International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Sensitivity of Time-Domain Heart Rate Variability Indices in Detecting Acute Dietary Autonomic Modulation: NN50 versus RMSSD

Harjot Kaur Sidhu[1], Gagneen Kaur Sandhu[2], Rachna Bachhel[3], Sneh Lata Garg[4]

[1]Department of Physiology, Government Medical College, Patiala, Punjab, India.
[2]Associate Professor, Department of Physiology, Government Medical College, Patiala, Punjab, India.
[3]Professor and Head, Department of Physiology, Government Medical College, Patiala, Punjab, India.
[4]Senior Resident, Department of Physiology, Government Medical College, Patiala, Punjab, India.

OPEN ACCESS

Corresponding Author:

Gagneen Kaur Sandhu
Associate Professor, Department of Physiology, Government Medical College, Patiala, Punjab, India.

Received: 05-02-2026
Accepted: 25-02-2026
Available online: 25-03-2026

ABSTRACT

Background: Time-domain heart rate variability (HRV) indices are commonly used to assess cardiac parasympathetic modulation during short-term recordings. RMSSD is widely recommended for this purpose; however, the sensitivity of NN50 in detecting acute autonomic modulation has not been adequately evaluated.
Objectives: To compare the sensitivity of NN50 and RMSSD in detecting acute dietary autonomic modulation using short-term HRV recordings in healthy young adults.
Methods: This methodological analysis was conducted on HRV data obtained from 100 healthy young adults who underwent 5-minute resting HRV recordings before and after an acute dietary autonomic stimulus. Time-domain HRV indices NN50 and RMSSD were analysed. Pre–post changes, sex-stratified differences, standardized effect sizes (Cohen's d and Hedges' g), and 95% confidence intervals were calculated to assess comparative sensitivity.
Results: NN50 showed a statistically significant increase following the intervention ($p = 0.015$) and demonstrated a larger standardized effect size with confidence intervals excluding zero. In contrast, RMSSD exhibited a non-significant change ($p = 0.120$) and smaller effect sizes with confidence intervals crossing zero. Sex-stratified analysis revealed greater NN50 responsiveness among females, while RMSSD showed no significant sex-related difference.
Conclusion: NN50 is a more sensitive time-domain HRV index than RMSSD for detecting acute dietary autonomic modulation in short-term recordings and should be considered alongside conventional measures in acute autonomic research.

Keywords*: Heart Rate Variability; Autonomic Nervous System; Parasympathetic Nervous System; Dietary Stimulants; Caffeine; NN50; RMSSD.*

INTRODUCTION

Heart rate variability (HRV) is a widely accepted non-invasive tool for assessing cardiac autonomic modulation and has been extensively applied in cardiovascular, physiological, and psychophysiological research. Among HRV indices, time-domain parameters are particularly useful in short-term recordings due to their computational simplicity and robust physiological interpretation.[1] However, not all time-domain indices demonstrate equivalent sensitivity in detecting acute autonomic perturbations, especially those induced by transient dietary or pharmacological interventions.

RMSSD (root mean square of successive differences of NN intervals) is commonly regarded as the preferred marker of short-term parasympathetic activity and is frequently recommended in guidelines and applied research.[1,2] In contrast, NN50, which represents the absolute count of successive NN interval differences exceeding 50 ms, has received comparatively less attention in contemporary HRV studies. Despite its close physiological association with vagal modulation, NN50 is often excluded due to concerns regarding its dependence on recording length and heart rate, rather than on demonstrated inferiority.[3]

Emerging evidence suggests that NN50 may be particularly responsive to rapid, short-term autonomic fluctuations. Unlike RMSSD, which reflects averaged short-term variability, NN50 captures discrete beat-to-beat changes and episodic parasympathetic bursts, potentially making it more sensitive to acute autonomic modulation under resting conditions.[4] This distinction becomes especially relevant in studies examining dietary stimulants such as caffeine, where autonomic effects may be subtle, transient, and not accompanied by overt hemodynamic changes.[2,5]

Despite widespread caffeine consumption, few studies have directly compared the performance of different time-domain HRV indices in detecting acute dietary autonomic responses within the same population. Most investigations focus on frequency-domain measures or rely primarily on RMSSD, potentially overlooking more sensitive time-domain markers. Furthermore, methodological evaluations of HRV index sensitivity in young, healthy adults—particularly from South Asian populations—remain scarce.

Therefore, the present study aimed to compare the sensitivity of NN50 and RMSSD in detecting acute dietary autonomic modulation using short-term HRV recordings. By examining pre–post changes, effect sizes, confidence intervals, and sex-stratified responses within the same dataset, this study seeks to identify the most responsive time-domain HRV marker for acute autonomic research and to inform optimal parameter selection in future physiological and nutritional studies.

METHODOLOGY

This study presents a secondary, pre-specified methodological analysis derived from a dataset of 100 healthy young adults aged 18–30 years who underwent short-term heart rate variability (HRV) assessment before and after a standardized acute dietary autonomic stimulus. Only time-domain HRV indices—NN50 and RMSSD—were analysed for the present comparison, as these parameters directly reflect short-term parasympathetic modulation.[1] HRV was recorded for 5 minutes in the supine resting state using a digital ECG system with beat-to-beat RR interval acquisition and validated HRV analysis software.

Pre–post changes were calculated for each parameter, and sensitivity was evaluated through statistical significance, magnitude of change, and standardized effect sizes. Paired t-tests were used to assess within-subject changes, while sex-stratified comparisons of Δ values were analysed using Welch's t-test. Cohen's d (between-group) and Hedges' g (pre–post, small-sample corrected) were calculated, with 95% confidence intervals estimated to determine the precision of effect size measures. A p-value <0.05 was considered statistically significant.

This methodological design allows direct comparison of the responsiveness of NN50 and RMSSD to acute autonomic modulation under controlled resting conditions.

RESULTS

Baseline values for the two time-domain HRV indices, NN50 and RMSSD were comparable between males and females, with no significant sex-related differences at baseline (p = 0.694 and p = 0.512, respectively), confirming suitability for a methodological sensitivity comparison. Further, the pre–post comparisons are important. NN50 demonstrated a statistically significant increase following the acute dietary autonomic stimulus (mean Δ +1.23 ± 5.92, p = 0.015). In contrast, RMSSD showed a non-significant increase (mean Δ +2.49 ± 14.41, p = 0.120), indicating weaker responsiveness under identical experimental conditions. (Table 1)

The comparative sensitivity analysis in Table 2 shows that NN50 exhibited a larger standardized effect size than RMSSD. For NN50, the overall pre–post effect size was Hedges' g = 0.21 with a 95% confidence interval (CI) of 0.05 to 0.37, indicating a small but consistent effect that did not cross zero. In contrast, RMSSD demonstrated a smaller effect (Hedges' g = 0.17) with a 95% CI of −0.04 to 0.38, crossing zero and therefore failing to meet the threshold for statistical reliability. Similarly, Cohen's d values also favoured NN50 over RMSSD (d = 0.39 vs 0.23), strengthening the conclusion that NN50 is more sensitive to detecting acute autonomic modulation.

Sex-stratified Δ values (Table 3) revealed that the increase in NN50 was greater among females (+2.63 ± 4.53) compared with males (+0.30 ± 6.97), with a statistically significant difference (p = 0.046) and a moderarate sex-specific effect size (Cohen's d = 0.39). RMSSD showed no significant sex-based difference in responsiveness (p = 0.268), and its effect size was small (d = 0.23), reinforcing the observation that RMSSD is less sensitive to acute modulation.

Across all analytic approaches—absolute change, statistical significance, effect size magnitude, confidence intervals, and sex-stratified comparison—NN50 consistently demonstrated superior sensitivity to acute autonomic modulation compared with RMSSD. RMSSD showed a trend toward increase but failed to reach statistical significance or produce effect sizes with confidence intervals excluding zero.

Table 1. Baseline Time-Domain HRV Parameters in the Study Population (N = 100) with Pre-Post Changes in NN50 and RMSSD Following Acute Dietary Intervention

Parameter		NN50 (count)	RMSSD (ms)
Baseline parameters (Mean ± SD)	Total	7.82 ± 11.05	18.45 ± 29.16
	Male	7.77 ± 10.80	18.08 ± 28.13
	Female	7.97 ± 11.43	19.11 ± 30.71
	p	0.694	0.512
Pre – Post intervention (Mean ± SD)	Pre	7.82 ± 11.05	18.45 ± 29.16
	Post	8.27 ± 12.28	20.70 ± 31.65
	Δ	5.92 ± 1.23+	14.41 ± 2.49+
	p	0.015	0.120

Table 2. Comparative Sensitivity of NN50 and RMSSD: Effect Size Analysis

Parameter	Mean Δ (Post–Pre)	Cohen's d	Hedges' g	CI %95 (Effect Size)	Significant Change
NN50	1.23+	0.39	0.21	0.37 – 0.05	Yes
RMSSD	2.49+	0.23	0.17	0.38 – 0.04−	No

Table 3. Sex-Stratified Sensitivity Analysis of NN50 and RMSSD

Parameter	Male Δ (Mean ± SD)	Female Δ (Mean ± SD)	p-value (Δ M vs F)	Cohen's d (Sex Effect)
NN50	6.97 ± 0.30+	4.53 ± 2.63+	0.046	0.39
RMSSD	10.71 ± 1.08+	17.98 ± 4.61+	0.268	0.23

DISCUSSION

The present methodological analysis compared the sensitivity of two widely used time-domain heart rate variability (HRV) indices—NN50 and RMSSD—in detecting acute dietary autonomic modulation. The key finding is that NN50 consistently demonstrated superior responsiveness compared with RMSSD across multiple analytic dimensions, including statistical significance, effect size magnitude, confidence interval behaviour, and sex-stratified analysis. These results highlight important methodological considerations for selecting HRV indices in short-term autonomic research, particularly in studies examining subtle, transient interventions.

NN50 showed a statistically significant pre–post increase, accompanied by a standardized effect size with a confidence interval excluding zero. In contrast, RMSSD exhibited a non-significant increase, and an effect size whose confidence interval crossed zero, indicating lower reliability in detecting acute autonomic change under identical recording conditions. From a methodological standpoint, this divergence underscores the distinction between sensitivity and popularity of HRV indices. While RMSSD is often favoured due to its mathematical robustness and relative independence from recording length, NN50 appears to be more responsive to brief autonomic perturbations.

This differential sensitivity can be attributed to the conceptual foundations of the two indices. RMSSD represents an averaged measure of short-term variability, effectively smoothing transient fluctuations. NN50, on the other hand, quantifies discrete beat-to-beat changes exceeding a defined threshold, making it particularly sensitive to episodic parasympathetic bursts. In contexts where autonomic modulation is subtle and transient—such as acute dietary interventions—NN50 may therefore capture meaningful changes that are attenuated or diluted in averaged measures.

The findings have direct implications for HRV study design and parameter selection. Current HRV guidelines acknowledge both RMSSD and NN50 as indices of parasympathetic modulation but provide limited guidance on their comparative performance in acute intervention settings.[1] Consequently, many studies default to RMSSD alone, potentially underestimating autonomic responsiveness. The present analysis suggests that reliance on RMSSD as the sole time-domain marker may lead to false-negative findings in short-term studies. Incorporating NN50 alongside RMSSD may improve detection sensitivity without increasing recording complexity. This is particularly relevant for nutritional, pharmacological, and psychophysiological studies, where interventions are brief and autonomic effects may not be accompanied by overt hemodynamic changes. Furthermore, effect size analysis reinforces the importance of moving beyond p-values when evaluating HRV indices. Although the absolute change in RMSSD was numerically larger than that of NN50, its standardized effect size was smaller and less reliable. This highlights the potential for misinterpretation when absolute changes are considered in isolation and supports the routine inclusion of effect size metrics in HRV research.

The sex-stratified analysis demonstrated a greater NN50 response among females, with a moderate effect size, whereas RMSSD showed no significant sex difference. While this observation should be interpreted cautiously, it suggests that NN50 may be more sensitive to sex-related differences in acute parasympathetic modulation. Existing literature indicates that females generally exhibit higher baseline vagal tone and greater autonomic flexibility, which may amplify NN50 responsiveness.[6] Importantly, the absence of consistent sex differences for RMSSD further supports the notion that NN50 may capture aspects of autonomic modulation that are not adequately reflected by averaged measures. Methodologically, this reinforces the value of including NN50 in studies where sex-based analysis is anticipated.

International studies examining acute autonomic modulation have reported mixed findings regarding time-domain HRV responsiveness. Some researchers demonstrated rapid parasympathetic responses to dietary stimuli that were more evident in beat-to-beat measures than in averaged indices.[3] Others have similarly reported that discrete vagal markers responded more readily than RMSSD in short-term settings.[4] However, direct methodological comparisons between NN50 and RMSSD remain scarce, particularly in young, healthy populations. Most contemporary studies, especially from Western cohorts, preferentially report RMSSD, often excluding NN50 altogether.[2] Data from South Asian populations are even more limited, with few studies exploring HRV index performance beyond standard parameters. The present study therefore contributes region-specific evidence supporting the methodological value of NN50 in acute autonomic research.

Beyond dietary interventions, the implications of these findings extend to other areas of autonomic research, including stress testing, mental workload assessment, and short-duration pharmacological studies. In such contexts, the choice of HRV index can influence not only statistical outcomes but also physiological interpretation and clinical inference. The present results suggest that NN50 should not be dismissed as an outdated or redundant parameter. Instead, it may serve as a complementary or even primary index in studies prioritising sensitivity to rapid autonomic change. This reappraisal aligns with recent calls for more nuanced HRV reporting practices that match index selection to research objectives rather than convention.

This methodological analysis has certain limitations. First, the findings are based on short-term resting HRV recordings and may not be generalisable to exercise or stress conditions. Second, habitual dietary patterns were not quantified, which may influence autonomic responsiveness. Third, while effect sizes and confidence intervals were calculated, the study was not powered specifically for methodological comparisons. Finally, only two time-domain indices were examined; inclusion of additional indices such as SD1 may provide further insight.

Future research should systematically compare HRV indices across diverse acute interventions, recording durations, and populations. Incorporating NN50 alongside RMSSD in short-term studies may enhance detection sensitivity and reduce false-negative findings. Longitudinal investigations examining whether NN50 predicts chronic autonomic adaptation better than RMSSD are also warranted. Standardised reporting frameworks that link HRV index selection to study objectives would further strengthen methodological rigour in autonomic research.

CONCLUSION

This methodological analysis demonstrates that NN50 is more sensitive than RMSSD in detecting acute autonomic modulation during short-term HRV assessment. Despite both indices reflecting parasympathetic activity, NN50 consistently showed greater responsiveness, larger standardized effect sizes, and more reliable confidence intervals. These findings suggest that reliance on RMSSD alone may underestimate subtle, transient autonomic changes in acute intervention studies. Incorporating NN50 alongside conventional time-domain measures can enhance analytical sensitivity and improve methodological robustness. Selection of HRV indices should therefore be guided by study objectives and intervention characteristics rather than convention, particularly in short-duration dietary or pharmacological research.

REFERENCES

1. Malik M, Bigger JT, Camm AJ, Kleiger RE, Malliani A, Moss AJ, et al. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Eur Heart J. 1996 Mar 1;17(3):354–81.
2. Koenig J, Jarczok MN, Kuhn W, Morsch K, Schäfer A, Hillecke TK, et al. Impact of Caffeine on Heart Rate Variability: A Systematic Review. J Caffeine Res. 2013 Mar;3(1):22–37.
3. Sondermeijer HP, van Marle AGJ, Kamen P, Krum H. Acute effects of caffeine on heart rate variability. Am J Cardiol. 2002 Oct 15;90(8):906–7.
4. Zimmermann-Viehoff F, Thayer J, Koenig J, Herrmann C, Weber CS, Deter HC. Short-term effects of espresso coffee on heart rate variability and blood pressure in habitual and non-habitual coffee consumers--a randomized crossover study. Nutr Neurosci. 2016 May;19(4):169–75.
5. Notarius CF, Floras JS. Caffeine Enhances Heart Rate Variability in Middle-Aged Healthy, But Not Heart Failure Subjects. J Caffeine Res. 2012 Jun;2(2):77–82.

6. Woo JM, Kim TS. Gender plays significant role in short-term heart rate variability. Appl Psychophysiol Biofeedback. 2015 Dec;40(4):297–303.