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Normal values and reproducibility of the real-time index of vagal tone in healthy humans: a multi-center study

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Abstract

Background The parasympathetic nervous system has been implicated in the pathogenesis of a number of gastrointestinal disorders including irritable bowel syndrome. Within the field, cardiometric parameters of parasympathetic/vagal tone are most commonly derived from time, or frequency, domain analysis of heart rate variability (HRV), yet it has limited temporal resolution. Cardiac vagal tone (CVT) is a non-invasive beat-to-beat measure of brainstem efferent vagal activity that overcomes many of the temporal limitations of HRV parameters. However, its normal values and reproducibility in healthy subjects are not fully described. The aim of this study was to address these knowledge gaps.

Methods 200 healthy subjects (106 males, median age 28 years, range 18-59 years) were evaluated across three study centers. After attachment of CVT recording equipment, 20 min of data (resting/no stimulation) was acquired. 30 subjects, selected at random, were restudied after 1 year.

Results The mean CVT was 9.5±4.16 linear vagal scale (LVS). Thus, the normal range (mean±2 standard deviations) for CVT based on this data was 1.9-17.8 LVS. CVT correlated negatively with heart rate (r=-0.6, P=0.001). CVT reproducibility over 1 year, as indexed by an intra-class correlational coefficient of 0.81 (95% confidence interval 0.64-0.91), was good.

Conclusions In healthy subjects, the normal range for CVT should be considered to be 1.9-17.8 LVS and is reproducible over 1 year. Future research utilizing CVT should refer to these values although further study is warranted in patient groups.

Keywords Autonomic nervous system, parasympathetic nervous system, cardiac vagal tone

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Introduction

Visceral pain is a common feature of many gastrointestinal (GI) disorders including inflammatory bowel disease (IBD) [1] and irritable bowel syndrome (IBS) [2]. Visceral pain is a major cause of healthcare seeking, disability and reduction in health-related quality of life [3]. The autonomic nervous system (ANS) is a bidirectional, hierarchically controlled brain-body nexus that integrates the external environment with the internal milieu and has been postulated to play a critical role in the genesis and modulation of visceral pain through its multiple interactions with the nociceptive system at the level of the periphery, spinal cord, brainstem, and forebrain [4]. Dysfunction of the ANS has been variably described in both IBD [5] and IBS [6]. The main neural substrate of the parasympathetic nervous system (PNS) is the vagus nerve containing both afferent and efferent pathways. In humans the vagus nerve innervates the entirety of the GI tract except the distal third of the colon [7]. The efferent vagus nerve is increasingly considered to play a critical role

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in the neuro-endocrine-immune axis within the gut through what is termed the cholinergic anti-inflammatory pathway [8]. Thus, the non-invasive measurement of vagal tone in humans is likely to remain high on the contemporaneous GI research agenda.

Cardiac vagal tone (CVT) is a putative measure of the influence of the PNS on the heart via the vagus nerve, which arises in the brainstem. During ventricular systole, baroreceptor discharge increases as a consequence of a transient increase in blood pressure [9]. This initiates a vago-vagal reflex, mediated through the medullary neurons of the nucleus of the solitary tract, thereby increasing vagal discharge from the brainstem. This reflex results in a reduction of the rate of spontaneous discharge of the sino-atrial node thus reducing heart rate (HR) through prolongation of the R-R interval [10]. There are a diverse array of factors that influence HR such as stress, respiratory rate, metabolic needs and blood pressure through homeostatic synergy of the broadly reciprocal actions of sympathetic nervous system (SNS) and PNS [11]. In humans, this aforementioned vagal response to increased baroreceptor discharge occurs within a time frame in the order of 240 msec, which is of sufficient celerity to influence the subsequent cardiac cycle [12]. In contrast, the SNS influence on HR occurs relatively slowly through dynamic changes largely mediated by alterations in peripheral vascular resistance. Thus, over short epochs beat-to-beat changes in HR have been postulated to be due to changes in PNS tone alone [13]. However, the direct measurement of human vagal tone is problematic. In animal models, direct measurement of vagal tone is possible through the invasive placement of electrodes directly into the brainstem or vagal afferents [14,15]. Clearly in *in vivo* human studies such an approach is not viable and therefore considerable effort has been afforded in identifying validated non-invasive surrogate markers of PNS tone.

Given that beat-to-beat changes in HR are largely due to PNS influences, various measures commonly derived from power spectral analysis of heart rate variability (HRV), amongst other statistical techniques, have been used as surrogate markers of PNS tone. Such measures are widely utilised across a plethora of scientific disciplines including gastroenterology research [16]. However, many of these methods of deriving PNS tone are notwithstanding limitations. Arguably the most important of these is their relatively poor temporal resolution [17]. Whilst this is not an issue in studies where prolonged recordings are made, erroneous conclusions maybe drawn from studies where changes in PNS tone over relatively short-time periods such as those when changes under 1 min represent the investigators' epoch of interest. It is likely that these limitations are a potential source of confounding and a source of the disparate results reported, particularly in IBS [18].

Given our basic physiological understanding of PNS influence on the heart it should technically be possible to deduce PNS activity non-invasively by measuring beat-to-beat changes in R-R interval. The adaptation of such an approach has been demonstrated to be successful in dogs [19]. Based on this principle, a relatively recent advance has been the development of a validated real-time beat-to-beat index of PNS tone known as CVT [20] (*vide infra* for a more detailed explanation). Recently, the measurement of CVT has been increasingly utilized across a range of research themes, particularly within visceral pain and GI research [21-24]. However, the normal baseline/resting range for CVT and its long-term reproducibility in healthy subjects of the parameter is incompletely understood and it is this significant knowledge gap that our multi-center study aimed to address.

Materials and methods

Subjects

200 healthy subjects took part in the study recruited from three study centers (133/200 at Barts & the London School of Medicine; 49/200 at the Catholic University of Leuven; and 18/200 at the University of Manchester). Subjects were non-smokers with no medical history and not taking any medications whatsoever, including the oral contraceptive pill. All pre-menopausal female participants were studied in the follicular phase of their menstrual cycle. Subjects were asked to refrain from caffeine and alcohol consumption for 24 h prior to the study. Reproducibility of CVT was evaluated in a randomly selected subgroup of 30/200 subjects after 1 year. All subjects gave written informed consent and the study was approved by the City and East London Ethics Committee 2.

ANS measures

Blood pressure: Mean digital arterial BP (MBP) was measured non-invasively using the validated photoplethysmographic technique (Portapres, Amsterdam, Netherlands) [25,26]. *Heart Rate, Cardiac Vagal Tone and Cardiac Sensitivity to the Baroreflex* - Electrocardiogram (ECG) electrodes (Ambu Blue Sensor P, Denmark) were placed in right and left sub-clavicular areas and cardiac apex. ECG was acquired digitally using the Neuroscope system (Medifit Instruments, Enfield, Essex, UK) at 5 kHz. The Neuroscope non-invasively measures two parameters of PNS activity thought to encompass efferent and afferent limb activity.

CVT

PNS efferent activity is given by CVT. The incoming QRS complex is compared to a template that is generated from the initial stages of ECG acquisition from that particular subject. If these two QRS complexes are sufficiently similar, voltage-gated oscillators within the instrument generate a 1 mV pulse, which is then fed into a two-limb circuit. The first limb, known as the high-pass limb, follows the incoming QRS signal exactly whereas the second limb, known as the low-pass limb, produces a damped rendition [27]. Thus the slower the rate of change of the incoming signal, i.e. the lower the HRV,

the closer the low-pass limb mimics the high-pass, resulting in a lower value. Conversely, the higher the HRV the more the low-pass limbs deviates from the high-pass limb thus producing a higher value. This process has been termed phase shift demodulation and is based uniquely among non-invasive measures of PNS tone. CVT is measured on a linear vagal scale (LVS) where 0 was derived from fully atropinized healthy human volunteers [20]. CVT has been demonstrated both a sensitive and specific measure of vagal tone and comparable to other HRV indices [27].

Cardiac sensitivity to the baroreflex (CSB)

Secondly, vagal baroreflex sensitivity incorporating PNS afferent and efferent activity was derived from CSB. CSB is derived from the incorporation of RR intervals with mean BP into an algorithm on a 10-sec cycle. Thus CSB does not have any units *per se* and is expressed as a ratio of change in R-R interval against change in unit BP (DRR/DmmHg). ANS parameters were recorded according to internationally agreed recommendations [28].

Protocol: All subjects were studied in the afternoon (from 1400-1600 h) in a temperature controlled (20-22°C), constantly lit, quiet laboratory. Subjects were reclined at 45° on a bed. After attachment of ANS recording equipment, 20 min of autonomic data (resting/no stimulation) was acquired. The mean/median values of the ANS parameters were derived from the middle 10 min of recording. The subjects that were restudied after 1 year had the protocol repeated based on subject availability at the London center.

Statistical analysis

Data distribution was analyzed using the D'Agostino-Pearson omnibus K2 normality test [29]. Results of quantitative data are presented either as median with interquartile ranges (IRQ), for non-normally distributed data, or mean ± standard deviation (SD) and range for parametric data. For quantitative data, differences between the groups were assessed using the Student's t-test in case of parametric data and using the Wilcoxon matched-pairs test in case of non-parametric data. Correlational analyses were performed using Pearson (r) or Spearman's (r_s) coefficient as appropriate. Agreement was assessed using a two-way, random effects, single measure intra-class correlational coefficient (ICC) model, the coefficient of reproducibility (CR) and Bland Altman plots. The CR is the maximum difference that is likely to occur between repeated measurements, and is defined as $1.96 \times \sqrt{\sum(d_2\text{-}d_1)^2/n\text{-}1}$, where d_2-d_1 is the difference between the first and second measurements and is quoted in the original units without transformation. Confidence intervals for the ICC and CR were calculated according to the methods of Scheffe [30]. ICC were interpreted according to suggestions made by Yen *et al* as: excellent (0.75-1), moderate (0.4-0.74), or poor (0-0.39) [31]. All tests were two-tailed, and P<0.05 was adopted as the statistical criterion. Analyses were performed using proprietary software (GraphPad Prism 5, CA, USA and SPSS 18, IBM, NY, USA).

Results

Subject characteristics

All 200 (106 males, median age 28 years, range 18-59 years) subjects completed the study. Subjects had mean body mass index of 25.7±0.38 kg/m^2. All subjects were in normal sinus rhythm. No adverse events or discomfort during the recording.

Baseline autonomic parameters

The mean ± SD of HR, MBP and CVT and median with IRQ of CSB are detailed in Table 1. The mean CVT was 9.5±4.16 LVS. Thus, the normal range (mean ±2 SD) for CVT based on this data is therefore 1.9-17.8 LVS. This is not different from a previously published study examining CVT which reported a normal range of 0-17.19 LVS (unpaired *t*-test, P=0.09) [32].

Inter-relationship of autonomic parameters

CVT and CSB correlated negatively with HR (r=–0.6 and r_s=–0.53, P=0.001 respectively). CVT and CSB correlated positively with one another (r_s=0.88, P=0.0001).

The influence of demographic variables on CVT

Age weakly correlated negatively with CVT (r=–0.36, P<0.0001) and CSB (r_s=–0.24, P=0.004) but there was no discernable effect of gender.

Stratification of CVT by age

In order to dichotomize age into a younger and older group, the raw ages of the subjects were first converted into *Z*-scores and then into *T*-scores (mean 50±10), a method similar to that described by Zobel *et al* [33,34]. The mean and

Table 1 The mean±SD of HR, MBP and CVT and median with IRQ of CVT and CSB measured at baseline

	HR (bpm)	MBP (mmHg)	CVT (LVS)	CSB (ΔRR/ΔmmHg)
Mean (±SD)		88.8±16.6	9.5±4.16	
Median (IRQ)	67.4 (61.6-75)			6.5 (4.4-10.6)

HR, heart rate; MBP, mean blood pressure; CVT, cardiac vagal tone; LVS, linear vagal scale; CSB, cardiac sensitivity baroreflex; SD, standard deviation; IRQ, interquartile

SD for the younger and the older age groups were 41.6±1.7 and 59.8±9.3 years, respectively (P<0.001). The mean CVT for the younger and older age groups were 9.5±0.5 and 8.8±0.7 LVS, respectively (P=0.4).

Reproducibility studies

The 30/200 subjects (18 males, median age 32 years, range 20-54 years) were representative of the initial cohort although the reproducibility cohort was older (Table 2).

Table 3 demonstrates the ICC and CR for the autonomic parameters that were measured for the thirty subjects that took part in both studies. In particular, CVT and CSB had excellent reproducibility. Furthermore, Bland-Altman plots for the differences in CVT between the two time points for the 30 subjects show that 29 of 30 points lie within ± 2 SD of the difference between measurements indicating that there was no bias or systematic error (Fig. 1).

Discussion

In this study we have demonstrated that the normal range for CVT, at rest, in the healthy population is at least 1.9-17.8 LVS and furthermore this parameter has excellent reproducibility over a period of 1 year. CVT has been demonstrated experimentally to be both a sensitive and specific proxy measurement of efferent vagal activity [27]. Many of the earlier studies regarding CVT, focussed purely on CVT change rather than absolute values [35]. More recently, Murray *et al* have examined absolute values of CVT in a population of 50 subjects, representing a mix and healthy controls and patients, although CVT was termed the cardiac parasympathetic index [32]. However, this study was only performed in 11 healthy volunteers (4 male) thus rendering the generalizability of these results somewhat limited. However, and to a degree reassuring, the normal range for CVT derived from our current study was 9.5 (1.9-17.8 LVS) which was not significantly different to the normal range reported by Murray *et al* of 0-17.19 LVS [32]. This is notwithstanding the additional limitations of Murray *et al* including a patient sample that were recruited from cardiology and cardiothoracic wards [32]. It has been well described that reduced HRV predicts poor outcomes in those with congestive cardiac failure, post myocardial infarction and mitral regurgitation [36-38]. Thus, the inclusion of in-patients, from a cardiological setting, may have confounded the results to a certain degree.

Over the recent past, CVT has been increasingly utilized, particularly in GI pain, nociceptive physiological and wider research in the field of psychosomatic medicine [21,39,40]. The ANS has provided an attractive candidate for linking psychological traits with physical symptoms as it integrates with regions of the brain associated with pain, interoception and behavior [4,41]. However, a considerable methodological draw back as Tillisch notes, has been "*the inability to measure the ANS in action throughout the body*" [42]. Arguably, within the literature, the most widely utilized proxy measures of autonomic tone in humans are surrogates derived from spectral analysis of heart rate variability (HRV) yet they are not without methodological limitations [18]. For instance,

Table 2 Demographic comparison of the initial study group (n=120) vs. the reproducibility cohort (n=30)

Variable	Initial study (n=200)	Reproducibility cohort (n=30)	P value
Gender (Male: female)	106:94	16:14	1
Age	28 years (range 18-59)	31 years (range 22-55)	0.04
BMI (kg/m^2 - mean±SD)	25.68±0.38	25.22±0.81	0.56

BMI, body mass index; SD, standard deviation

Table 3 Reproducibility, as assessed by ICC and the CR, of the autonomic parameters at 1 year demonstrating that CVT and CSB have excellent reproducibility

	Study 1 Mean (±SD)	Study 2 Mean (±SD)	ICC (95% confidence interval)	CR (95% confidence interval)
HR (bpm)	66±1.9	67.6±1.7	0.53 (0.22-0.74)	18.8 (-17-20)
MBP (mmHg)	78.2±2.2	83.2±1.84	0.71 (0.47-0.85)	16.6 (-11.6-21.7)
CVT (LVS)	10.09±0.9	9.67±0.79	0.81 (0.64-0.91)	5.7 (-6.2-5.37)
CSB (ΔRR/ΔmmHg)	7.61±0.75	7.07±0.64	0.8 (0.65-0.9)	4.5 (-5.1-4)

ICC, intra-class correlation coefficients; CR, coefficient reproducibility; CVT, cardiac vagal tone; CSB, cardiac sensitivity baroreflex; SD, standard deviation; HR, heart rate; MBP, mean blood pressure

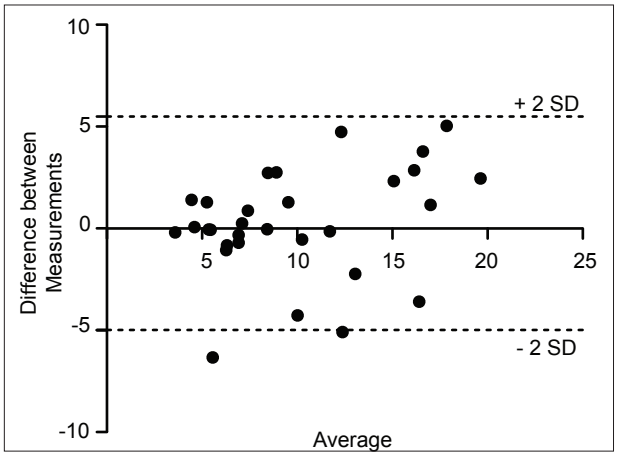

Figure 1 Bland-Altman plot of the reproducibility of CVT measurements. 29 out of the 30 measurements lie within ±2 standard deviations of the differences between measurements suggesting that there was no bias or systematic error and that the parameter of CVT is reproducible at a period of 1 year

many of the validated measures of HRV are inaccurate in epochs of less than 1 min, which should therefore limit experimental design, although this is not always the case [18]. Not surprisingly therefore, many studies using HRV, across a diverse array of academic disciplines, have reported disappointingly inconsistent results. CVT however transcends many of these difficulties, allowing researchers to interrogate and study smaller epochs of interest, which may, in future, further its applications in GI research. A recent example of this is a study by Paine *et al*, who demonstrated for the first time co-activation of the SNS and PNS in response to a painful visceral stimulus using CVT and CSB [22]. The traditional and dogmatic understanding would have predicted a classical "fight or flight" response in response to an acutely painful stimulus, being characterized by SNS activation with concomitant PNS withdrawal. These data highlight the knowledge gaps that remain in our understanding of the response of the ANS to acutely stressful stimuli.

This study is not without its limitations. Firstly, we did not formally assess the regularity with which individuals undertook physical exercise. It is not known whether exercise and fitness levels influence CVT *per se*. However, a number of previous studies have demonstrated that cardiovascular fitness influences autonomic control of heart rate and therefore, by extension, vagal tone [43,44,45]. In addition, we chose to study subjects after one year so no comment can be made as to whether CVT is reproducible over the short (hours to days) or medium (weeks to months) term. Although ANS function is incompletely evaluated using this technique, for instance with concomitant measurement of more established measures of time or frequency domain measures of HRV, the enhanced temporal resolution has facilitated our groups and others to describe changes in these PNS parameters to various somatic and visceral stimuli. However, the results described herein cannot be extrapolated to patients groups as we have only studied "supernormal" subjects for our normative data. Further research is warranted to objectively and systematically comparing these techniques to established measures in the clinical environment particularly in patients with GI disorders such as IBD or IBS.

The ANS has been the focus of research across a number of disciplines in gastroenterology including IBD, functional GI disorders and hepatology [46]. However, differing study designs, for instance comparing baseline/resting autonomic tone and response to a stimulus, makes the meaningful interpretation and comparison a significant challenge both to the basic scientist and clinician. This is notwithstanding the bewildering and complex array of autonomic parameters reported across the literature based on both frequency and time domain analysis of HRV. CVT in its most simple terms reduces these challenges to a degree in that its interpretation is more intuitive and its phraseology is arguably easier to understand. Given these assertions, in combination with the aforementioned temporal benefits, it is an attractive parameter in GI research, which may allow the further delineation and refinement of our understanding and the contribution of the PNS to the pathophysiology of many GI disorders. Nevertheless, it remains largely unresolved whether the measurement of ANS measures derived from cardiac chronotropy truly reflect the degree of autonomic tone within the gut and to the best of our knowledge has not been systematically studied (Fig. 2). Whilst this controversy, to the best of our knowledge, has not been objectively determined, measurement of rectal mucosal blood flow (RMBF) by laser Doppler flowmetry has been used

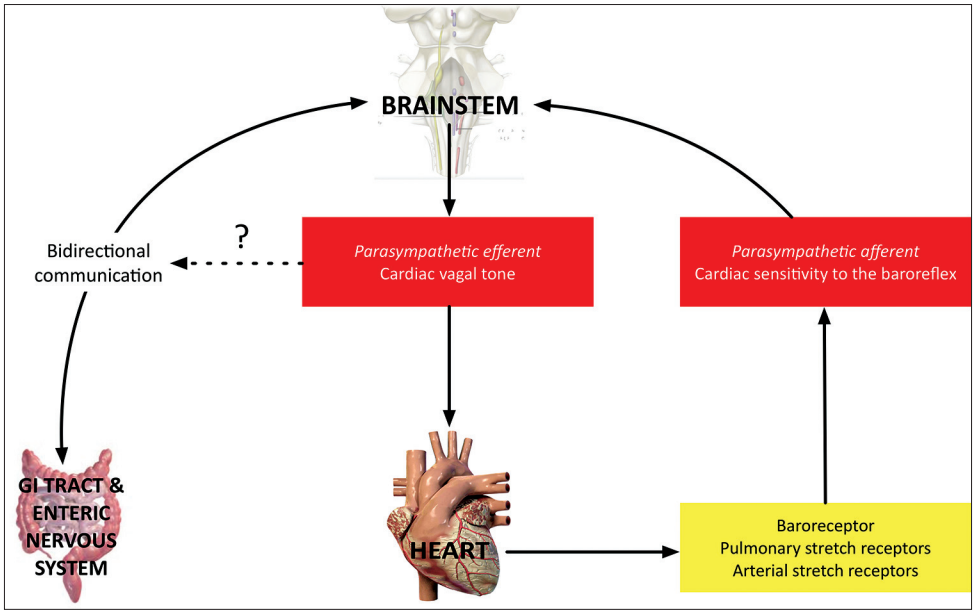

Figure 2 A highly schematic representation of the efferent limb of parasympathetic /vagal tone arising in the brainstem (cardiac vagal tone), its interaction with the heart and subsequently the afferent limb (cardiac sensitivity to the baroreflex). Whether these parameters, derived from cardiac chronotropic measures reflect the bidirectional communication between the parasympathetic nervous system and the gastrointestinal tract remains to be determined

in a number of studies [47]. RMBF reflects the level of activity of autonomic innervation specifically to the rectum, although it must be noted that this technique does have a high degree of intra-operator variability. To date, RMBF and cardiac derived autonomic parameters have not been systematically compared and remains, in our opinion, a key knowledge gap within the field.

As mentioned, autonomic dysfunction has been variably associated with a number of functional GI disorders, including IBS. In a recent narrative review, Masurak *et al* reported that the majority of studies in the literature demonstrated no difference in HRV parameters when the IBS population was compared to healthy controls [48]. However, when IBS patients are sub-classified according to predominant bowel habit, and the presence of psycho-affective disorders, they are associated with changes in autonomic functioning, particularly in the PNS. Whilst CVT *per se* has not been measured in patients with IBS, we have recently reported a study in patients with functional chest pain of presumed esophageal origin, where at baseline, patients had lower CVT (5.5±0.84 vs. 11.76±1.6, P=0.003) [49].

In conclusion, we have defined the normal range of CVT in a relatively large population of normal healthy subjects. Over a period of 1 year, CVT is a reproducible measure of PNS tone.

Summary Box

What is already known:

- Abnormalities within the parasympathetic nervous system have been proposed across a number of gastrointestinal disorders including the prevalent irritable bowel syndrome
- Parasympathetic nervous system parameters can be difficult to measure, interpret and reproduce
- Herein we present a novel non-invasive measure of efferent parasympathetic nervous system tone, known as cardiac vagal tone

What the new findings are:

- Cardiac vagal tone, in comparison to more traditional values derived from heart rate variability, has enhanced temporal resolution and is increasingly being used in gastrointestinal research
- In a large group of healthy volunteers across 3 study centers, we described the normal range of cardiac vagal tone and demonstrate its reproducibility at 1 year
- Further research is now warranted to examine the salience of this parameter in patient cohorts, such as those with irritable bowel syndrome

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