different HR levels by applying eight correction procedures, multiplying or dividing HRV parameters by the mean RR-interval (RR_{avg}) to the power 0.5–16. Data collected from 1288 patients in The Finnish Cardiovascular Study (FINCAVAS), who satisfied the inclusion criteria, was used for the analyses. HRV parameters (RMSSD, VLF Power and LF Power) were calculated from 2-min segment in the rest phase before exercise and 2-min recovery period immediately after peak exercise. Area under the receiver operating characteristic curve (AUC) was used to determine the predictive performance for each parameter with and without HR corrections in rest and recovery phases. The division of HRV parameters by segment's RR_{avg} to the power 2 ($HRV_{DIV\text{-}2}$) showed the highest predictive performance under the rest phase (RMSSD: 0.67/0.66; VLF Power: 0.70/0.62; LF Power: 0.79/0.65; cardiac mortality/non-cardiac mortality) with minimum correlation to HR ($r = -0.15$ to 0.15). In the recovery phase, Kaplan-Meier (KM) survival analysis revealed good risk stratification capacity at $HRV_{DIV\text{-}2}$ in both groups (cardiac and non-cardiac mortality). Although higher powers of correction ($HRV_{DIV\text{-}4}$and $HRV_{DIV\text{-}8}$) improved predictive performance during recovery, they induced an increased positive correlation to HR. Thus, we inferred that predictive capacity of HRV during rest and recovery is augmented when its dependence on HR is weakened by applying appropriate correction procedures.

Keywords: heart rate correction, heart rate variability, receiver operating characteristics, Kaplan-Meier, FINCAVAS

INTRODUCTION

Heart rate (HR) recovery and heart rate variability (HRV) have been used by researchers for assessing the role of autonomic regulation in predicting all-cause and cardiovascular mortality (Freeman et al., 2006). The prognostic capabilities of HR response to exercise and after exercise have been well-documented (Lauer et al., 1996; Cole et al., 1999; Lipinski et al., 2004; Jouven et al., 2005; Kiviniemi et al., 2011) and reviewed by Freeman et al. (2006). Increased sympathetic and decreased parasympathetic activities have been associated with an enhanced risk of sudden death or the vulnerability to ventricular arrhythmias (Lahiri et al., 2008). Subdued time- and frequency-domain HRV indices have been linked with increased risk of mortality in the Framingham cohort (Tsuji et al., 1994), survivors of acute myocardial infarction (MI) (Kleiger et al., 1990; Kiviniemi et al., 2007) and cardiovascular morbidity and mortality (Zuanetti et al., 1996). However, studies determining the prognostic capacity of exercise induced short-term HRV have been sparse and inconsistent. Leino et al. (2010) concluded that none of the HRV indices were good predictors of mortality during peak exercise or recovery phase. In a study by Dewey et al. (2007), a greater short-term HRV during recovery post exercise was associated with an increased risk for all-cause and cardiovascular mortality. This is in contrast to observations made in resting HRV, which implies higher RR-interval variability is associated with better prognosis (Dekker et al., 2000; Leino et al., 2010).

Nieminen et al. (2007) justified that the non-linear inverse relationship between RR interval and HR could be the cause for misinterpretation when comparing subjects with different HR levels and this has been concurred by other researchers (Chiu et al., 2003; Sacha and Pluta, 2005; Sacha et al., 2005; Virtanen et al., 2007; Bailón et al., 2011). Possible physiological mechanisms involved have also been probed (Perini and Veicteinas, 2003; Goldberger et al., 2006). The non-linear relation between HR and HRV has been addressed by Sacha and Pluta (2008) and correction methods have been suggested to strengthen or weaken the influence of HR (Sacha et al., 2013a; Sacha, 2013). By determining whether decreasing dependence on HR improves the prognostic capacity of HRV, we sought to establish the influence of HR in predicting mortality risk. The aim of our study was to scrutinize these correction techniques and their influence on the predictive capacity of cardiac and non-cardiac mortality in the Finnish Cardiovascular Study (FINCAVAS) cohort.

MATERIALS AND METHODS

PATIENT POPULATION AND FOLLOW-UP

A total of 2212 consecutive patients, who were referred by a physician and willing to undergo exercise stress tests at the Tampere University Hospital, were recruited between 2001 and 2004 for FINCAVAS. Informed consent was obtained from all the participants prior to the interview. Measurements were conducted as stipulated in the Declaration of Helsinki and the study protocol was approved by the Ethical Committee of the Hospital District of Pirkanmaa, Finland. In addition to raw electrocardiograph (ECG), descriptive information, medical history and habitual lifestyle of each patient were recorded. More detailed information regarding the patient population and sample size determination is described elsewhere (Nieminen et al., 2006). Of these, 1288 patients satisfied the inclusion criteria for this study with good quality HRV measurements for at least 2 min during rest phase, immediately prior to exercise, and 2 min during post-exercise recovery immediately after maximum effort.

The follow-up data consisted of information related to causes of death and was collected in 2011. The information for the follow-up was obtained from Causes of Death Register and has been shown to be reliable (Pajunen et al., 2005). The follow-up yielded 66 cardiac deaths and 94 non-cardiac deaths, while the remaining 1128 patients constituted the survival group.

EXERCISE TESTING PROTOCOL

The prognoses of mortality were analyzed using HRV indices obtained from 2 min segments during rest phase before exercise and 2 min recovery immediately after maximal exercise. Resting ECG was measured in the supine position prior to exercise. The exercise stress test was then performed on a bicycle ergometer with electrical brakes and the Mason-Likar modified lead system

Table 1 | Baseline characteristics of the study population, classified into survival, cardiac, and non-cardiac mortality groups.

	Survival group ($N = 1128$)	Mortality group ($N = 160$)		
		Cardiac mortality ($N = 66$)	Non-cardiac mortality ($N = 94$)	*p*-value
INDIVIDUAL FACTORS				
Age (years)	54.3 ± 12.6	61.6 ± 10.9	64.1 ± 10.5	0.145
Gender (males, %)	699 (62.0)	42 (78.8)	58 (61.7)	0.020
BMI	27.4 ± 4.5	28.9 ± 4.7	27.0 ± 3.9	0.004
Smoking (yes, %)	317 (28.1)	20 (30.3)	32 (34.0)	0.622
CRI (%)	82.8 ± 24.4	62.3 ± 30.1	73.5 ± 29.8	0.021
Resting heart rate (bpm)	63.3 ± 11.3	64.8 ± 13.9	64.5 ± 12.5	0.656
SAP at rest (mmHg)	135.8 ± 18.5	134.4 ± 21.1	136.3 ± 20.2	0.563
DAP at rest (mmHg)	79.7 ± 9.6	78.1 ± 9.9	77.3 ± 12.2	0.675
Maximum heart rate (bpm)	149.1 ± 25.7	125.6 ± 27.2	132.1 ± 26.5	0.106
SAP peak exercise (mmHg)	196.2 ± 28.6	179.7 ± 32.9	184.8 ± 27.8	0.296
DAP peak exercise (mmHg)	92.4 ± 12.3	88.2 ± 12.2	87.7 ± 13.4	0.813
CLINICAL CONDITION				
CHD (yes, %)	360 (31.9)	30 (45.5)	32 (34.0)	0.146
MI (yes, %)	226 (20.0)	24 (36.4)	22 (23.4)	0.075
Diabetes (yes, %)	128 (11.3)	15 (22.7)	14 (14.9)	0.208
MEDICATION				
ACE inhibitors (yes, %)	235 (20.8)	26 (39.4)	21 (22.3)	0.020
Beta blockers (yes, %)	639 (56.6)	56 (84.8)	70 (74.5)	0.116
Calcium channel blockers (yes, %)	179 (15.9)	17 (25.8)	19 (20.2)	0.412
Diuretics (yes, %)	180 (16.0)	20 (30.3)	28 (29.8)	0.945
Lipid medication (yes, %)	443 (39.3)	39 (59.1)	44 (46.8)	0.127
Nitrates (yes, %)	357 (31.6)	32 (48.5)	44 (46.8)	0.208

Values are expressed as Mean ± SD or number of subjects (%). BMI, body mass index; CRI, chronotropic response index; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; CHD, coronary heart disease; MI, myocardial infarction.

(Mason and Likar, 1966) was used for the ECG data acquisition. Initial work load and increments were defined based on patient's age, gender, body mass index (BMI) and physical activity. Starting work load varied between 20 and 30 W and the stepwise increments ranged between 10 and 30 W every minute. ECG and HR were measured continuously during the test. Tests were sign- and symptom-limited with the recommended criteria for termination whereas in the case of post-MI patients, the upper limit for HR was set at 120–130 beats per minute (bpm). The chronotropic response index (CRI), which represents the chronotropic response to exercise, was evaluated as CRI $= 100 \times$ (peak HR $-$ resting HR)/(220 $-$ age $-$ resting HR) (Kiviniemi et al., 2011). CRI $< 80\%$ was defined as low reserve capacity (Lauer et al., 1996). Measurement during the recovery phase was performed in the sitting position, immediately after exercise.

HRV MEASUREMENT

ECG was recorded at a sampling frequency of 500 Hz using CardioSoft exercise ECG system (Version 4.14, GE Healthcare, Freiburg, Germany) and was analyzed using Modified CASE software (GE Healthcare, Freiburg, Germany). After producing the RR-interval tachogram, the data was preprocessed to remove abnormal intervals and artifacts before they were divided into shorter segments based on the stages of rest and recovery. HRV parameters were determined using the Kubios HRV analysis software (Tarvainen et al., 2014). All intervals were resampled using cubic spline interpolation at 4 Hz. Linear and smoothness prior (smoothing parameter, $\lambda = 500$) detrending were performed prior to calculating time-domain parameters. The fast Fourier transform (FFT) spectrum was computed with a window width of 240 samples, which corresponds to the length of 1 min segment with 4 Hz resampling rate. A 50% overlapping window was used for longer segments. Mean RR intervals (RR_{avg}) were calculated from each segment individually for the HR correction procedure.

Post-exercise recovery is marked by sympathetic withdrawal and parasympathetic reactivation. Sympathetic activation and attenuated parasympathetic recovery are significantly associated with adverse prognosis. The parameters included for examination were chosen based on previous HRV studies on mortality prediction and its outcomes. Of the spectral measures, low frequency (0.04–0.15 Hz, LF) power has been found to increase during exercise in normal subjects and reflects both sympathetic and vagal influences (Malliani et al., 1991). In addition, higher log LF power during recovery significantly predicted increased risk of all-cause and cardiovascular mortality (Dewey et al., 2007). Bigger et al. (1993) demonstrated that spectral measures from short segments (2–15 min) correlated significantly with those computed using 24-h periods. Bernardi et al. (1996) indicated that very low frequency (0.0033–0.04 Hz, VLF) power fluctuations were highly dependent on changes in physical activity, rather than preconceived notion of reflecting autonomic tone and thereby, emphasized the importance of activity as a confounding factor. Therefore, VLF power was evaluated due to its independent risk stratification property for all-cause mortality in patients

Table 2 | Association of individual factors, clinical conditions and medication to cardiac and non-cardiac mortality based on univariate Cox regression.

	Cardiac mortality ($N = 66$)		Non-cardiac mortality ($N = 94$)	
	RR (95% CI)	***p*-value**	**RR (95% CI)**	***p*-value**
INDIVIDUAL FACTORS				
Age $\geq$ 60 years	2.33 (1.43–3.80)	< 0.001	3.01 (1.98–4.58)	< 0.001
Gender (male)	2.27 (1.26–4.09)	< 0.05	0.98 (0.65–1.48)	0.91
BMI $\geq$ 25	1.40 (0.76–2.56)	0.001	1.08 (0.68–1.73)	0.47
Smoking (yes)	1.10 (0.65–1.85)	0.13	1.29 (0.84–1.98)	0.21
CRI $\leq 80\%$	3.95 (2.25–6.93)	< 0.001	2.02 (1.33–3.08)	< 0.001
CRI $\leq 39\%$	4.98 (2.76–8.99)	< 0.001	2.63 (1.43–4.82)	< 0.001
$HR_{rest} \geq 80$ bpm	0.59 (0.32–1.06)	0.08	0.70 (0.44–1.17)	0.13
$HR_{max} \leq 120$ bpm	3.69 (2.27–6.00)	< 0.001	2.12 (1.37–3.27)	< 0.001
CLINICAL CONDITIONS				
CHD (yes)	1.72 (1.06–2.78)	< 0.05	1.05 (0.68–1.60)	0.84
MI (yes)	2.18 (1.32–3.60)	< 0.001	1.16 (0.72–1.86)	0.55
Diabetes (yes)	2.16 (1.21–3.84)	< 0.05	1.29 (0.73–2.27)	0.38
MEDICATION				
ACE inhibitors (yes)	2.37 (1.45–3.89)	< 0.001	1.06 (0.65–1.73)	0.81
Beta blockers (yes)	3.95 (2.02–7.75)	< 0.001	2.03 (1.28–3.23)	< 0.05
Calcium channel blockers (yes)	1.76 (1.01–3.05)	< 0.05	1.29 (0.78–2.13)	0.33
Diuretics (yes)	2.09 (1.24–3.54)	< 0.05	2.09 (1.34–3.25)	< 0.05
Lipid medication (yes)	2.11 (1.29–3.45)	< 0.05	1.29 (0.86–1.93)	0.22
Nitrates (yes)	1.87 (1.16–3.03)	< 0.05	1.70 (1.13–2.55)	< 0.05

CI, confidence interval; RR, relative risk; BMI, body mass index; CRI, chronotropic response index; HR_{rest}, resting heart rate; HR_{max}, maximum heart rate achieved during peak exercise; CHD, coronary heart disease; MI, myocardial infarction.

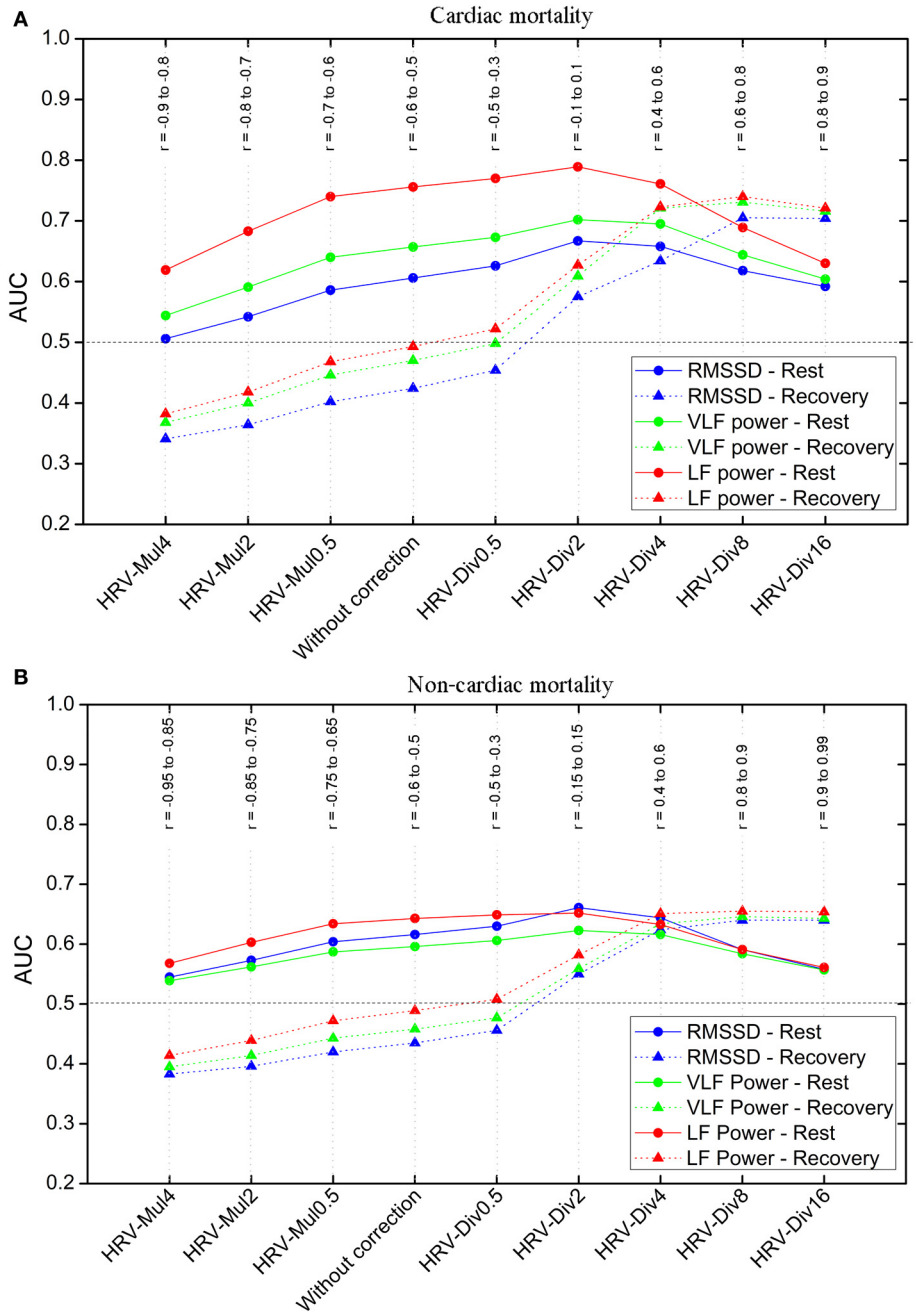

FIGURE 1 | Predictive performance of heart rate variability (HRV) parameters for: (A) cardiac mortality and (B) non-cardiac mortality groups. Area under the receiver operating characteristics curves (AUC) and correlation coefficients (r), between HRV parameters and HR, for different correction methods during rest and recovery after exercise. AUC > 0.5 indicates that higher heart rate variability (HRV) is associated with better prognosis and AUC < 0.5 indicates higher HRV is associated with worse prognosis.

with acute MI (Bigger et al., 1993). Although high frequency (0.15–0.4 Hz, HF) power has been frequently used to measure parasympathetic tone in resting HRV, interpreting values during recovery after exercise is complicated due to tonic autonomic activity and residual adrenergic activity (Dewey et al., 2007). Goldberger et al. (2006) demonstrated that short-term (as small as 30 s windows) root mean squared difference of successive RR intervals (RMSSD), which represents high frequency variations in HR, is adequate for measuring parasympathetic reactivation in recovery phase.

HR CORRECTION

As described by Sacha et al. (2013a), the HRV dependence on HR is strengthened or weakened by multiplying or dividing the HRV indices by the corresponding segment's RR_{avg}, respectively. In addition to normal determination of HRV indices,

eight other classes for the indices were assessed in this study: $HRV_{MUL\text{-}0.5}$—multiplying HRV indices by RR_{avg} to the power 0.5; $HRV_{MUL\text{-}2}$—multiplying HRV indices by RR_{avg} to the power 2; $HRV_{MUL\text{-}4}$—multiplying HRV indices by RR_{avg} to the power 4; $HRV_{DIV\text{-}0.5}$—dividing HRV indices by RR_{avg} to the power 0.5; $HRV_{DIV\text{-}2}$—dividing HRV indices by RR_{avg} to the power 2; $HRV_{DIV\text{-}4}$—dividing HRV indices by RR_{avg} to the power 4; $HRV_{DIV\text{-}8}$—dividing HRV indices by RR_{avg} to the power 8; and $HRV_{DIV\text{-}16}$—dividing HRV indices by RR_{avg} to the power 16. With these classes, different levels of dependence/independence to HR were attained and can be considered significant in determining the contribution of HR in prognosis of cardiac and non-cardiac mortalities.

STATISTICAL ANALYSES

The relative risks for cardiac and non-cardiac mortality were assessed for individual characteristics, clinical condition and medication using univariate Cox models. The measure of the predictive power for different HR correction methods for each segment was computed using area under the receiver operating characteristics (ROC) curve. Spearman's rank correlation was performed to determine the degree of correspondence to HR. The cut-off points for Kaplan-Meier (KM) survival analyses were defined from the ROC analyses for each segment. The point of highest overall predictive performance (average of sensitivity and specificity) was chosen as the cut-off to distinguish mortality and survival groups based on HRV observed in the patient population. It has to be noted that these cut-off points were not optimized in order to preserve uniformity during comparisons. The Log-rank chi-square estimates were then used to evaluate the significance of the correction methods based on this classification.

RESULTS

During the follow-up of the patients who satisfied the inclusion criteria, 66 cardiac deaths were recorded, which included 31 sudden cardiac deaths, with a mean follow-up time of 54 months (min: 4.8 days; max: 99.5 months). 94 patients died of non-cardiovascular causes between 1.2 and 110.7 months of follow-up (mean: 60.2 months). The baseline characteristics, clinical conditions and medications used by patients who suffered cardiac and non-cardiac deaths are listed in **Table 1**.

The univariate Cox regression results for various factors associated with cardiac and non-cardiac mortality are presented in **Table 2**. The relative risk (RR) of cardiac death was significantly higher in males [$RR = 2.27$, 95% confidence interval (CI) = 1.26–4.09, $p < 0.05$]. Age ≥ 60 years was a risk factor for cardiac ($RR = 2.33$, 95% $CI = 1.43$–3.80, $p < 0.001$) and non-cardiac ($RR = 3.01$, 95% $CI = 1.98$–4.58, $p < 0.001$) mortality. Clinical conditions were significantly associated with risk of cardiac death. Medication such as ACE inhibitors ($RR = 2.37$, 95% $CI = 1.45$–3.89) and beta blockers ($RR = 3.95$, 95% $CI = 2.02$–7.75) were significantly associated with increased risk of cardiac mortality ($p < 0.001$).

The area under the ROC curve (AUC) for HR was found to be 0.57/0.70 (rest/recovery) for cardiac mortality and 0.53/0.64 for non-cardiac mortality, implying that HR is a better predictor during recovery than during rest phase. The AUC for RMSSD, VLF and LF power, calculated under different correction methods during rest and recovery phases are presented in **Figures 1A,B**. Correlation with HR (r, presented in **Figure 1**) indicated increasing dependence or independence of HRV to HR, based on the method of correction used. AUC > 0.5 suggested that higher HRV are indicative of better prognosis. $HRV_{DIV\text{-}2}$, which revealed minimum correlation to HR, was the best predictor for both outcomes (cardiac and non-cardiac mortality) in the rest phase. However, during recovery, higher standard HRV (i.e., HRV without correction) was associated with worse prognosis (AUC $<$ 0.5), as seen in **Figure 1**. In addition, similar associations were observed for HRV parameters multiplied by different powers of RR_{avg} ($HRV_{MUL\text{-}0.5}$, $HRV_{MUL\text{-}2,\ and}$ $HRV_{MUL\text{-}4}$). Conversely, after division by higher powers of RR_{avg} (i.e., for $HRV_{DIV\text{-}2}$, $HRV_{DIV\text{-}4}$, $HRV_{DIV\text{-}8}$, and $HRV_{DIV\text{-}16}$), higher HRV was associated with better prognosis (AUC > 0.5). Though higher orders of correction resulted in better predictive capacity, it also induced moderate/strong positive correlation to HR (in the case of $HRV_{DIV\text{-}4}$, $HRV_{DIV\text{-}8}$, and $HRV_{DIV\text{-}16}$).

These results were further corroborated by KM survival analysis. Log-rank estimates at different degrees of correction for both cardiac and non-cardiac mortality are presented in **Table 3**. $HRV_{MUL\text{-}4}$ and $HRV_{DIV\text{-}16}$ were excluded due to their very strong correlation to HR. Mortality prediction was most significant for $HRV_{DIV\text{-}2}$ in the rest phase. During recovery, the division of HRV by higher powers of RR_{avg} resulted in better risk stratification for cardiac and non-cardiac deaths. Although $HRV_{DIV\text{-}4}$ and $HRV_{DIV\text{-}8}$ exhibited better predictive powers during recovery, the HRV indices exhibited strong positive correlation to HR ($r = 0.6$

Table 3 | Chi-square values for Kaplan-Meier analyses under different heart rate correction methods for cardiac and non-cardiac mortality.

Parameter	$HRV_{MUL\text{-}2}$	Without correction	$HRV_{DIV\text{-}2}$	$HRV_{DIV\text{-}4}$	$HRV_{DIV\text{-}8}$
CARDIAC MORTALITY					
Two minute resting period prior to exercise					
RMSSD	14.10**	11.36**	43.47**	25.22**	11.37**
VLF power	9.90*	21.43**	27.56**	27.84**	15.56**
LF power	33.84**	61.65**	75.37**	50.60**	25.38**
Two minute recovery period post exercise					
RMSSD	15.88**	7.93**	16.98**	30.77**	35.84**
VLF power	13.56**	4.81**	21.38**	48.48**	42.57**
LF power	5.50*	12.72**	20.09**	41.77**	52.71**
NON-CARDIAC MORTALITY					
Two minute resting period prior to exercise					
RMSSD	8.97*	16.82**	26.64**	21.46**	10.09**
VLF power	7.63*	15.54**	19.16**	19.05**	10.44**
LF power	16.17**	21.24**	24.13**	17.73**	12.46**
Two minute recovery period post exercise					
RMSSD	18.60**	7.83*	4.59*	16.09**	21.21**
VLF power	9.56*	3.95*	5.08*	19.22**	29.24**
LF power	4.43*	2.49	8.61*	28.39**	26.01**

Significance is denoted by $^{*}p < 0.05$ and $^{**}p < 0.001$.

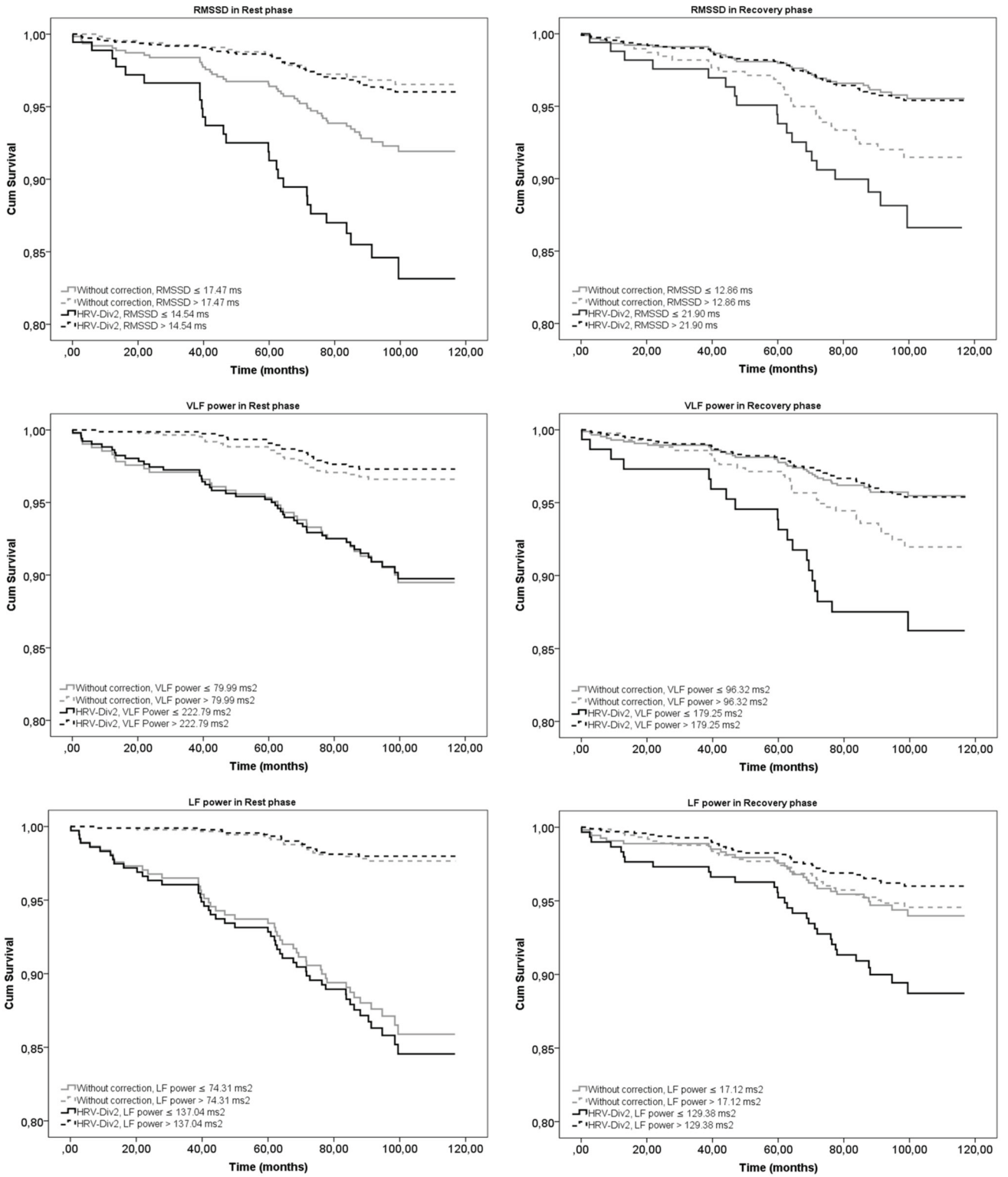

FIGURE 2 | Kaplan-Meier (KM) survival curves for prediction of cardiac mortality using heart rate variability (HRV) parameters at rest and recovery after exercise. Curves in gray represent HRV indices without correction and in black indicate the survival estimates for the best correction with minimum dependence on heart rate (HRV_{DIV-2}).

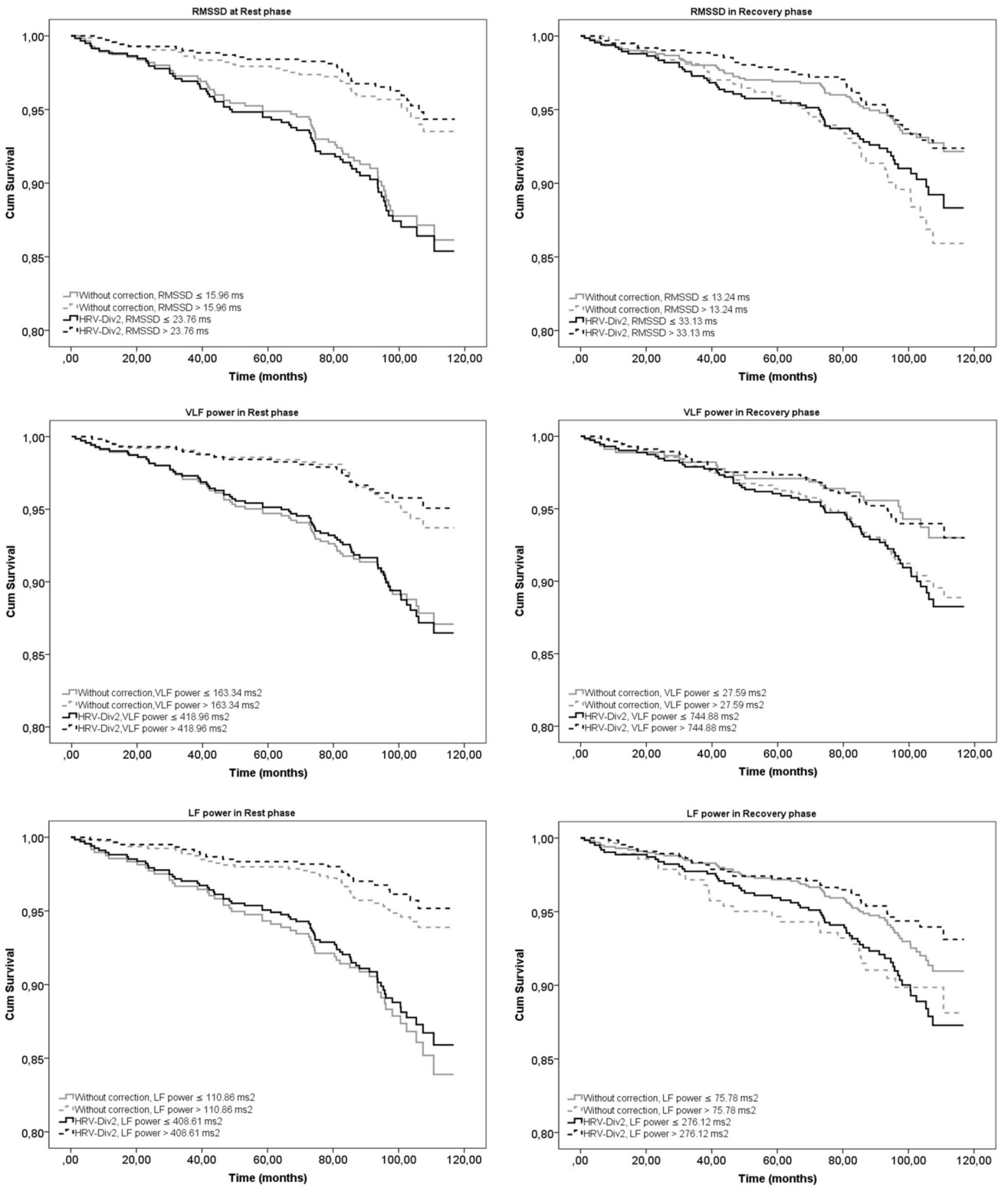

FIGURE 3 | Kaplan-Meier (KM) survival curves for prediction of non-cardiac mortality using heart rate variability (HRV) parameters at rest and recovery after exercise. Curves in gray represent HRV indices without correction and in black indicate the survival estimates for the best correction with minimum dependence on heart rate (HRV_{DIV-2}).

to 0.9 across both groups, as shown in **Figure 1**) at these correction levels. On the contrary, $HRV_{DIV\text{-}2}$ was a good predictor of cardiac ($p < 0.001$) and non-cardiac ($p < 0.05$) mortality during recovery, with minimum influence of HR ($r = -0.15$ to 0.15). **Figures 2**, **3** represent the survival curves for $HRV_{DIV\text{-}2}$ during rest and recovery.

DISCUSSION

The HRV indices computed from RR-interval measurements correlated with HR as a result of the non-linear relationship between the RR-interval and instantaneous HR (Chiu et al., 2003; Sacha and Pluta, 2005; Sacha et al., 2005; Nieminen et al., 2007; Virtanen et al., 2007; Bailón et al., 2011). Higher variability during rest and lower variability during recovery were associated with better prognosis, and this corresponds to observations made by Dewey et al. (2007). Our results indicate that the predictive capacity of HRV at rest was highest when the correlation to HR was minimum ($HRV_{DIV\text{-}2}$, $r = -0.15$ to 0.15), suggesting that exclusion of HR influence on resting HRV improved prognostic capacity for cardiac and non-cardiac mortality. Since HR is a poor predictor at rest, removal of HR influence perchance resulted in improved prognostic capacity. On the contrary, HR during recovery phase exhibited significant risk stratification for both outcomes. Thus, increasing HRV's dependence on HR enhanced its predictive capacity (observed in $HRV_{DIV\text{-}4}$ and $HRV_{DIV\text{-}8}$). However, higher degrees of correction produced moderate/strong positive correlation to HR, similar to observations made by Sacha et al. (2013c), Sacha (2014). To attain true independence, the correction technique that yields HRV least influenced by HR, needs to be identified. In our study, $HRV_{DIV\text{-}2}$ demonstrated improvement in predictability of mortality risk during recovery phase with minimum dependence on HR.

However, conclusive evidence could not be established to distinguish between cardiac and non-cardiac related deaths. This is in contrast to findings by Sacha et al. (2013b), who suggested that increasing the HRV dependence on HR resulted in greater predictive ability for cardiac death and increasing its independence indicated greater predictive power for non-cardiac death. One possible explanation could be that the study population analyzed by Sacha and coworkers comprised only post-MI patients whereas the current study included more heterogeneous patient material.

This study suffered certain limitations. First, the risk factors for individual, clinical conditions and medication were not modeled to determine their contribution toward mortality prediction. By including these variables to the analyses, a more definite conclusion on the cause of mortality could have been established. Second, the patients were not controlled for the type of medication prescribed. The effects of beta blockers and nitrates have been known to affect HR, which could have an effect on the results of HR correction. However, the purpose of the current study was to evaluate the effects of HR correction methods in mortality prediction and therefore, these issues need to be considered in future studies.

CONCLUSION

The findings of this study indicate that the predictive power of HRV parameters for both cardiac and non-cardiac mortality is augmented when its dependence on HR is weakened during rest and recovery. In addition, when HR is a good predictor, increasing HRV's dependence on HR further enhances the risk stratification for both modes of death.

AUTHOR CONTRIBUTIONS

The study was conceptualized by Tuomo Nieminen, Kjell Nikus, Terho Lehtimäki, Mika Kähönen, and Jari Viik. Data acquisition and analysis was performed by Paruthi Pradhapan, Mika P. Tarvainen, Rami Lehtinen, and Jari Viik. All authors contributed equally in drafting and revising the manuscript.

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Heart rate variability in patients being treated for dengue viral infection: new insights from mathematical correction of heart rate

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Introduction: Severe dengue hemorrhagic fever (DHF) is a viral infection that acts to increase permeability of capillaries, resulting in internal hemorrhage. Linear frequency domain Fourier spectral analysis represents the most published noninvasive tool for diagnosing and assessing health status via calculated heart rate variability (HRV). As such, HRV may be useful in assessing clinical status in DHF patients, but is prone to erroneous results and conclusions due to the influence of the average HR during the time period of HRV assessment (defined as the "prevailing" HR). We tested the hypothesis that alterations in HRV calculated with linear frequency analysis would be minimal when mathematically corrected for prevailing HR following dengue viral infection.

Methods: Male ($N = 16$) and female ($N = 11$) patients between the ages of 6 months and 15 years of age (10 ± 6 SD years) were tracked through the progression of the dengue viral infection with treatment following the abatement of a fever (defervescence). Electrocardiographic recordings were collected and analyzed for HRV.

Results: High frequency (HF), low frequency (LF), and LF/HF ratio were unaffected by correction for prevailing HR.

Conclusion: HRV corrected for changes in HR did not alter the interpretation of our data. Therefore, we conclude that cardiac parasympathetic activity (based on HF frequency) is responsible for the majority of the HR reduction following defervescence in patients with dengue viral infection.

Keywords: dengue hemorrhagic fever, heart rate variability, high power frequency, parasympathetic nervous system, autonomic balance

INTRODUCTION

Heart rate variability (HRV) represents a noninvasive "vital sign" that can be easily calculated in real-time from the R-to-R interval of the electrocardiogram (ECG). Low HRV has been recognized as reflecting more severe pathophysiology as reported in ICU patients (Winchell and Hoyt, 1996; Grogan et al., 2005; Morris et al., 2006; Norris et al., 2008; Ryan et al., 2008), experimental human models of hemorrhage (Convertino et al., 2008; Cooke et al., 2008; Ryan et al., 2010), and following injury in trauma (Cooke et al., 2006a,b; Cancio et al., 2008; Ong et al., 2008; King et al., 2009), while high HRV has been used as an indication of improved health. However, these reported results were based on linear analyses of HR calculated in the time and frequency domains. Importantly, linear frequency analysis of HRV is significantly affected by both physiological and mathematical factors as a result of the nonlinear relationship between R -R intervals and HR (Sacha and Pluta, 2005). As such, the use of HRV metrics to provide an accurate assessment of the effectiveness in the treatment of hemorrhage relies on the assumption that the mathematical influence of is the average HR during the time period of HRV assessment [defined as the "prevailing" HR] on HRV is not present or has been corrected. This assumption is reasonable when HR is not different between clinical populations of comparison or over time in the same patient population. But in the absence of mathematical adjustment of differences in HR by dividing R-R intervals by the corresponding average R-R, accurate interpretation of changes in HRV may be severely compromised (Sacha and Pluta, 2005, 2008; Billman, 2011, 2013b; Sacha, 2013; Sacha et al., 2013a,b).

Severe dengue hemorrhagic fever (DHF) is a viral infection that acts to increase permeability of capillaries, resulting in internal hemorrhage. The diagnosis and management of dengue vascular permeability syndrome has been one of the greatest challenges over the past fifty plus years since dengue shock syndrome was first described (Cohen and Halstead, 1966; Halstead et al., 1970). Since HR decreases from the time of patient hospital admission to the time of discharge (Yacoub et al., 2012), we considered the potential use of HRV with confounding changes in HR as an opportunity to determine the usefulness of HRV to assess the effectiveness of in-hospital treatment and to provide clearer insight into the physiology underlying the association of changes in HR with the recovery from DHF.

In the present investigation, we had the unique opportunity to monitor R-to-R interval measurements from patients with dengue viral infection during their hospitalization in order to capture HRV during the progression of the disease and treatment following the day of the abatement of a fever (defervescence). This approach provided the opportunity to assess the mathematical

influence of HR on HRV in a patient population with internal hemorrhage by comparing linear measures of HRV with and without mathematical correction as a potential indicator of the effectiveness of treatment. Although Sacha and co-workers (Sacha and Pluta, 2005, 2008; Sacha et al., 2013b) have recently examined the relationship between average HR and indices of HRV under baseline conditions and compared methods to correct HRV for HR, the effects of HR on HRV during dengue fever (DF) disease progression and treatment remained to be determined. We hypothesized that alterations in HRV calculated with linear frequency analysis would be minimal or eliminated when mathematically corrected for changing HR under these unique conditions.

MATERIALS AND METHODS

Male ($N = 16$) and ($N = 11$) female patients between the ages of 6 months and 15 years of age (10 ± 6 SD years) who were admitted to the Queen Sirikit National Institute of Child Health (QSNICH) with fever and suspected dengue were eligible for enrollment. Exclusion criteria for the study included known chronic conditions (e.g., liver and renal disease, malignancy, thalassemia). Informed consent from a parent or guardian was provided for all study procedures. The study was approved by the hospital Institutional Review Board, the Thai Ministry of Public Health, the US Army Surgeon General, and the University of Massachusetts Medical School. In order to track the progression of the dengue viral infection with treatment following defervescence, we used data collected on days 0 (defervescence), 1, and 2. Day 0 ranged from 0 to 3 days (mean 1 ± 0.9 SD days) following admission to the hospital. All data used in this study were collected in the morning (07.00–10.00) while patients were in the supine position (i.e., hospital bed).

Electrocardiographic recordings were collected in using a Nexfin (BMEye, Amsterdam, the Netherlands) at a sampling rate of 1000 Hz and exported at a rate of 200 Hz to a computer-based data acquisition software package (WinDAQ, Dataq Instruments, Akron, OH). The ECG waveforms were imported into data analysis software (WinCPRS, Absolute Aliens, Turku, Finland) using a Labview application for automatic R-wave detection. Due to the 200-Hz sampling rate, a smoothing filter of a 5-point running average was applied to the ECG data to provide clear peaks for R-wave generation. This filter application produced 0.5–1.0 s of data to be cut from each of the ECG waveforms. All signals were manually scanned for noise and missing R-wave detection. ECG recordings were discarded if they contained less than five minutes of data, more than one ectopic beat during any 5-min time span, or contained electromechanical noise or interference. Aberrant beats in the ECG recording were interpolated, most occurring from calibration or patient movement.

HRV measurements were assessed with analysis of R-R intervals (the time between the two successive R waves in ECG) using frequency domain methods obtained from 300-s continuous recordings with the least amount of aberrant beats. Using WinCPRS software, the following metrics were obtained according to a previously described approach (Ryan et al., 2010) RRI, heart rate (HR), RRI low frequency power (LF), RRI high frequency power (HF), and LF/HF ratio. However, HRV measurements have been shown to be significantly associated with HR due to both physiological and mathematical reasons. In order to remove mathematical bias from our HRV calculations, we used the HR correction methodology previously described by Sacha et al.(Sacha and Pluta, 2005; Sacha, 2013; Sacha et al., 2013a). Removal of this mathematical bias was achieved by the division of the SD of R-R interval (RRSD) by average R-R interval and HRV indices (LF and HF) by the average R-R interval (in seconds) squared. Corrected LF/HF ratio was calculated from corrected LF and corrected HF. After this initial mathematical correction was made, the relationships between resting HR and HRV indices (SD of R-R interval, LF variability, and HF variability) were evaluated by linear regression analysis. The resulting coefficient of determination (r^2) value (i.e., $r^2 = 0.38$) from the regression analysis was interpreted as the % change in HRV due to the prevailing HR. All reported coefficients of determination correspond to the RRSD/HR relationship. Therefore, comparison of r^2-values before and after mathematical correction for prevailing HR, allowed for tracking of how prevailing HR influenced HRV during dengue viral infection. All data are presented as mean ± SD. An ANOVA with repeated measures was used for comparison between fever days.

RESULTS

The comparison of corrected and uncorrected HRV parameters following defervescence is presented in **Table 1**. By day 2, HR decreased from 98 ± 12 to 81 ± 9 beats per minute and RRI increased from 623 ± 73 to 750 ± 78 ms. Uncorrected HF and LF variability increased on Day 2 while LF/HF ratio decreased ($P < 0.001$). After correction for prevailing HR, corrected HF and LF variability were still increased, and LF/HF ratio was still decreased ($P < 0.001$) each of the 2 days following defervescence (**Table 1**). At defervescence (Day 0), HR accounted for ∼40% ($r^2 = 0.38$) of the variability (based on RRSD/HR relationship) before correction for HR and ∼30% ($r^2 = 0.28$) of the variability after correction for HR (normalized unit following HR correction). By Day 2 prevailing HR accounted for ∼7% prior to application of HR correction and less than 1% after correction.

To compare the absolute changes in HRV between Day 0 and Days 1 and 2 in a quantitative fashion, changes in HRV indices were calculated as percent changes (**Table 2**). Application of HR correction did not influence the interpretation of HF, LF, and LF/HF ratio on days 1 and 2. Specifically, uncorrected HF increased by 424 ± 120% and corrected HF increased by 377 ± 134% on day 2 ($p = 0.45$). By day 2, uncorrected LF increased by 425 ± 83% and corrected LF increased slightly less to 327 ± 96% ($P < 0.05$).

DISCUSSION

In general, alteration in HRV offers a clinically useful and quantifiable measure of alteration in the physiologic state of the human body. The most published HRV assessment technique for diagnosing infection is the frequency domain Fourier spectral analysis. This method; however, may be prone to erroneous results and conclusions from data due to the influence of prevailing HR. We demonstrated that the HR correction methodology used in this study was an effective way to examine HRV alterations and autonomic balance, independent of prevailing HR. The following indices of HRV were determined: (1) vagal cardiac

Table 1 | Heart rate variability indices with and without correction for prevailing HR.

Uncorrected	RRI (ms)	r^2 (HR to RRSD)	HR (b/min)	HF (ms^2)	LF (ms^2)	LF/HF
Day 0	623 ± 74	0.38*	98 ± 12	194 ± 495	249 ± 381	8.2 ± 9.6
Day 1	707 ± 86	0.34	87 ± 11	405 ± 571	622 ± 751	4.1 ± 4.5
Day 2	751 ± 78#	0.07#	81 ± 9#	824 ± 1146#	1060 ± 1052#	2.4 ± 2.0#
Corrected		**r^2 (HR to RRSD)**	**HR (b/min)**	**HF**	**LF**	**LF/HF**
Day 0	–	0.28	98 ± 12	0.0003 ± 0.0008	0.0005 ± 0.0007	8.2 ± 9.6
Day 1	–	0.19	87 ± 11	0.0007 ± 0.0009	0.001 ± 0.001	4.1 ± 4.5
Day 2	–	0.004#	81 ± 9#	0.0014 ± 0.0019#	0.002 ± 0.001#	2.4 ± 2.0#

*Note that HR accounted for 38% ($r^2 = 0.38$) of the variability before correction for HR and 28% ($r^2 = 0.28$) following HR correction.

#Denotes significant differences between Day 0 and Day 2. Values are presented as mean ± SD.

parasympathetic activity as HF component of R-R interval variability (HF, 0.15–0.40 Hz), (2) LF component (0.04–0.15 Hz), and LF/HF ratio. The interpretation of LF/HF ration as a marker of autonomic balance has been recently questioned (Billman, 2013a,b) and has been shown to reflect major parasympathetic activity (∼50%) and some sympathetic activity (Randall et al., 1991).

The present study investigated the effects of HR responses in dengue viral infected patients on HRV with and without correction for the baseline HR. Our major findings are (1) correcting HRV did not affect the direction of change in HRV parameters and (2) correcting HRV allowed for more accurate assessment of possible sympathetic (SNS) and parasympathetic nervous systems (PSNA) contributions to HRV. While we hypothesized that alterations in HRV would be minimal when corrected for prevailing HR, our data suggest that there is an autonomic regulatory basis for the HRV alterations observed with dengue viral infection independent of the influence of HR. HRV uncorrected and corrected for changes in HR revealed that cardiac parasympathetic activity likely plays major role of the HR changes following defervescence.

La-Orkhun et al. (2011) assessed HRV as an index of autonomic function in patients with DF, and found no significant changes in various time and frequency domain metrics of HRV at least 24 h after defervescence and follow-up conducted at least 14 days after defervescence. Since monitoring was performed 2 weeks after hospital discharge, it is unlikely that changes in HRV during the critical phase of illness would have been detected in their study. As such, we are the first to report HRV analysis in patients during in- hospital treatment for dengue viral infection that demonstrated significant reductions in HRV.

Several studies have examined the usefulness of HRV analysis for early diagnosis and prognosis of viral infections, particularly in neonates and infants at risk of developing septic shock (Griffin and Moorman, 2001; Griffin et al., 2004, 2005). In their studies, it was reported that abnormal HR with reduced variability and transient decelerations preceded neonatal/infant sepsis. In a study on 81 patients, Chen and Kuo showed that septic patients who subsequently developed shock had lower LF/HF ratio with respect to patients who did not develop sepsis (Chen and Kuo, 2007). In our study, the LF/HF ratio showed a progressive reduction during recovery from dengue infection (**Table 1**) with and without HR correction. While, it has been suggested the decreases in LF/HF ratio correspond to shift toward parasympathetic dominance (Eckberg, 1997), autonomic balance has been recently interrogated (Billman, 2013b). These adjustments in autonomic regulation are consistent with our observations in the HR response and RRI returning toward baseline values with in-hospital resuscitative treatment in DHF patients.

Table 2 | Percent changes in HRV parameters following Day 0 (defervescence) with and without HR correction.

	Day 1		Day 2	
	Uncorrected %	Corrected %	Uncorrected %	Corrected %
HF	209 ± 43	189 ± 76	424 ± 120	377 ± 134
LF	249 ± 90	237 ± 74	425 ± 83	327 ± 96*
LF/HF ratio	50 ± 34	50 ± 34	29 ± 23	29 ± 23

*Denotes significant differences in uncorrected and corrected.

After correction for prevailing HR, LF variability was still significantly increased (both $P < 0.01$) on day 2 following defervescence. Furthermore, when corrected for prevailing HR, the percent change in LF variability was slightly reduced from uncorrected values of 425% to corrected values of 327%. Houle and Billman (1999) and co-workers demonstrated that the LF component of the HR power spectrum probably results from an interaction of the sympathetic and PSNA and, as such, does not precisely reveal changes in the sympathetic activity (Randall et al., 1991). These data further support our interpretation that reductions in HR following defervescence are mediated by increased cardiac parasympathetic activity and not reductions in sympathetic drive.

In conclusion, we showed that uncorrected and corrected HRV does not alter the interpretation of the potential contributions of parasympathetic and sympathetic activity in patients with dengue viral infection during their hospitalization. Additionally, HRV uncorrected and corrected for changes in HR suggest that cardiac parasympathetic activity plays an important role in HR changes following defervescence. Furthermore, the HR correction methodology employed in this study provided a unique opportunity to delineate the physiological changes in HR during treatment of dengue viral infection.

DISCLAIMER

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting

the views of the Department of the Army or the Department of Defense.

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New methods for the analysis of heartbeat behavior in risk stratification

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Developing better methods for risk stratification for tachyarrhythmic sudden cardiac remains a major challenge for physicians and scientists. Since the transition from sinus rhythm to ventricular tachycardia/fibrillation happens by different mechanisms in different people, it is unrealistic to think that a single measure will be adequate to provide a good index for risk stratification. We analyze the dynamical properties of ventricular premature complexes over 24 h in an effort to understand the underlying mechanisms of ventricular arrhythmias and to better understand the arrhythmias that occur in individual patients. Two dimensional density plots, called heartprints, correlate characteristic features of the dynamics of premature ventricular complexes and the sinus rate. Heartprints show distinctive characteristics in individual patients. Based on a better understanding of the natures of transitions from sinus rhythm to sudden cardiac and the mechanisms of arrhythmia prior to cardiac arrest, it should be possible to develop better methods for risk stratification.

Keywords: cardiac arrhythmias, sudden cardiac death, ventricular tachycardia, non-linear dynamics, parasystole, early after depolarization

INTRODUCTION

Cardiac arrhythmias occur when the normal mechanisms of cardiac initiation and propagation no longer prevail and abnormal patterns of cardiac activity occur over some or all regions of the heart. Because of its clinical importance, the transition to tachyarrhythmic sudden cardiac death (for convenience, we use SCD here to indicate tachyarrhythmic sudden cardiac death) has attracted a large amount of attention. In particular, since tachyarrhythmias can generally be terminated by an implantable cardioverter-defibrillator (ICD), developing better means of predicting who will experience spontaneous transitions to tachyarrhythmias is a major focus for research. A large numbers of factors have been proposed that can be derived non-invasively from the electrocardiogram (ECG), but to date, none appear adequate (Huikuri et al., 2001; Goldberger et al., 2008, 2011). Many patients who would benefit from an ICD do not receive one, and many who receive one do not benefit. In view of the cost of ICDs and the potential complications of their use, the cost effectiveness of ICD use has been questioned (Tung et al., 2008).

A recent review identified several roadblocks to risk stratification (Goldberger et al., 2011). In the following we outline an approach that is complementary to current approaches. We argue that by relating the problem of risk stratification to basic science questions of dynamics and physiology, it should be possible to understand the physiology of individuals better and in this fashion develop better means for risk stratification.

We first briefly mention several risk factors that have been proposed. Then we describe an approach for analyzing arrhythmias in which there are frequent premature ventricular complexes (PVCs) that may help to identify the mechanisms generating those complexes. Finally, we consider the basic science question of analysis of the transition from sinus rhythm to tachycardia, and indicate how the various measures might be useful in helping to predict individuals at risk.

RISK FACTORS FROM CLINICAL STUDIES

Goldberger et al. (2008) provide an excellent survey of risk factors for SCD with extensive literature citations. We only provide selected references.

Variables that reflect parasympathetic and sympathetic activity play a prominent role in risk stratification for SCD (Barron and Lesh, 1996; La Rovere et al., 1998). In healthy hearts, there are typically wide fluctuations in the normal sinus rhythm over the course of the day. Various measures have been used to document reduction of these fluctuations by analyzing the SDs of the fluctuations in time, the power spectra of the heart rate, and other statistical measures derived from non-linear dynamics (Voss et al., 2009). The heart rate turbulence, reflecting the fluctuations in the sinus rhythm following a PVC, is also a reflection of parasympathetic-sympathetic function (Schmidt et al., 1999). Since less healthy hearts display lower levels of fluctuations of the sinus rate many of these measures are an indirect reflection of ventricular function. Higher levels of sympathetic activity may be pro-arrhythmogenic since they might predispose the heart to PVCs arising from early after depolarizations (EADs; Zipes, 1991) or other mechanisms.

A second class of risk factors for SCD relate directly to cardiac anatomy and physiology. The most important of these is the left ventricular ejection fraction (LVEF), where low LVEF (<30–35%) is often used to identify candidates for an ICD (Bardy, 2005). Another important risk factor reflecting cardiac physiology is inducibility of ventricular tachycardia (VT) using programmed electrical stimulation, since a positive test indicates an anatomical

substrate for VT (Vandepol et al., 1980; Wellens et al., 1985; Josephson, 2008). Another test that has attracted great attention recently is T-wave alternans, where an elevated level of alternation of T-waves at higher pacing rates or sinus rates reflects higher risk (Rosenbaum et al., 1994; Koller et al., 1998; Sato et al., 2006; Qu et al., 2010). Additional risk factors include an increased width of terminal portions of the QRS complex on the signaled-averaged ECG (Gomes et al., 2001), greater frequency of PVCs (Carrim and Khan, 2005), and long QT intervals (Zipes, 1991; Fermini and Fossa, 2003) all of which confer a higher risk of SCD.

THE HEARTPRINT AND MECHANISMS FOR PVCs

From the previous analysis of risk factors for SCD, it is clear that we have not yet identified a single factor – or even combination of factors – that are adequate for risk stratification for SCD. The large number of factors investigated to date, all of which are associated with an increased risk, suggest that there may be many potential mechanisms for transitions from sinus rate to tachyarrhythmias. A given factor would be expected to be useful as a predictor for a certain mechanism but not others. Consequently, we believe that an important step in the development of better risk stratification markers for SCD will be to develop methods that are useful for identifying specific physiological mechanisms in the individual that might be underlying arrhythmias (Glass and Lerma, 2006).

In order to do this, we have proposed a method, called the heartprint to capture dynamical features of ventricular premature ventricular complexes (PVCs) in Holter recording as a function of sinus period (NN) see **Figures 1** and **2** (Schulte-Frohlinde et al., 2002). Heartprints give a visual display of the qualitative and quantitative features of the dynamics over the entire 24-h period. A heartprint is a way to represent dependencies between the NN interval and (i) the ectopic beat interval (between two V beats, or VV interval), (ii) the number of intervening beats (NIB) between two consecutive PVCs, and (iii) the coupling interval (CI) from a sinus beat to the next PVC. The ordinate of the 3 grayscale plots in the heartprint is the NN interval. The incidence of the VV intervals, NIB values, and the CI are indicated in the grayscale plots, respectively, where the relative frequency of occurrence is indicated by the shading, (e.g., black is associated with the highest incidence). The plots above the grayscale plots give the histograms of the VV intervals, the NIB values, and the CI, respectively. The histogram to the left of the grayscale plots gives the histogram of NN values.

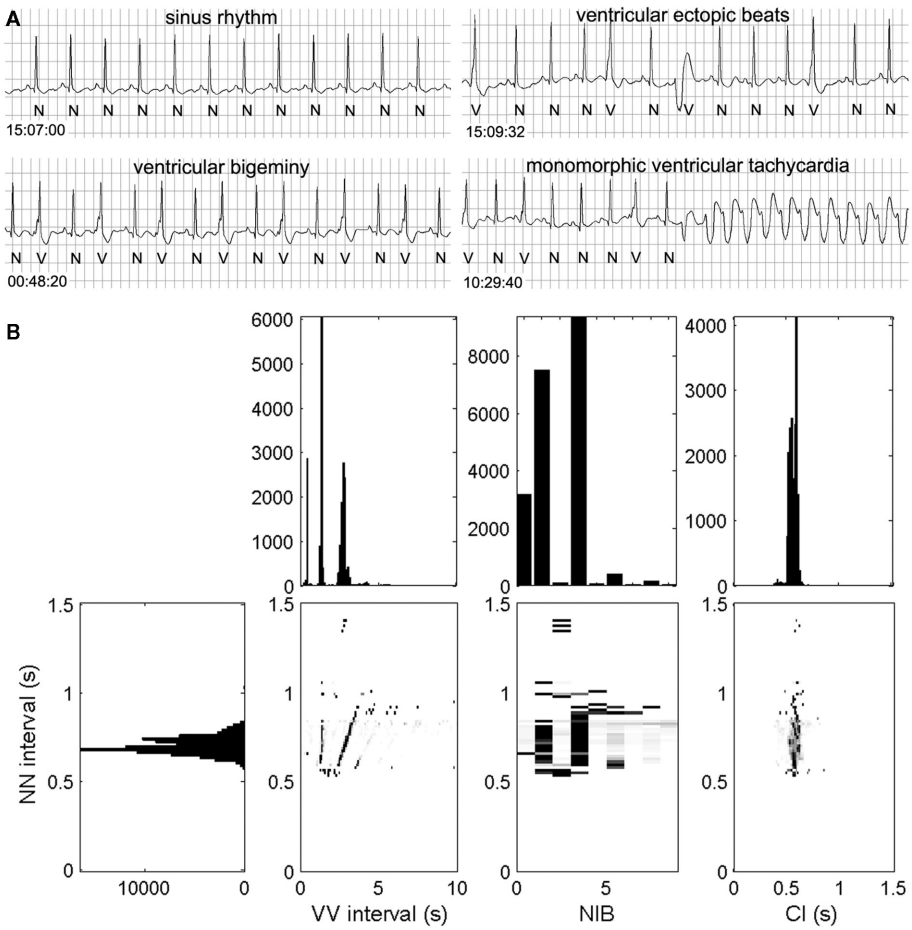

FIGURE 1 | Rhythm strips (A) and heartprint (B) for a male patient with unknown clinical history. Heartprint shows sinus rates with NN intervals between 0.5 and 1 s, the number of intervening beats between two ectopic beats (NIB) were mostly 0, 1 and 3, and the coupling interval (CI) was relatively fixed. Case 44 from the PhysioNet Sudden Cardiac Death Data Base (Goldberger et al., 2000). For abbreviations, see text.

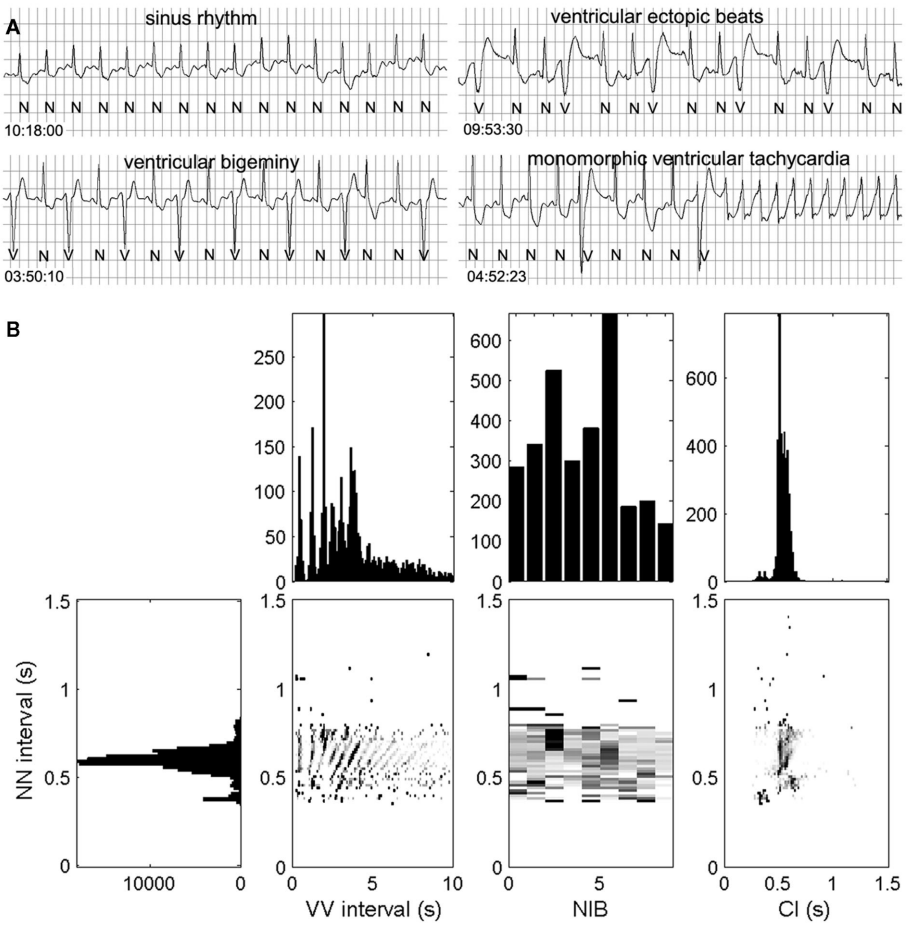

FIGURE 2 | Rhythm strip (A) and the heartprint (B) for an 80-year-old male patient with unknown clinical history. Heartprint shows that NN intervals were between 0.4 and 0.8 s with a wide range of NIB values and coupling interval (CI) was highly variable. Case 48 from the PhysioNet Sudden Cardiac Death Data Base (Goldberger et al., 2000).

In **Figures 1** and **2**, we show ECG traces and heartprints for two different patients from the PhysioNet Sudden Cardiac Death Database (Goldberger et al., 2000). The dynamical features of the arrhythmias are very different, potentially implying that different mechanisms may be underlying the arrhythmia in each patients. If the mechanisms of the PVCs are independent of the mechanism for the transition to tachycardia, then understanding the mechanisms of the PVCs might be an interesting academic exercise, but it would be of little practical importance. But in the current records, the presence of the PVCs immediately prior to the tachycardia, may reflect an underlying causal relationship.

In previous work we have found some striking patterns of PVC occurrence that reflect on the mechanism. In one set of patients with long QT syndrome, the CIs were strongly peaked and there were frequent bigeminal episodes in which there is an alternation between sinus beats and PVCs (Lerma et al., 2007). This triad of features is consistent with long QT syndrome, and might of itself be a novel marker for SCD. Record 44 shares some of these characteristics, but has a confounding feature of two morphologies of PVCs. Another different striking set of characteristics found in some patients are variable CIs, but strong dependence of the pattern of NIBs as a function of the sinus rate, consistent with theoretical predictions based on a mechanism in which there is an independent ectopic (parasystolic) ventricular focus (Schulte-Frohlinde et al., 2002).

Although in a limited number of circumstances, such as the two mentioned above, we can propose physiological mechanisms that are consistent with the features of the heartprint, in most of the heartprint records that we have examined, we do not yet know how to decode the underlying mechanisms. Despite a significant literature on the interpretation of complex arrhythmias based on comparatively short rhythm strips (Pick and Langendorf, 1979), there is a need for developing better ways of identifying mechanisms of arrhythmia based on Holter tape or other long term data.

THE TRANSITION TO VT

We view the transition to VT as a problem both in clinical medicine and in basic science. Our perspective derives from the mathematical field of non-linear dynamics (Guckenheimer and Holmes, 1983). Non-linear dynamics focuses on qualitative features of dynamics. The classification of different types of cardiac

arrhythmias by clinicians combined with the development of mathematical models for dynamics and transitions observed in certain arrhythmias such as atrio-ventricular heart block fully support the relevance of non-linear dynamics to understand mechanisms and dynamics in the heart (Glass et al., 1987a,b). Although classification of transitions between sinus rhythm and tachycardias is not yet nearly as developed, there have been a large number of proposals, many enriched by the interplay between clinical medicine and basic science. We briefly summarize several of these mechanisms.

(i) Induction of monomorphic VT by programmed electrical stimulation. A classic electrophysiologic approach to risk stratification involves the induction of monomorphic VT by delivery of 1–3 premature stimuli in patients with an anatomically based reentrant pathway (Vandepol et al., 1980; Wellens et al., 1985; Josephson, 2008). The successful induction of VT with the same morphology as observed in an ambulatory ECG can provide an anatomical target for ablation. Outside of the electrophysiology laboratory, the VT might be induced by a PVC generated by some other mechanism. This type of arrhythmia is common in patients with a prior myocardial infarction. It can be hemodynamically stable with a well defined exit point for the reentrant pathway into the ventricle. Thus, it can potentially be detected and treated. Although in principle the reentrant pathway could also lead to isolated PVCs, it is not clear how to identify this mechanism for PVCs.

(ii) Induction of reentrant tachycardia associated with spiral waves by delivery of a single pulse in the vulnerable period. The classical concept of PVCs falling on the T-wave of the electrocardiogram (R on T) has a counterpart in the theoretical and experimental studies that demonstrate the initiation of spiral waves by the delivery of a stimulus during the repolarization phase of a propagating action potential (Witkowski et al., 1998). In the heart, a PVC could be generated by some physiologic mechanism. In the theoretical literature, there is often the assumption that the rotating spiral would be associated with monomorphic VT, but it seems likely to us, that in a heterogeneous heart the spiral might drift or even break up, leading to a polymorphic VT or fibrillation.

(iii) VT induced by an accelerated ectopic focus. One of the classic examples is slow monomorphic VT originating from the right ventricular outflow tract (Belhassen, 2005). This is typically benign, even though it might be associated with a large number of PVCs per day. Typically the coupling intervals of PVCs to the preceding sinus beat are long. However, variants have been described in which the coupling intervals are shorter, and may be associated with initiation of dangerous polymorphic VT (Viskin and Antzelevitch, 2005).

(iv) Initiation of ventricular tachycardia by break up of propagating waves in the context of alternating action potential duration (T-wave alternans). The identification of alternation of action potential duration in experimental and mathematical models has a rich mathematical development from analysis of the action potential restitution curve (Guevara et al., 1984; Koller et al., 1998; Sato et al., 2006; Qu et al., 2010). In spatially heterogeneous systems, action potentials with a short duration might be locally blocked leading to wave break and initiation of reentrant arrhythmias. Fenton et al. (2002) have documented several different ways in which regularly propagating spiral waves in mathematical models of cardiac tissue in which alternans is prominent can become unstable leading to reentrant dynamics similar to those that are believed to underlie ventricular fibrillation.

(v) Initiation of polymorphic VT in the setting of genetic abnormalities or drug effects that lead to long QT syndrome and EADs. It is well known that a long QT interval can arise as a consequence of genetic abnormalities in potassium channels (Sanguinetti and Tristani-Firouzi, 2006) or as an unwanted side effect of medication (Fermini and Fossa, 2003) and is an important risk factor for sudden death. Recent theoretical and experimental studies (Sato et al., 2009; Auerbach et al., 2011) have documented the way in which EADs can be generated and lead to reentrant tachycardia. Experimental studies of arrhythmias induced by potassium channel (I_{kr}) blocking drugs show induction of couplets or triplets that could stand as an experimental model of arrhythmogenic sites (Kim et al., 2009).

(vi) Initiation of reentry in hearts with heterogeneity and reduced coupling. Fibrosis or sarcoidosis is sometimes found on autopsy of subjects with sudden cardiac death (Fabre and Sheppard, 2006). These clinical observations have a counterpart in experimental and theoretical models of heart cells in tissue culture demonstrating the spontaneous initiation of reentrant arrhythmias in situations of increased heterogeneity and reduced coupling (Bub et al., 1998, 2002).

This listing is not meant to be exhaustive but rather illustrative. Although listed separately, more than one mechanism might operate together in an individual patient. For example, a monomorphic reentrant tachycardia associated with anatomical reentry could degenerate into ventricular fibrillation as a consequence of heterogeneity and/or blockage secondary to alternans. Further, although we list induction of VT by R on T and EADs separately, EADs may be simply be one mechanism for generating R on T.

ANALYSIS OF ARRHYTHMIA AND DYNAMICS IN MODEL SYSTEMS AND IN INDIVIDUAL PATIENTS

We believe that the above discussion provides a foundation and suggests a strategy for developing better methods for risk stratification based on a better understanding of the individual physiology, combined with a better understanding of the mechanisms of transition to tachyarrhythmias. What is necessary is to develop better methods for characterization of physiological mechanisms of arrhythmia in patients and to correlate that with outcome data.

In order to make progress we suggest three parallel research paths.

MATHEMATICAL ANALYSIS OF TRANSITION TO TACHYCARDIA IN MODEL SYSTEMS

Experimental studies in model cardiac systems have demonstrated a variety of circumstances that lead to spontaneous transition to reentrant rhythms including variation of the coupling between cells (Bub et al., 2002), addition of drugs that modify ionic

channels leading to long action potential durations and EADs (Sato et al., 2009), modifications of geometry leading to heterogeneities (Auerbach et al., 2011), addition of drugs that lead to alternation of action potential duration, and instabilities during rapid propagation (Qu et al., 2010). As is clear, this is a very active and rich area for basic science research. As our ability to modify geometry and physiology in tissue culture improves, there should continue to be strong progress.

CLASSIFICATION OF ARRHYTHMIAS IN PEOPLE BASED ON HOLTER RECORDS AND ADDITIONAL CLINICAL DATA

Electrocardiographic data from the human heart can be readily collected and constitute a vast store of data that remains incompletely understood. From an examination of the records in **Figures 1** and **2**, it is clear that individuals have very different characteristics, and that standard methods for PVC arrhythmia analysis which rely largely on counting frequencies of PVCs do not address fine details that reflect the mechanism (Carrim and Khan, 2005). Yet, since the Cardiac Arrhythmia Suppression Trial it has been clear that a simple consideration and manipulation of the frequency of PVCs is not adequate (Echt et al., 1991). Developing deeper insight into the classification of mechanisms of arrhythmia remains a challenge for the future.

MODELING OF THE ARRHYTHMIAS IN INDIVIDUAL PATIENTS

Several different styles of model can provide insight into arrhythmia mechanisms. Simplified models of parasystole can be used to explain subtle dynamical details of the occurrence of PVCs in selected patients (Moe et al., 1977; Schulte-Frohlinde et al., 2001, 2002). The essential feature is to carry out quantitative models over extended periods of time (e.g., hours), taking into account the possibility that cardiac parameters may change as a consequence of activity, drugs, or other environmental factors. A complementary style of analysis involves detailed simulations based on realistic anatomical data (Aguado-Sierra et al., 2011). Although modeling realistic anatomies is still extremely difficult, there is rapid development of imaging methods and computer power. Yet, development of realistic models based on clinical data that are capable of predictive value for susceptibility to arrhythmia and transition to VT still seems to be remote. However, collaborations between basic scientists, engineers, and clinicians in this direction remains an important future direction.

CONCLUSION

In their recent review, Goldberger et al. (2011) raised and rejected the possibility that "better risk stratification for SCD is unachievable." Although the problem is clearly difficult, we have argued that progress should be possible by adopting a complementary approach to risk stratification compared to current clinical approaches. Based on the notion that there are multiple routes for the transition from sinus rhythm to tachyarrhythmic SCD, we would not expect a single risk factor to be adequate. Even taking multiple factors, each of which might be useful for different individuals, would not be adequate to predict risk. However, an absence of those factors, might be a useful predictor of low risk (as has been found; Goldberger et al., 2011).

We argue for the analysis of mechanisms of arrhythmias in individual patients as a strategy to better develop methods of risk stratification. Combination of such a classic approach (Pick and Langendorf, 1979) with modern methods of computer analysis and simulation may yet provide insight into disease as well as new practical methods for risk stratification.

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