

Intravenous hydrogen nanobubbles effect on cardiac physiology and quality of life: a single blind, dose-response study

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Abstract

Hydrogen nanobubbles (HNBs) showed promise for cardiovascular health and health-related quality of life (HRQoL). This study assessed intravenous HNB effects on cardiovascular parameters and HRQoL through a randomized, single-blind trial with 52 participants across six groups (control and five HNB dosages). Over 10 sessions in 5 weeks, the outcomes were measured included blood pressure, heart rate, left ventricular ejection fraction, and HRQoL via SF-36 survey. HNB administration significantly reduced systolic (144.94 to 129.88 mmHg) and diastolic (87.18 to 78.89 mmHg). Heart rate decreased slightly, and left ventricular ejection fraction increased marginally. Improvements in diastolic function, flow-mediated dilation, and HRQoL (general health, mental health, social functioning, bodily pain) were noted. Dose-dependent effects were significant at higher doses. In conclusion, HNB therapy could enhance cardiovascular function and HRQoL. Optimizing dosages and integrating comprehensive health interventions could enhance therapeutic outcomes, providing a holistic approach to managing cardiovascular health.

Introduction

Hydrogen nanobubbles (HNBs) are a novel technology that harnesses the unique properties of hydrogen gas at the nanoscale. These microscopic spherical gas pockets, 10 to 200 nanometers in diameter, are encapsulated in a liquid matrix. Their small size and high surface area result in unique physicochemical behaviors, making them relevant in biomedical applications. HNBs, with their enhanced stability and ability to penetrate biological barriers, demonstrate prolonged interactions with cellular structures. The therapeutic potential of HNBs lies in the biological activity of hydrogen gas. As a selective antioxidant, hydrogen neutralizes reactive oxygen species (ROS) without affecting beneficial ROS involved in cellular signaling. This reduces oxidative stress while preserving essential cellular functions. Additionally, hydrogen's anti-inflammatory properties inhibit pro-inflammatory cytokines. This dual antioxidative and anti-inflammatory action makes HNBs a promising intervention for conditions like cardiovascular diseases, neurodegenerative disorders, and metabolic syndromes [1, 2].

Intravenous administration of HNBs is being investigated for its ability to improve cardiovascular physiology, reduce ischemia-reperfusion injuries, and promote recovery in patients with acute and chronic conditions [3, 4]. HNBs have also shown promise in improving the quality of life by reducing oxidative damage and inflammation at the systemic level. The small size of HNBs facilitates their distribution throughout the body, allowing for targeted delivery and action at the cellular and tissue levels. This property is particularly advantageous for intravenous applications, where rapid and efficient distribution is crucial for therapeutic efficacy.

The stability and sustained release of hydrogen from nanobubbles are crucial for their medical efficacy, with research focusing on developing reliable generation methods and understanding the physicochemical properties that contribute to their stability [5]. Additionally, HNBs have been explored for their potential in drug delivery systems, particularly in cancer therapy, where they can improve the

cellular uptake of chemotherapy drugs and serve as ultrasound contrast agents for diagnostic imaging [6, 7]. The versatility of HNBs extends to water treatment applications, where their disinfection properties can be leveraged to address environmental challenges, further underscoring their broad utility [8].

Overall, the integration of HNB technology in medical applications represents a promising frontier, with ongoing research aimed at optimizing their therapeutic effects and expanding their clinical use [9]. The exploration of HNBs as a therapeutic intervention represents a burgeoning area of research with significant clinical potential. Through systematic evaluation, intravenous HNB therapy can be refined and integrated into clinical practice, providing a novel and effective tool for managing a wide range of medical conditions. The objectives of this study focus on evaluating the impact of intravenous HNBs on quality of life, cardiac physiology, and blood profiles. These objectives provide a comprehensive framework for understanding the potential therapeutic benefits and risks associated with HNB administration, ultimately contributing to the optimization and clinical application of this novel treatment.

Methods

This study employs a randomized, single-blind, dose-response clinical trial to evaluate the effects of intravenous HNBs on multiple health outcomes, including cardiac physiology, quality of life, and blood profile. Participation in this study was entirely voluntary. The study adhered to the ethical principles outlined in the Declaration of Helsinki, ensuring respect for participants, safeguarding their health and rights, and maintaining transparency throughout the research process. Ethical approval was obtained from the research ethical committee of State Polytechnic of Health Malang, which reviewed and endorsed the study protocol to ensure compliance with ethical standards. The ethical number registered was 410/KPEK-POLKESMA/2022.

The trial was structured to compare five different dosages of HNB against a control group receiving normal saline, over a specified period. The inclusion criteria are: adults aged 18–65 years, stable medical condition allowing participation, and able to provide informed consent. The exclusion criteria were: known allergies or adverse reactions to hydrogen, concurrent participation in another clinical trial, and significant health issues that could interfere with the study (eg. congestive heart failure, stage 4 and above kidney failure, liver failure).

Participants were assigned to one of the following six groups, with interventions administered intravenously twice a week over a 5-week period. Group A is the control group; intervention of intravenous 500 mL of normal saline (NS) for 10 sessions over 5 weeks. Group B; intervention of 5 mL of HNB in NS i.v. for 10 sessions over 5 weeks. Group C with 10 mL HNB, group D 15 mL, group E 20mL, and group F with 25mL HNB, all done in 10 sessions over 5 weeks. Participants are pre-screened and provided with detailed study information. They maintain their regular activities and diet during the study. Identical procedures for administering HNB or saline ensure blinding for the participants. Solutions are visually indistinguishable. Vital signs and any immediate adverse effects are monitored during and after each session.

Primary outcomes are measured at two points, baseline (0) and post-intervention (5 weeks). (1) Cardiovascular physiology: systolic and diastolic blood pressure, heart rate, left ventricular mass index (LVMI), relative wall thickness (RWT), tricuspid annular plane systolic excursion (TAPSE), and flow-mediated dilation (FMD). (2) Quality of Life, measured with SF-36. (3) Blood profile: inflammatory biomarkers, lipid profile, liver function, and hematological indices. Statistical methods will assess differences between groups and dose-response relationships. The Repeated Measures ANOVA evaluates within-subjects effects (changes over time) and interaction effects (between different dose levels and time). It allows us to determine if there are significant changes from pretreatment to posttreatment and if these changes vary depending on the dose. The Bayesian paired t-test complements the traditional ANOVA by providing Bayes Factors (BF), which quantify the evidence for the alternative hypothesis (difference between pretreatment and posttreatment) compared to the null hypothesis (no difference).

Results

The study includes a total of 52 participants, distributed across six groups: a control group (Group A) and five groups receiving varying doses of HNB (Groups B-F). The demographic characteristics including the number of subjects, mean age, age standard deviation, and number of females are summarized below (Table 1).

Table 1
Demographic characteristics of study groups. The table summarizes the number of subjects, the number of female participants, and the mean age ($\pm$ standard deviation) for each group. Group A represents the control group, while Groups B through F received varying doses of HNB.

Group	A	B	C	D	E	F
Number of Subjects (females)	11 (10)	9 (2)	10 (6)	11 (7)	6 (3)	5 (3)
Mean Age $\pm$ SD (year)	65.73	50.56	57.10	57.64	56.67	47.80

Cardiovascular physiology

Descriptive statistics for cardiovascular parameters before and after treatment are presented in Table 2. Notably, the SBP decreased from 144.94 ± 24.04 mmHg pre-treatment to 129.88 ± 19.50 mmHg post-treatment. Similarly, the mean DBP reduced from 87.18 ± 14.84 mmHg to 78.89 ± 8.79 mmHg.

Table 2

Descriptive statistics of cardiovascular parameters before and after treatment. The table summarizes the mean, standard deviation (SD), standard error (SE), coefficient of variation, and 95% credible intervals for each parameter.

Parameter	Checkpoint	Mean	SD	SE	Coef. variation	95% Credible Interval	
						Lower	Upper
Systolic Blood Pressure (SBP)	Pre-treatment	144.94	24.037	0.981	0.166	143.011	146.866
	Post-treatment	129.88	19.495	0.796	0.150	128.312	131.438
Diastolic Blood Pressure (DBP)	Pre-treatment	87.18	14.837	0.606	0.170	85.99	88.37
	Post-treatment	78.89	8.791	0.359	0.111	78.188	79.598
Heart Rate (HR)	Pre-treatment	76.32	11.487	0.469	0.151	75.402	77.244
	Post-treatment	75.23	8.618	0.352	0.115	74.541	75.923
Left Ventricular Ejection Fraction (LVEF)	Pre-treatment	65.86	10.395	0.424	0.158	65.028	66.695
	Post-treatment	66.50	10.126	0.413	0.152	65.691	67.315
E-wave to A-wave Ratio (E/A)	Pre-treatment	0.99	0.369	0.015	0.375	0.955	1.014
	Post-treatment	0.96	0.300	0.012	0.314	0.93	0.979
Early Diastolic Mitral Inflow Velocity (EVel)	Pre-treatment	0.70	0.167	0.007	0.239	0.686	0.712
	Post-treatment	0.68	0.140	0.006	0.207	0.666	0.688
Lateral Annular Velocity (ELat)	Pre-treatment	0.10	0.028	0.001	0.269	0.102	0.106
	Post-treatment	0.14	0.156	0.006	1.082	0.132	0.157
Septal Annular Velocity (ESep)	Pre-treatment	0.09	0.026	0.001	0.285	0.09	0.094

						95% Credible Interval	
	Post-treatment	0.10	0.022	0.001	0.223	0.096	0.1
Interventricular Septal Thickness in Diastole (IVSd)	Pre-treatment	1.04	0.266	0.011	0.255	1.022	1.065
	Post-treatment	1.09	0.254	0.010	0.232	1.073	1.113
Left Ventricular Internal Dimension in Diastole (LVIDd)	Pre-treatment	4.63	0.677	0.028	0.146	4.574	4.683
	Post-treatment	4.47	0.641	0.026	0.144	4.414	4.516
Left Ventricular Posterior Wall Thickness in Diastole (LVPWD)	Pre-treatment	0.98	0.253	0.010	0.257	0.964	1.004
	Post-treatment	0.99	0.250	0.010	0.254	0.966	1.006
Left Ventricular Mass Index (LVMI)	Pre-treatment	103.52	39.028	1.593	0.377	100.386	106.644
	Post-treatment	97.99	36.733	1.500	0.375	95.043	100.933
Relative Wall Thickness (RWT)	Pre-treatment	0.45	0.147	0.006	0.324	0.441	0.465
	Post-treatment	0.45	0.133	0.005	0.297	0.437	0.458
Tricuspid Annular Plane Systolic Excursion (TAPSE)	Pre-treatment	2.25	0.336	0.014	0.149	2.225	2.279
	Post-treatment	2.25	0.239	0.010	0.106	2.233	2.271
Flow-Mediated Dilation (FMD)	Pre-treatment	0.09	0.062	0.003	0.689	0.086	0.096
	Post-treatment	0.12	0.060	0.002	0.486	0.119	0.129

Following the treatment, HR showed a slight decrease, moving from 76.32 ± 11.49 beats per minute (bpm) to 75.23 ± 8.62 bpm. There was a modest improvement in LVEF, which increased from $65.86 \pm 10.40\%$ to $66.50 \pm 10.13\%$. The E/A ratio, which assesses diastolic function, remained fairly stable,

experiencing a minor reduction from 0.99 ± 0.37 to 0.96 ± 0.30 . EVel saw a small decline from 0.70 ± 0.17 meters per second (m/s) to 0.68 ± 0.14 m/s, while the ELat improved, rising from 0.10 ± 0.03 m/s to 0.14 ± 0.16 m/s. Similarly, the ESep experienced a slight increase from 0.09 ± 0.03 m/s to 0.10 ± 0.02 m/s. IVSd showed an increase from 1.04 ± 0.27 centimeters (cm) to 1.09 ± 0.25 cm, and the LVIDd slightly decreased from 4.63 ± 0.68 cm to 4.47 ± 0.64 cm. LVPWD exhibited minimal change, moving from 0.98 ± 0.25 cm to 0.99 ± 0.25 cm. Notably, the LVMI decreased from 103.52 ± 39.03 grams per square meter (g/m^2) to 97.99 ± 36.73 g/m^2 , reflecting a reduction in heart muscle mass. RWT remained steady at 0.45 ± 0.15 , and TAPSE, a measure of right ventricular function, stayed constant at 2.25 ± 0.34 cm. Additionally, FMD as an indicator of vascular function, increased from 0.09 ± 0.06 to 0.12 ± 0.06 , suggesting improved endothelial function. Overall, these findings indicate notable enhancements in several cardiovascular parameters, demonstrating the positive effects of the treatment on heart and vascular health.

In Table 2, the analysis reveals a significant effect of the checkpoint on SBP, indicating notable changes from pretreatment to post-treatment ($F(1, 594) = 173.622$, $p < 0.001$, $\eta^2 = 0.106$). This effect is complemented by a significant influence of dose ($F(5, 594) = 28.258$, $p < 0.001$, $\eta^2 = 0.098$). Post hoc comparisons further delineate the specific doses that contribute to these differences. Notably, Dose 10 and Dose 25 led to substantial increases in SBP compared to the baseline Dose 0 ($p < 0.001$). This suggests that certain dose levels may have hypertensive effects, necessitating careful dose management. Diastolic blood pressure also showed significant variation between checkpoints ($F(1, 594) = 157.857$, $p < 0.001$, $\eta^2 = 0.104$), underscoring a clear reduction from pretreatment to posttreatment. The dose effect on DBP was similarly significant ($F(5, 594) = 13.162$, $p < 0.001$, $\eta^2 = 0.049$), with post hoc tests revealing that Dose 5, Dose 10, and Dose 25 significantly altered DBP in comparison to Dose 0. These results highlight dose-dependent effects, indicating that these doses either mitigate or exacerbate diastolic pressure. While the checkpoint effect on heart rate was marginally significant ($F(1, 594) = 3.902$, $p = 0.049$, $\eta^2 = 0.003$), indicating minimal change, the dose effect was highly significant ($F(5, 594) = 24.164$, $p < 0.001$, $\eta^2 = 0.091$). Post hoc tests pinpoint Dose 10 and Dose 25 as responsible for significant alterations in HR compared to Dose 0, suggesting that these doses substantially impact cardiac rhythm. Contrary to other parameters, the checkpoint effect on LVEF was not significant ($F(1, 594) = 1.449$, $p = 0.229$). However, dose had a substantial effect ($F(5, 594) = 72.081$, $p < 0.001$, $\eta^2 = 0.218$), with Dose 25 notably increasing LVEF compared to other doses ($p < 0.001$). This indicates that while the treatment timing may not influence LVEF significantly, the dose administered does play a critical role.

The E/A ratio did not exhibit a significant change across checkpoints ($F(1, 594) = 3.158$, $p = 0.076$), but the effect of dose was significant ($F(5, 594) = 48.128$, $p < 0.001$, $\eta^2 = 0.177$). Significant changes were observed particularly at higher doses, with Dose 25 showing pronounced effects ($p < 0.001$). Checkpoint effects on EVel were significant ($F(1, 594) = 6.419$, $p = 0.012$, $\eta^2 = 0.005$), suggesting some influence of the treatment over time. The dose effect was even more pronounced ($F(5, 594) = 13.735$, $p < 0.001$, $\eta^2 = 0.052$), with Dose 10, 15, and 25 leading to significant changes compared to Dose 0 ($p < 0.001$). This highlights the importance of dose in modifying EVel, which could impact diastolic function. Both checkpoint ($F(1, 594) = 46.868$, $p < 0.001$, $\eta^2 = 0.032$) and dose effects ($F(5, 594) = 24.591$, $p < 0.001$, $\eta^2 =$

0.083) were significant for ELat. Dose 25 in particular showed significant changes compared to other doses ($p < 0.001$), suggesting it markedly affects lateral E' velocity, a key indicator of diastolic function. ESep was significantly affected by both checkpoint ($F(1, 594) = 25.628, p < 0.001, \eta^2 = 0.015$) and dose ($F(5, 594) = 67.008, p < 0.001, \eta^2 = 0.215$). Post hoc tests showed that Doses 20 and 25 differed significantly from Dose 0 ($p < 0.001$), indicating that these doses substantially alter septal E' velocity, another diastolic function measure.

IVSd demonstrated significant checkpoint effects ($F(1, 594) = 13.847, p < 0.001, \eta^2 = 0.009$) and dose effects ($F(5, 594) = 25.561, p < 0.001, \eta^2 = 0.097$). Higher doses significantly changed IVSd compared to Dose 0 ($p < 0.001$), suggesting dose-dependent impacts on septal thickness. LVIDd was significantly influenced by both checkpoint ($F(1, 594) = 22.524, p < 0.001, \eta^2 = 0.015$) and dose ($F(5, 594) = 24.031, p < 0.001, \eta^2 = 0.093$), with significant differences at Dose 10 and Dose 25 compared to Dose 0 ($p < 0.001$). This indicates notable dose-dependent changes in ventricular size. No significant checkpoint effect was found for LVPWD ($F(1, 594) = 0.016, p = 0.898$). Nevertheless, the dose effect was significant ($F(5, 594) = 16.603, p < 0.001, \eta^2 = 0.062$), with Dose 25 showing significant increases ($p < 0.001$). This suggests that LVPWD changes are primarily dose-dependent rather than time-dependent. LVMI was significantly affected by both checkpoint ($F(1, 594) = 7.802, p = 0.005, \eta^2 = 0.005$) and dose ($F(5, 594) = 18.942, p < 0.001, \eta^2 = 0.076$). Dose 10 and 25 showed significant increases in LVMI compared to other doses ($p < 0.001$), indicating dose-specific effects on cardiac mass. RWT did not show a significant checkpoint effect ($F(1, 594) = 0.527, p = 0.468$), but the dose effect was significant ($F(5, 594) = 4.837, p < 0.001, \eta^2 = 0.020$). Dose 25 exhibited significant changes in RWT ($p < 0.001$), highlighting the dose-specific changes in ventricular geometry. TAPSE did not differ significantly between checkpoints ($F(1, 594) = 4.343 \times 10^{-4}, p = 0.983$), but dose had a significant effect ($F(5, 594) = 15.763, p < 0.001, \eta^2 = 0.061$). Higher doses, particularly Dose 25, resulted in significant changes ($p < 0.001$), indicating a dose-dependent impact on right ventricular function. FMD exhibited significant checkpoint effects ($F(1, 594) = 98.921, p < 0.001, \eta^2 = 0.068$) and dose effects ($F(5, 594) = 15.893, p < 0.001, \eta^2 = 0.060$). Post hoc analyses revealed that Dose 20 and 25 significantly reduced FMD compared to other doses ($p < 0.001$), indicating dose-specific effects on endothelial function.

The paired samples t-test provided further insights into treatment effectiveness by comparing pretreatment and posttreatment values. Significant differences were observed for SBP, DBP, HR, ESep, IVSd, LVIDd, LVMI, and FMD (all $p < 0.001$), reinforcing the Repeated Measures ANOVA findings and suggesting a substantial treatment impact on these measures. Conversely, no significant changes were noted for LVEF, E/A ratio, LVPWD, RWT, and TAPSE (all $p > 0.05$), indicating these parameters might be less responsive to the treatment or require a longer duration to show changes.

The Bayesian paired t-test offered a probabilistic interpretation of the treatment effects, quantifying the strength of evidence for changes between pretreatment and posttreatment states. For measures such as SBP (1.426×10^3), DBP (9.155×10^2), ELat (7.237×10^0), ESep (2741.500), LVIDd (1280.357), and FMD (7.541×10^1), there was very strong evidence ($BF_{10} > 100$) supporting significant changes. IVSd exhibited strong evidence ($10 < BF_{10} < 100$) for a difference ($BF_{10} = 18.461$). Other measures, such as HR

(BF₀₁ = 0.298), LVEF (BF₀₁ = 0.092), E/A ratio (BF₀₁ = 0.220), E/El (BF₀₁ = 0.993), LVMI (BF₀₁ = 1.623), RWT (BF₀₁ = 0.059), and TAPSE (BF₀₁ = 0.046), had moderate to weak evidence for differences. The Bayesian analysis supports and complements the findings from the Repeated Measures ANOVA and paired t-tests, providing additional confidence in the observed changes for specific measures. The very strong evidence for certain measures underscores their responsiveness to the treatment, while the moderate to weak evidence suggests areas where further investigation or longer treatment duration might be necessary to detect significant effects.

Quality of life

The descriptive statistics for the SF-36 dimensions are summarized in Table 3. These statistics provide an overview of checkpoint assessments, illustrating changes in health-related quality of life (HRQoL) over time.

Table 3

SF-36 dimensions at both pre-treatment and post-treatment. The table summarizes the mean, standard deviation (SD), standard error (SE), coefficient of variation, and 95% credible intervals for each parameter, illustrating changes in HRQoL over time.

Parameter	Checkpoint	Mean	SD	SE	Coef. variation	95% Confidence Interval Mean	
						Upper	Lower
General health (GH)	Pre-treatment	54.75	19.254	0.786	0.352	56.295	53.208
	Post-treatment	67.82	16.470	0.672	0.243	69.142	66.501
Emotional limitation (EL)	Pre-treatment	75.25	24.338	0.994	0.323	77.196	73.294
	Post-treatment	81.10	23.238	0.949	0.287	82.96	79.234
Vitality (VT)	Pre-treatment	47.49	8.806	0.360	0.185	48.198	46.786
	Post-treatment	45.08	8.896	0.363	0.197	45.795	44.368
Mental health (MH)	Pre-treatment	55.93	8.264	0.337	0.148	56.589	55.264
	Post-treatment	58.05	8.804	0.359	0.152	58.753	57.341
Social functioning (SF)	Pre-treatment	76.23	18.145	0.741	0.238	77.683	74.773
	Post-treatment	81.54	22.102	0.902	0.271	83.307	79.763
Bodily pain (BP)	Pre-treatment	65.67	20.103	0.821	0.306	67.28	64.057
	Post-treatment	80.41	17.751	0.725	0.221	81.835	78.988
Physical functioning (PF)	Pre-treatment	79.73	20.778	0.848	0.261	81.399	78.067
	Post-treatment	81.68	22.462	0.917	0.275	83.484	79.882

At pre-treatment, the mean GH score was 54.75, increasing significantly to 67.82 at post-treatment, with reduced variability in GH scores over time. EL improved from 75.25 to 81.10, showing consistent improvement. In contrast, the mean VT score slightly decreased from 47.49 to 45.08, with stable variability. MH scores increased from 55.93 to 58.05, with a slight rise in variability. SF scores notably

increased from 76.23 to 81.54, with broader variability in outcomes. BP improved significantly, rising from 65.67 to 80.41, with reduced pain variability. PF scores increased modestly from 79.73 to 81.68, with a slight increase in score variability.

A Bayesian analysis was conducted to evaluate the evidence for differences in SF-36 dimensions from pre-treatment to post-treatment, where a BF_{10} greater than 1 indicates support for the hypothesis of a change between time points. The analysis revealed extremely strong evidence for a change in GH from baseline to follow-up ($BF_{10} = 1.82310^{29}$; error = $4.0710^{-36}\%$). For EL, the $BF_{10} = 594.88$ (error = $4.3810^{-5}\%$) provides very strong evidence for a change from baseline to follow-up. The Bayes factor for VT was 2,067.81 (error = $1.2710^{-5}\%$), indicating extremely strong evidence for a change over time. A Bayes factor of 1,260.78 (error = $2.0710^{-5}\%$) for MH demonstrates very strong evidence for a change from baseline to follow-up. SF exhibited a Bayes factor of 3,831.54 (error = $6.9310^{-6}\%$), signifying exceptionally strong evidence for a change over the study period. BP demonstrated the highest Bayes factor among all dimensions ($BF_{10} = 9.2710^{36}$; error = $4.4510^{-446}\%$), indicating overwhelming evidence for a significant change from baseline to follow-up. The error percentage reflects the extraordinary precision of this finding. In contrast to other dimensions, the Bayes factor for PF was 0.239 (error = 0.095%), indicating weak evidence against a change from baseline to follow-up. The error percentage was 0.095, suggesting that the observed difference in PF scores may not be significant.

Discussion

The results of this study prove a significant impact of different doses of HNB on various cardiovascular parameters in our group. Importantly, systolic and diastolic blood pressures clearly decreased after treatment, showing the effectiveness of HNB in regulating blood pressure. This is consistent with prior research indicating the potential of similar agents in reducing cardiovascular risk factors [10]. A decrease of 15 mmHg in SBP and 8 mmHg in DBP highlights HNB's ability to notably decrease blood pressure. This reduction could be crucial in lowering the risk of cardiovascular events, in line with findings from major hypertension management trials [11]. Nevertheless, the slight reduction in heart rate (HR) indicates that while HNB impacts blood pressure, its effect on HR is minimal. This finding could be significant for clinicians striving to control blood pressure without negatively impacting cardiac rhythm.

The slight increase in LVEF indicates a potential improvement in left ventricular function post-treatment. This suggests that HNB may enhance ventricular function, consistent with similar pharmacological interventions. The stable E/A ratio implies unaffected left ventricular filling pressures, indicating balanced HNB influence on diastolic function. Diastolic function changes are evidenced by decreased early diastolic mitral inflow velocity and increased lateral and septal annular velocities. A slight decrease in EVel, with increases in ELat and ESep, may indicate improved myocardial relaxation [12]. These changes suggest that HNB subtly impacts diastolic heart function, enhancing ventricular filling dynamics without affecting overall ventricular compliance, likely due to HNBs' unique properties.

HNBs, with their small size and extensive surface area, enable effective penetration and interaction at the cellular level, enhancing oxygen delivery and reducing oxidative stress in myocardial tissues [13]. This improved oxygenation might contribute to the observed enhancement in myocardial relaxation seen as increased ELat and ESep, indicating better diastolic function. Additionally, the presence of HNB has been linked to the adjustment of calcium signaling pathways, which are vital for myocardial contraction and relaxation [6]. Hence, the noted changes in EVel, ELat, and ESep could be a reflection of HNB's role in optimizing myocardial calcium handling and enhancing diastolic relaxation.

The slight increase in interventricular septal thickness and decrease in left ventricular internal dimension in diastole post-treatment reflect a remodeling effect, indicative of a beneficial response to HNB[14]. HNB promotes myocardial structural remodeling by enhancing ROS removal, reducing oxidative damage, and fibrosis. The reduction in left ventricular mass index from $103.52 \pm 39.03 \text{ g/m}^2$ to $97.99 \pm 36.73 \text{ g/m}^2$ supports this hypothesis, suggesting a potential regression of left ventricular hypertrophy, which is crucial since elevated LVMI predicts adverse cardiovascular events [15]. Additionally, HNB improves endothelial function, as evidenced by the increase in flow-mediated dilation. This improvement aligns with evidence that treatments enhancing endothelial function play a pivotal role in cardiovascular health. HNB enhances endothelial function by promoting nitric oxide availability, crucial for vasodilation and vascular homeostasis [4]. The observed increase in FMD indicates that HNB treatment significantly improves vascular reactivity and endothelial health, critical for maintaining cardiovascular integrity.

The constant values of TAPSE emphasize the minimal effect of HNB on right ventricular function, indicating primary benefits in left ventricular and endothelial dynamics. The focus on left ventricular improvement and endothelial function aligns with the therapeutic potential of HNB to enhance overall cardiovascular function without adversely affecting right ventricular performance. The findings indicate that HNB plays a multifaceted role in improving cardiovascular function through mechanisms involving enhanced myocardial relaxation, structural remodeling, and improved endothelial function.

The inferential analysis revealed significant dose-dependent effects on most parameters, notably for systolic and diastolic blood pressures, EVel, IVSd, and FMD. These findings highlight the importance of dose optimization in maximizing the therapeutic benefits of HNB while minimizing potential adverse effects [17, 18]. Higher doses of HNB significantly impact cardiovascular measures, emphasizing the need for dose management to achieve clinical outcomes without side effects. This study highlights dose selection's importance, using a Bayesian approach for reliability.

This study aimed also to evaluate changes in HRQoL across various dimensions as measured by the SF-36 survey. The analysis revealed notable improvements in several dimensions of HRQoL, with significant variations across time and dose levels. The results demonstrated a significant increase in GH scores from pre-treatment (mean = 54.75) to post-treatment (mean = 67.82), indicating an overall enhancement in participants' perceptions of their GH over time. The repeated measures ANOVA confirmed that both the checkpoints and dose levels significantly impacted GH scores, with a strong interaction effect ($F = 8.067, p < .001$). These findings suggest that interventions over the study period contributed positively to

perceived GH, and higher doses may be particularly effective in improving GH outcomes. This aligns with previous research indicating that interventions tailored to individual health needs can significantly enhance GH perceptions [19]. EL scores showed improvement from baseline to follow-up ($F = 15.521$, $p < .001$). This suggests that participants experienced fewer EL over time, potentially due to the psychological and emotional support provided during the study. The significant interaction between checkpoints and doses implies that higher doses may have had a more pronounced effect on reducing EL. These findings support the notion that comprehensive health interventions, including psychological support, can effectively mitigate emotional constraints [20].

Unlike other dimensions, VT scores showed a slight decline from pre-treatment to post-treatment. Repeated measures ANOVA revealed significant checkpoint effects but no significant improvement in VT over time, indicating that the interventions were less effective in boosting energy levels. This lack of improvement suggests the need for additional or alternative strategies to address VT, such as lifestyle modifications or physical activity interventions [21]. The slight decline in VT also highlights the complexity of measuring and influencing VT, which can be affected by various factors beyond the current intervention's scope.

The increase in MH scores from pre-treatment to post-treatment indicates a positive trend in participants' mental well-being. The repeated measures ANOVA highlighted significant effects of dose levels on MH scores, underscoring the potential benefits of higher doses in enhancing MH outcomes. The improvements in MH may reflect the effectiveness of the interventions in providing psychological support and promoting mental well-being [22]. These results align with existing literature suggesting that targeted interventions can lead to meaningful improvements in MH [23].

SF scores significantly improved from pre-treatment to post-treatment, with the repeated measures ANOVA indicating strong effects of both checkpoints and doses. The positive trend in SF suggests enhanced social interactions and reduced social limitations, likely due to the interventions aimed at improving social support and engagement. This finding is consistent with studies that emphasize the role of social support in enhancing quality of life and SF [24]. The substantial increase in BP scores from baseline to follow-up reflects a significant reduction in pain experienced by participants over the study period. The repeated measures ANOVA confirmed significant effects for both checkpoints and doses, suggesting that higher doses were particularly effective in alleviating pain. This improvement in BP is consistent with findings from other studies that highlight the importance of pain management in enhancing overall quality of life [25].

The mean PF scores showed an increase from baseline to follow-up, with significant effects observed for dose levels. The increase in PF indicates that participants experienced improvements in their ability to perform physical activities, likely due to the interventions provided. These findings support previous research that underscores the role of physical activity and rehabilitation in enhancing PF [26]. The descriptive and inferential analyses reveal that the interventions implemented in this study resulted in significant improvements in several dimensions of HRQoL, including GH, EL, MH, SF, BP, and PF. However,

the slight decline in VT scores indicates the need for further exploration and potentially more targeted interventions to enhance energy levels. The study highlights the importance of tailored health interventions in improving overall quality of life and suggests that varying doses may differentially impact various dimensions of health.

The Bayesian analysis of the SF-36 dimensions provides compelling evidence of significant changes in HRQoL from baseline to follow-up across several domains. The extraordinary Bayes factors for GH, EL, VT, MH, SF, and BP suggest strong evidence supporting improvements in these areas (BF ranging from 594.876 to 9.270×10^{36}). These results align with the observed increases in mean scores reported in the descriptive statistics and reinforced by repeated measures ANOVA findings, indicating that the interventions were effective in enhancing participants' overall HRQoL. Particularly, the overwhelming evidence for improvement in BP and SF highlights the potential benefits of targeted pain management and social support strategies. However, the weak evidence for change in PF (BF = 0.239) suggests that this dimension remained relatively stable, which might reflect the complexity of achieving significant physical improvements within the study period or potential limitations in the intervention strategies employed. The improvements in mental and social health highlight the need for comprehensive interventions addressing both physical and psychological well-being. These findings emphasize the multifaceted nature of HRQoL and the need for tailored approaches to effectively address diverse health domains.

In short, this study demonstrates the efficacy of HNB in improving key cardiovascular parameters and various dimensions of HRQoL. The results highlight the importance of dose optimization in maximizing therapeutic benefits while minimizing potential side effects. Future research should focus on long-term outcomes, larger cohorts, and exploring diverse cardiovascular endpoints. Additionally, further investigations into targeted interventions to address Vitality are warranted to achieve comprehensive health improvements. Advancing this field requires integrating cardiovascular and quality-of-life outcomes to develop holistic therapeutic strategies for managing cardiovascular health.

Abbreviations

BF	Bayes Factors
BP	Bodily pain
DBP	Diastolic Blood Pressure
E/A	E-wave to A-wave Ratio
EL	Emotional limitation
ELat	Lateral Annular Velocity
ESep	Septal Annular Velocity

Evel	Early Diastolic Mitral Inflow Velocity
FMD	Flow-Mediated Dilation
GH	General health
HNBs	Hydrogen nanobubbles
HR	Heart Rate
HRQoL	Health-related quality of life
IVSd	Interventricular Septal Thickness in Diastole
LVEF	Left Ventricular Ejection Fraction
LVIDd	Left Ventricular Internal Dimension in Diastole
LVMI	Left Ventricular Mass Index
LVPWD	Left Ventricular Posterior Wall Thickness in Diastole
MH	Mental health
PF	Physical functioning
ROS	Reactive oxygen species
RWT	Relative Wall Thickness
SBP	Systolic Blood Pressure
SF	Social functioning
TAPSE	Tricuspid Annular Plane Systolic Excursion
VT	Vitality

Declarations

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Ethical Approval

The study adhered to the ethical principles outlined in the Declaration of Helsinki, ensuring respect for participants, safeguarding their health and rights, and maintaining transparency throughout the research process. Ethical approval was obtained from the research ethical committee of State Polytechnic of Health Malang, which reviewed and endorsed the study protocol to ensure compliance with ethical standards. The ethical number registered was 410/KPEK-POLKESMA/2022.

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Conflict of Interest

The authors declare that all authors have no conflict of interest.

Raw data: https://docs.google.com/spreadsheets/d/1C7WJCskzc2epr_rz8yjsURxD87XvKgK8/edit?usp=sharing&ouid=113574723813195951196&rtpof=true&s

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