Aging Affects the Response of Heart Rate Variability Autonomic Indices to Atropine and Isoproteronol

Kenneth M. Madden[1], Wayne C. Levy[2] and John R. Stratton[2]

[1]Department of Medicine, University of British Columbia, Vancouver, BC, Canada. [2]Department of Medicine, Division of Cardiology, Seattle Veterans Affairs Medical Center and the University of Washington, Seattle, Washington.

Abstract

Background: Normalized ratios of portions of the power frequency spectrum of heart rate (HR) are commonly used to gain insight into cardiac "sympathovagal balance." Whether aging, which alters both sympathtetic and parasympathetic activities, influences these measures has not been well characterized.

Objectives: We examined the ability of normalized ratios of the power frequency spectrum of heart rate to describe autonomic activity at the sinus node in older and younger adults during conditions of sympathetic predominance.

Methods: 20 older (mean age 70.0 ± 1.5 years) and 24 younger (mean age 25.4 ± 0.9 years) normal adults were screened by history, physical examination, blood work (CBC, electrolytes, creatinine, liver function tests), ECG, exercise tolerance test, echocardiogram and myocardial perfusion scan (if >65 years old). A 2-channel Holter was used to monitor heart rate. Total (TP), low frequency (LF) and high frequency power (HF) were obtained by Fast Fourier Transform. Intravenous atropine (2 boluses of 0.01 mg/kg) was followed by isoproterenol infusions of 7 and 21 ng/kg/min to tilt the "sympathovagal balance" to the sympathetic nervous system side.

Results: Normalized HF power gave expected results in response to atropine only in younger subjects. Changes in normalized LF power had a much stronger correlation with changes in heart rate in older as opposed to younger subjects.

Conclusions: The response of normalized power ratios to atropine and isoproterenol varies between different age groups.

Keywords: heart rate variability, autonomic nervous system, isoproterenol, atropine, sympathetic nervous system, parasympathetic nervous system

Background

Aging of the autonomic nervous system can have profound implications for cardiovascular health, and can be influenced by numerous disease processes. Consequently, physiological investigators have long sought noninvasive indicies to determine the relative contributions of sympathetic and parasympathetic effects on the sinus node[1–3]. A variety of noninvasive measures have been postulated, most utilizing different measures of cardiovascular variability[4].

Low frequency oscillations in heart rate (LF, 0.04 to 0.15 Hz) have been found to have significant parasympathetic and sympathetic components while high frequency oscillations in heart rate (HF, 0.15 to 0.4 Hz) are due primarily to the parasympathetic arm of the autonomic nervous system[5]. Investigators have attempted to use normalized ratios of the areas under power frequency spectra curves to extract measures of sympathetic or parasympathetic contributions to modulation of heart rate under different physiological conditions[1]. Even if one ignores the various concerns raised about the validity of this approach[4, 6], the behaviour of the normalized LF (NLF), the normalized HF (NHF) and the LF/HF power ratio under various autonomic conditions has primarily been examined in young subjects only[1–3]. It is well established that aging is associated with significant sympathetic upregulation[7], while overall vagal tone to the sinus node decreases with age[8]. Since there are many factors that

Correspondence: Kenneth M. Madden, M.D., Room 7185, Gordon and Leslie Diamond Health Care Centre, 2775 Laurel St., Vancouver, BC, Canada, V5Z 1M9. Tel: 604-875-4931; Fax: 604-875-5696; Email: kmmadden@interchange.ubc.ca

contribute to heart rate variability that are external to the autonomic nervous system[4], we predicted that normalized ratios would most accurately reflect changes in vagal and sympathetic modulation of heart rate in populations where these influences are greatest. Consequently, we hypothesized that normalized ratios would most accurately capture vagal changes in younger subjects and sympathetic changes in older subjects. By first administering atropine and then isoproterenol we sought to create a state of progressive sympathetic predominance that would produce predictable changes in NLF, NHF and the LF/HF power ratio, as illustrated in Figure 1.

Materials and Methods

Subjects

Subjects were excluded if they had any history of angina, myocardial infarction, stroke, hypertension, chronic pulmonary disease, diabetes, current medication use (prescription or over the counter), current smoking, or exercise-limiting orthopedic impairment. Entry requirements included normal blood pressure (BP), physical examination, resting electrocardiogram, hematocrit, fasting blood glucose, total cholesterol, and creatinine. Subjects had to have normal M-mode and two-dimensional echocardiograms showing no more than mild valvular regurgitation, and a normal Bruce protocol treadmill maximal exercise stress test that was enhanced by myocardial perfusion imaging in all subjects over 65 years of age. We recruited a total of 44 healthy adults (24 younger and 20 older subjects). There were 8 younger female and 7 older female subjects. Older subjects were defined as persons with an age greater than or equal to 65 years. See Table 1 for subject characteristics.

This study was approved by the Human Subjects Committee of the University of Washington, and all subjects gave informed consent.

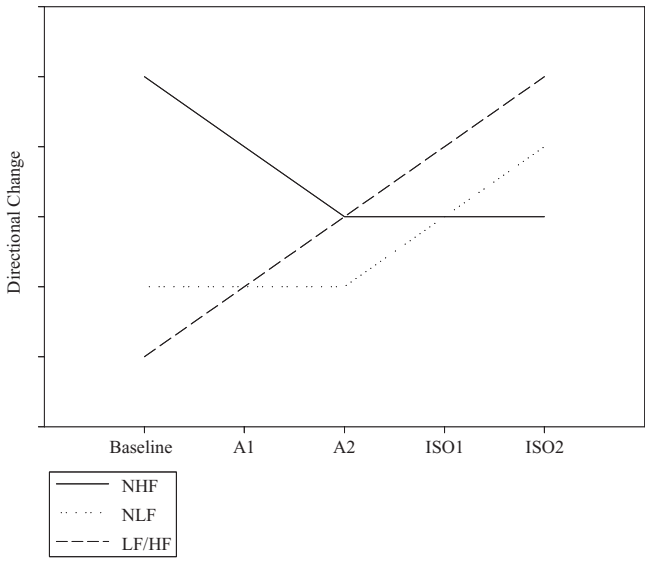

Figure 1. A demonstration of the expected directional changes in normalized high frequency (NHF), normalized low frequency (NLF) and low frequency over high frequency (LF/HF) power ratios with intravenous atropine (0.01 mg/kg bolus X 2, A1 and A2) and isoproterenol (7 ng/kg/min followed by 21 ng/kg/min; ISO1 and ISO2). Directional changes are expressed in arbitrary normalized power units (nU).

Study protocol

All subjects were examined in the fasting state. All study sessions were performed with the subject supine and occurred between 10 AM and noon for all subjects to avoid bias due to circadian rhythms in heart rate variability. Each subject was supine for 45 minutes prior to the start of data collection in order to reach steady state. Progressive doses of atropine (two at 0.01 mg/kg, each one given 6 minutes prior to data collection) were followed by continuous isoproterenol infusions (a beta-1 agonist) of 7 and 21 ng/kg/min for 14 minutes prior to data collection each in order to tilt the "sympathovagal balance" to the sympathetic side. Atropine is a vagolytic agent while isoproterenol is a beta-receptor agonist which acts primarily to increase heart rate, cardiac contractility and vasodilatation[9]. We chose doses of atropine[10] and isoproterenol[11] that were not expected to elevate heart rate beyond 80% of maximum, for safety reasons. A 14-minute isoproterenol infusion was chosen since this duration has been shown to be necessary to achieve steady-state concentrations[11].

Data collection and processing

Each subject had heart rate monitored on a beat-to-beat basis by a 2-channel Holter monitor (Spacelabs, United States) during rest and during drug infusions. 2-channel electrocardiogram signals were recorded for 10 minutes at baseline, after each atropine bolus and during each isoproterenol infusion. Using a metronome we paced respiration at 0.25 Hz in order to reduce the effects of vagally-mediated respiratory sinus arrythmia on the low frequency range of the frequency analysis[12].

Table 1. Subject characteristics.

	Younger Subjects	Older Subjects	P value
Age (years)	25.4 ± 0.9	70.0 ± 1.5	<0.001*
Weight (kg)	78.3 ± 4.3	73.1 ± 4.0	0.402
Height (cm)	168.8 ± 2.7	169.4 ± 2.9	0.894
Body surface area (m^2)	1.91 ± 0.07	1.85 ± 0.07	0.541
Body mass index (kg/m^2)	27.2 ± 0.9	25.3 ± 0.6	0.121

Characteristics of the subjects studied (age, weight, height, body surface area and body mass index) are expressed as mean ± standard error. The symbol * designates a significant effect of atropine/isoproterenol ($p < 0.05$) as per t-test for independent measures.

Power frequency spectra were calculated using a Fast Fourier Transform using a Spacelabs FT2000 (Spacelabs, United States). The Spacelabs FT2000 samples an analog electrocardiogram signal at 500 Hz and converts it into a digital signal, which is an appropriate sampling rate for spectral analysis[13]. Each heart rate signal was manually examined on a beat-by-beat basis to label each beat as normal, artifact or premature ventricular contraction. The Spacelabs FT2000 uses a linear interpolation algorithm for all premature ventricular contractions as is commonly used in the literature[14]. Each digitized data set of beat-to-beat heart rate was converted into a power frequency spectra using a Fast Fourier Transform. It is well established that the length of the dataset collected can affect the area calculated under the power frequency spectra; consequently all recordings were 10 minutes in length[13]. The low frequency (LF) power was calculated as the area under the power frequency spectra between 0.04 and 0.15 Hz. High frequency (HF) power was the area under the power frequency spectra between 0.15 and 0.4 Hz. Total frequency (TF) power is the area between 0.04 and 0.4 Hz[1,13]. This method of analysis is in accordance with published guidelines[13].

Normalized ratios of power frequency spectra were calculated as described in the literature[1], in order to attempt to separate out the influence of the sympathetic and parasympathetic nervous systems on "sympathovagal balance." Normalized high frequency power (NHF) was calculated as HF over TF power[1]. Normalized low frequency power (NLF) was calculated as LF over TF power[1]. "Sympathovagal balance" was calculated by the ratio of LF to HF power (LF/HF power ratio)[1]. If these ratios are accurately able to determine changes in sympathetic tone (NLF), parasympathetic tone (NHF), or "sympathovagal balance" (LF/HF power ratio) at the sinus node one would expect them to change in predictable directions in response to atropine and isoproterenol, as shown in Figure 1.

Statistical analysis

All data analysis was done in a blinded fashion. A two factor mixed design analysis of variance (ANOVA) involving one independent measure (age group) and one repeated measure was used to determine the effect of atropine and isoproteronol on the various autonomic measures. The effects of atropine and isoproteronol on the various autonomic measures within each age group were determined by one-way ANOVA with repeated measures. Mauchly's Test of Sphericity was used prior to each ANOVA for repeated measures and a Greenhouse-Geisser correction was applied to counteract violations of sphericity[15,16]. Correlations were calculated using the Pearson coefficient. A value of $p \leq 0.05$ was considered significant for all analyses.

Results

Absolute power frequency spectral powers

As expected, progressive doses of atropine and increasing isoproterenol infusion caused a significant drop in RR Interval in both young ($p < 0.0001$) and older ($p < 0.0001$) groups. Absolute values for LF, HF and TF power decreased with each atropine bolus and continued to drop with isoproterenol infusion in both age groups (See Table 2).

Normalized low frequency power (Fig. 2)

Progressive doses of atropine and isoproterenol infusions resulted in significant changes in

Table 2. Hemodynamic changes and changes in absolute power during atropine and isoproterenol.

	Resting	Atropine dose #1	Atropine dose #2	ISO infusion #1	ISO infusion #2	P value
RR Interval						
Young	856 ± 64	643 ± 18	556 ± 10	469 ± 10	408 ± 8	<0.001*
Old	923 ± 32	679 ± 25	580 ± 34	510 ± 50	483 ± 13	<0.001*
SBP						
Young	122 ± 2	126 ± 3	128 ± 3	127 ± 4	131 ± 4	0.038*
Old	126 ± 4	126 ± 5	122 ± 4	127 ± 4	132 ± 6	0.179
DBP						
Young	73 ± 2	78 ± 2	79 ± 2	72 ± 3	62 ± 2	<0.001*
Old	74 ± 3	76 ± 2	75 ± 3	73 ± 2	68 ± 3	0.002*
MAP						
Young	89 ± 1	94 ± 2	95 ± 3	90 ± 3	85 ± 2	<0.000*
Old	92 ± 3	93 ± 3	91 ± 3	91 ± 2	89 ± 4	0.415
LF power						
Young	1288 ± 371	70 ± 18	14 ± 1	22 ± 6	22 ± 10	<0.001*
Old	269 ± 59	26 ± 4	18 ± 3	12 ± 2	11 ± 1	<0.001*
HF power						
Young	899 ± 249	13 ± 5	3 ± 1	6 ± 4	3 ± 1	<0.001*
Old	85 ± 16	25 ± 10	15 ± 6	10 ± 7	2 ± 1	0.012*
TF power						
Young	3674 ± 742	307 ± 76	72 ± 9	85 ± 13	62 ± 8	<0.001*
Old	1155 ± 314	174 ± 36	97 ± 20	98 ± 27	48 ± 9	<0.001*

The effect of each atropine bolus (0.01 mg/kg) and both isoproterenol (ISO) infusions (7 ng/kg/min followed by 21 ng/kg/min) on RR interval (ms), systolic blood pressure (SBP, mm of Hg), diastolic blood pressure (DBP, mg of Hg), mean blood pressure (MAP, mm of Hg), low frequency power (LF, ms^2), high frequency power (HF, ms^2), and total power (TF, ms^2). All means are presented ± standard error. The symbol * designates a significant effect of atropine/isoproterenol ($p < 0.05$) as per Analysis of Variance with repeated measures.

NLF power in both young ($p = 0.040$) and older ($p = 0.009$) subjects. Both younger and older subjects showed no difference in the NLF response to atropine ($p = 0.528$, 2-way ANOVA) or isoproterenol ($p = 0.473$, see Fig. 2). The change in heart rate with the second infusion of isoproterenol (compared to post-second atropine bolus) did not significantly correlate with the change in NLF in young subjects ($r = 0.19$, $p = 0.59$) but had a strong correlation in older subjects ($r = 0.81$, $p = 0.01$).

Normalized high frequency power (Fig. 3)

As shown in Table 2 and Figure 3, NHF showed a significant change with progressive atropine and isoproterenol infusions in both young ($p < 0.001$) and older ($p = 0.026$) subjects. Young subjects demonstrated a decrease in NHF with each atropine bolus and a subsequent plateau with the isoproterenol infusions (Fig. 3). Older subjects showed an increase in NHF with atropine, followed by a decrease with the isoproterenol infusions (Fig. 3). The NHF response showed a significant difference when the two age groups were compared with both atropine ($p = 0.003$) and isoproterenol ($p = 0.038$). The change in heart rate after the second dose of atropine (compared to baseline) did not significantly correlate with the change in NHF in either young ($r = 0.49$, $p = 0.13$) or older ($r = 0.13$, $p = 0.764$) subjects.

LF/HF power ratio (Fig. 4)

After both atropine and isoproterenol were used to heavily tilt "sympathovagal balance" to the sympathetic side, the LF/HF power ratio was an unmeasurable singularity (a denominator equal to zero) in 1 subject (1 young, 0 older) after the first atropine bolus, 5 subjects (3 young, 2 older) after the second atropine bolus, 8 subjects (5 young, 3 older) at the end of the first isoproterenol

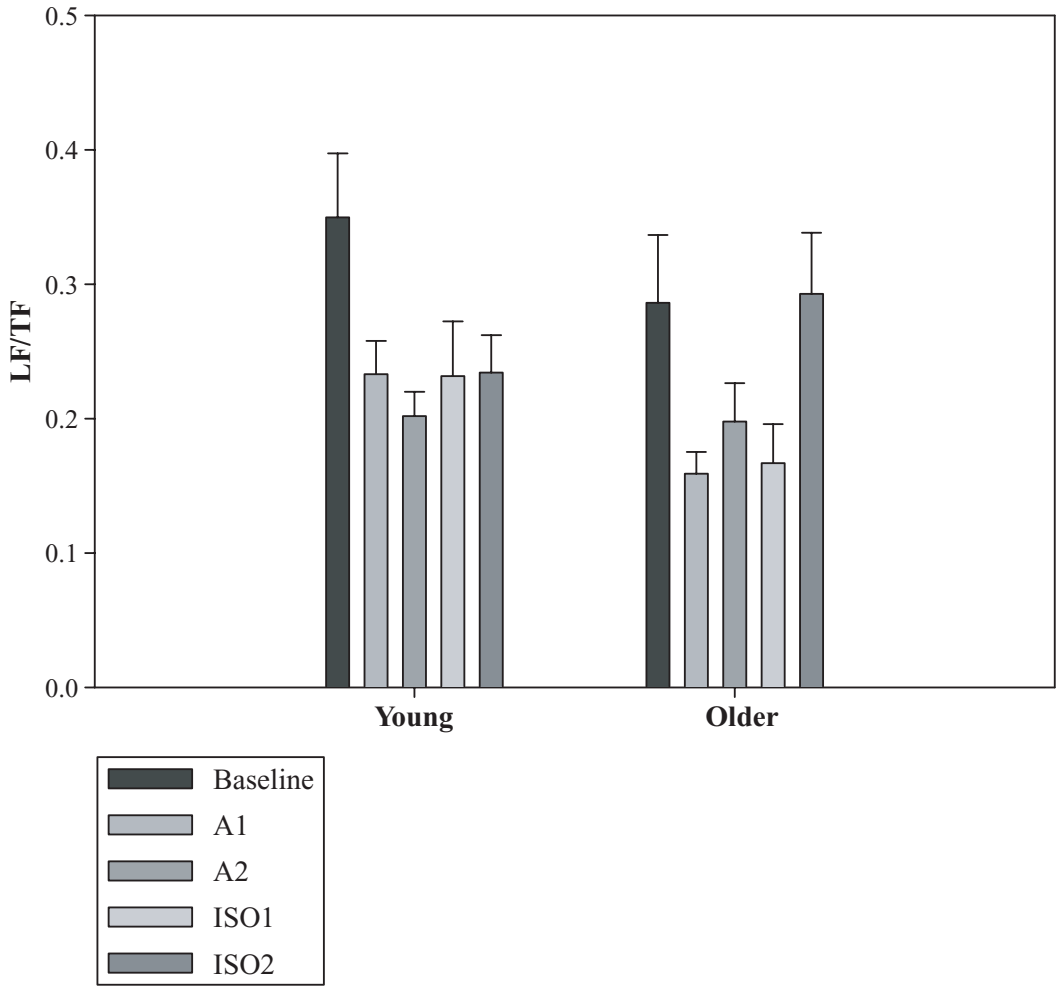

Figure 2. The effects of intravenous atropine (0.01 mg/kg bolus X 2, A1 and A2) and isoproterenol (7 ng/kg/min followed by 21 ng/kg/min; ISO1 and ISO2) on the normalized low frequency power (NLF), calculated by low frequency power/total power in both young and older subjects. Both younger and older subjects demonstrated a decrease in NLF with atropine. NHF demonstrated an increase with isoproterenol infusion in older subjects only. Error bars represent 95% confidence intervals.

infusion and 6 subjects (2 younger and 6 older) after the second isoproterenol infusion. Due to the forced exclusion of these subjects from the analysis, atropine and isoproterenol resulted in no significant change in "sympathovagal balance" in either young ($p = 0.369$) or older ($p = 0.319$) subjects as seen in Table 2 and Figure 4.

Hemodynamic changes with atropine and isoproterenol

As shown in Table 2, progressive boluses of atropine resulted in a statistically significant increase in heart rate, mean BP, diastolic BP and systolic BP in young subjects but no significant increase in systolic BP or mean BP in older subjects. While the increases observed in the various pressures were statistically significant, they were quite minimal in size (Table 2).

Discussion

We examined the behaviour of NLF, NHF and LF/HF power ratios in younger and older subjects during pharmacologically-induced sympathetic predominance at the sinus node. Our findings were congruent with the present literature with respect to questions surrounding their validity. Our study demonstrates that the change in NHF with atropine and isoproterenol varies with the age group being studied, a novel finding. To our knowledge, this is the first time that an age-related difference in the response of NHF to autonomic manipulation has been documented, demonstrating potentially important limitations of these measures. If NHF, NLF and LF/HF power ratios were accurately detecting progressive sympathetic predominance at the sinus node, then they should

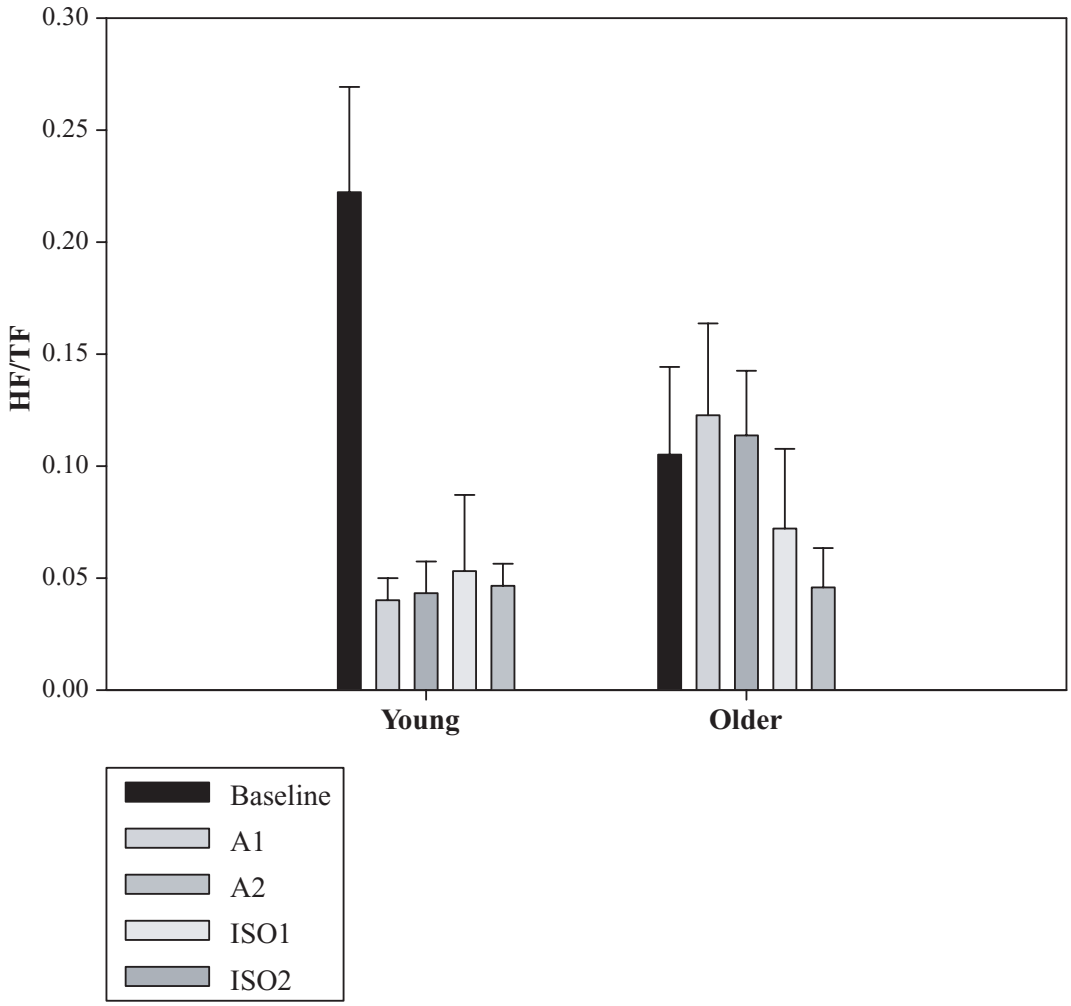

Figure 3. The effects of intravenous atropine (0.01 mg/kg bolus X 2, A1 and A2) and isoproterenol (7 ng/kg/min followed by 21 ng/kg/min; ISO1 and ISO2) on the normalized high frequency power (NHF) , calculated by high frequency power/total power in both young and older subjects. Young subjects demonstrated appropriate changes in NHF, while older subjects NHF increased with atropine and then decreased with isoproterenol. Error bars represent 95% confidence intervals.

have demonstrated specific directional (increase or decrease) changes with progressive doses of atropine and isoproterenol, as shown in Figure 1. Contrary to these expectations, appropriate changes were demonstrated in the NHF ratio only in younger subjects. Also changes in NHF (a measure of vagal activity) seemed to only correlate with changes in HR in younger subjects, while changes in NLF (a measure of sympathetic activity) seemed to only correlate with changes in HR in older subjects.

NLF power ratio

NLF did not appear to provide a reasonable qualitative measure of sympathetic tone at the sinus node equally in both the younger and older age groups. It is well-established that both the parasympathetic and sympathetic components of the autonomic nervous system contribute to low frequency power[17,18], but the use of the NLF as a marker of sympathetic tone at the sinus node assumes that the sympathetic contribution to low frequency power predominates[1,5,17,19,20]. But our study demonstrated little correlation between NLF and HR in younger subjects with the administration in atropine and isoproterenol, suggesting that changes in vagal tone overwhelmed low frequency oscillations due to the sympathetic nervous system. This is in agreement with previous studies demonstrating that isoproterenol infusion in young subjects (after similar doses of atropine as the present study) does not result in an increase in NLF power[21].

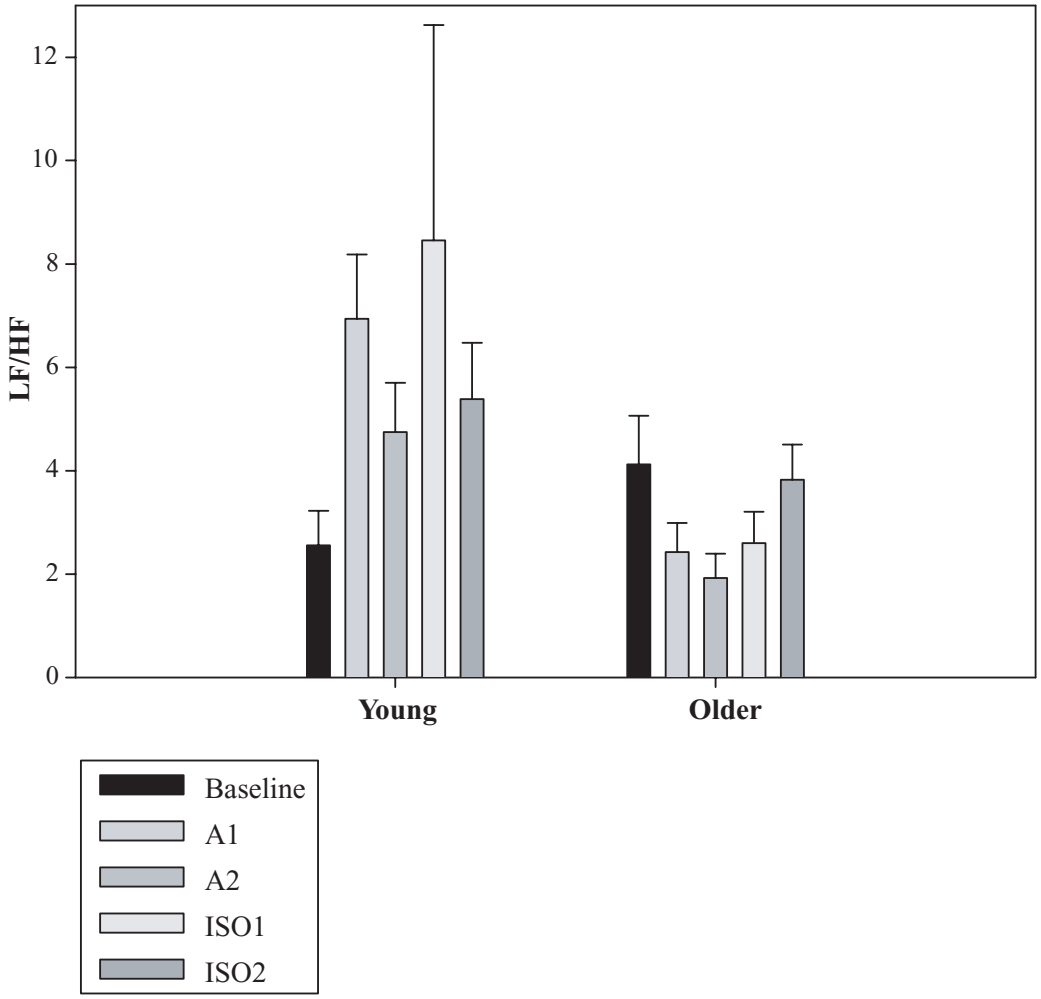

Figure 4. Changes in LF/HF power with intravenous atropine (0.01 mg/kg Bolus X 2, A1 and A2) and isoproterenol (7 ng/kg/min followed by 21 ng/kg/min; ISO1 and ISO2), calculated by low frequency power/high frequency power in both young and older subjects. Confidence intervals are quite high due to high subject dropout from the analysis due to a zero denominator. Error bars represent 95% confidence intervals.

In addition, other studies have shown a paradoxical increase[22,23] or no change[22] in low frequency power with sympathetic blockade (propranolol) and preganglionic cardiac sympathetic blockade with high spinal anesthesia has also been shown to have no impact on the NLF[24,25]. Overall, our results in younger subjects are in concordance with the established literature with regards to the lack of utility of the NLF as a measure of cardiac sympathetic activity.

Contrary to the above results, older subjects demonstrated quite a good correlation between NLF and heart rate during atropine and isoproterenol infusion, a result that was not seen in the younger population, suggesting that NLF might actually provide a quantitative measure of sympathetic influences on the sinus node in older subjects. Some have suggested that normalized power ratios are better at reflecting changes in the sympathetic nervous system in situations where "cardiac sympathetic drive is activated"[4] supported by work in both canine[26] and human[27] models. Since the aging process itself is associated with adrenergic upregulation[7], the sympathetic component to NLF might not have been as obscured by the atropine-induced drop in vagal contributions to low frequency power as it was in younger subjects.

NHF power ratio

NHF provided a reasonable qualitative measure of vagal activity at the sinus node in younger subjects as demonstrated by a decrease with atropine and subsequent plateau during the

isoproterenol infusions. However, older subjects demonstrated a paradoxical increase in NHF with atropine and a steady decrease with isoproterenol (Fig. 3). The NHF showed weak correlations with the shortening of the RR interval after two boluses of atropine in both young and older subjects. This suggests that while the NHF can indicate qualitative changes in younger subjects, it is not appropriately used as a quantitative index of vagal tone in any age group. This is in concordance with previous literature in young subjects showing poor correlations between NHF and the increase in heart rate with a maximal dose of atropine (gold standard measure of vagal tone)[19].

LF/HF power ratio

Our study showed that in 8 of the subjects studied, "sympathovagal balance" became an immeasurable singularity (high power became zero) under the influence of progressive sympathetic predominance. When the remaining subjects were analysed, there was no statistically significant changes in "sympathovagal balance" in either age group during simultaneous administration of atropine and isoproterenol, probably due to the small remaining number of subjects. Previous studies of younger subjects have questioned the validity of using the LF/HF ratio either due to theroretical grounds[6], due to the high variability in the index amoung different subjects under similar autonomic conditions[28] or due to the failure of pharmacologic sympathetic stimulation (with isoproterenol) to increase the ratio[28]. However, we found that not only did LF/HF power not respond appropriately to pharmacologic changes in the autonomic outflow to the sinus node, it actually became impossible to measure (zero denominator) in one-third of the subjects studied. An infinite "sympathovagal balance" means either there was complete vagal withdrawal (very unlikely at the doses administered) or that there was maximal sympathetic outflow to the heart. The fact that RR-interval continued to rise in subjects after they had developed an "infinite" LF/HF ratio suggests that in some subjects this measure ceases to provide insight into the relative autonomic contributions at the sinus node in situations of relative sympathetic (versus vagal) predominance. This indicates that the use of the LF/HF ratio in situations of simultaneous vagal withdrawl and sympathetic stimulation will likely result in uninterpretable results in many subjects.

Limitations

One limitation is the relatively large number of subjects who could not be included in the LF/HF power analysis due to a zero denominator. However, this still does not change the primary result of the study with regards to the LF/HF power; in a large percentage of both young and older subjects this measure will cease to provide useful information in situations of combined sympathetic predominance and vagal withdrawl. It is also possible that some of the changes in NLF, NHF and LF/HF power might have been due to arterial baroreflex responses to changes in blood pressure with atropine and isoproterenol. However, this is unlikely given the small magnitude of the pressure differences involved (see Table 2).

Conclusions

The ability of NLF, NHF and the LF/HF power ratios to assess changes in autonomic tone has severe limitations. These in part relate to the age of the subjects being studied. Our study demonstrated that NLF is a better qualitative and quantitative measure of sympathetic tone to the sinus node in older subjects while NHF appears more valid as a qualitative indicator of vagal tone in younger subjects only. LF/HF had even more limitations in both younger and older subjects, becoming an unmeasurable singularity in one-third of subjects during simultaneous administration of atropine and isoproterenol.

Our results suggest that the use of these particular cardiovascular variability indices to measure autonomic modulation of heart rate needs to consider the age of the subjects being studied. Given the significant effect age had on the applicability of these ratios, investigations that attempt comparisons between groups (such as a therapy group and a control group) need to be approached with caution. These ratios appear to be much better suited to investigating changes in autonomic activity within the same group, such as in study designs where the subjects act as there own controls (such as where heart rate variability measurements are taken before and after an intervention). However, even in this setting, ratios such as NLF and LHF are going be better

indicators of changes in sympathetic tone in older subjects and of changes in parasympathetic tone in younger subjects.

Competing Interests

The authors declare that they have no competing interests.

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