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Research article

Cold water stimulation and its effect on heart rate variability: A short time fourier transform analysis

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Abstract

This study utilizes cold water consumption as a method of vagus nerve stimulation, investigating how body mass index (BMI) and repeated stimulation affect heart rate variability (HRV) in both time and frequency domains. HRV was measured in 18 subjects (mean age 21.3 ± 1.6 years) over 90 minutes, divided into three equal phases: resting (baseline), first stimulation (FSP), and second stimulation (SSP). Fourier and Short-Time Fourier Transform (STFT) analyses were used to extract the static and time-varying frequency components of HRV. For the normal body mass index (BMI) group, stimulation resulted in statistically significant changes in frequency band power densities compared to their baseline measurements ($p<0.05$), suggesting that drinking cold water may activate vagal pathways and alter HRV components. For underweight subjects, the very low frequency (VLF) band power density of HRV decreased with stimulation (3.0%) and further depressed with repeated stimulation (6.5%). Low frequency (LF) power density stayed relatively constant with the first stimulation but still increased by 4.1% by shifting of VLF power into this band after the second stimulation, and finally, high frequency (HF) band power density increased after each stimulation up to 2.9%, suggesting that cold water intake shifted the HRV frequency spectrum to higher frequencies in all bands. For normal-weight subjects, after the first stimulation, the LF band power was shifted into both VLF and HF bands. With repetition, the shift was only towards the high frequencies, suggesting that repetition of cold-water intake affects the HRV frequency distribution, and only repetition shifts power to higher frequencies. For obese subjects, the power density shift is towards the low frequencies (5.0% in VLF), and repeated stimulation could only partially recover this shift (4.3%). In all BMI groups, the LF/HF ratio was decreased and was more prominent after the first stimulation. The maximum LF/HF reduction was observed in obese individuals (7.1%), followed by the subsequent highest decline in the normal BMI group (5.2%). This study also highlighted STFT as an effective tool for analyzing temporal changes in HRV, displaying distinct spectral power distribution patterns for each BMI group. Underweight and normal-weight subjects demonstrated an initial increase in HF power density, whereas overweight subjects exhibited a muted response.

Keywords: *BMI; heart rate variability; short time fourier transformation; spectral analysis of heart rate variability; vagus nerve stimulation; wavelet spectrogram*

1. Introduction

Heart rate (HR) is the heartbeat frequency, and it is calculated using the interval between consecutive R waves (RR intervals); heart rate is increased (shorter beat intervals) by the sympathetic system, whereas a lower heart rate (longer beat intervals) is associated with the parasympathetic nervous system. Heart Rate Variability (HRV) is the variation of RR intervals, calculated as the difference in consecutive RR intervals. HRV is expressed in milliseconds (Aro et al., 2025; Belica et al., 2025). HRV is critical in understanding the balance between the parasympathetic and sympathetic nervous systems and is used in evaluating the proper functioning of the autonomic nervous system. The interaction between the sympathetic and

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parasympathetic autonomic nervous systems is associated with HRV (Zhong et al., 2006; Qin et al., 2025).

Factors influencing HR and HRV include respiration rate, cardiac autonomic tone, and physiological responses to external stimuli. Body mass index (BMI) also affects the autonomic nervous system (Molfino et al., 2009; Struven et al., 2021). The effect of BMI on HRV is also observed in preschool children (Speer et al., 2021).

Amekran et al. (2024) described the standard HRV frequency bands to be used in studying the extent of vagus nerve activation: very low frequency (VLF), low frequency (LF), and high frequency (HF). These frequency bands are tabulated in Table 1 (Kwon et al., 2018). Irurzun et al. (2025) used the spectral analysis of HRV to study how the autonomic nervous system regulates heart rate, whereas Grinevich and Chemeris (2023) studied HRV frequency components obtained by Fourier Transformation, particularly focusing on the HF and LF bands Muhajir et al. (2022) showed that the parasympathetic nervous system controls HF, while LF and VLF are controlled by sympathetic nervous system activities. Additionally, VLF was shown to be related to the sympathovagal system and also influences thermoregulation (Claydon et al., 2008; Shaffer and Ginsberg, 2017; Usui and Nishida, 2017; Ocalan et al., 2023).

Table 1
Frequency bands used in HRV studies.

	Hz
VLF	[0.0033 - 0.04]
LF	[0.04 - 0.15]
HF	[0.15 - 0.4]

While studying HRV, due to the delayed and suspended response of the autonomic nervous system, measurements should last at least 5 minutes (Nunan et al., 2010). Similarly, vagus nerve stimulation exhibits changes in time, and using at least 5-minute measurements is needed to detect its early and late effects on the nervous system (Hartikainen et al., 1998).

The vagus nerve, the principal component of the parasympathetic nervous system, is the longest cranial nerve in the human body and plays a central role in regulating various physiological functions (Howland, 2014). Vagus nerve stimulation (VNS) is considered an important method in modern medicine, for treating various health issues. Research shows positive effects of VNS on conditions like epilepsy, treatment-resistant depression, anxiety disorders, and digestive disorders. In particular, VNS was highlighted for its effectiveness in reducing the frequency and severity of seizures in epilepsy treatment (Fisher et al., 1997). In depression treatment, VNS was shown to alleviate patients' symptoms and improve quality of life (George et al., 2005). VNS was also reported to have positive outcomes on anxiety disorders (Bremner et al., 2020). Since the vagus nerve also plays a critical role in the digestive system, VNS was investigated as a potential treatment method for managing digestive disorders (Murray et al., 2021). These findings indicated that vagus nerve stimulation provides broad health benefits. Vagal tone, a key indicator of parasympathetic activity, reflects the functional state of the vagus nerve, and it is closely associated with the modulation of HRV (Ocalan et al., 2023).

The vagus nerve can be stimulated in various ways, including electrical stimulation, cold application, breathing exercises, vocal vibrations, and physical exercises (Stanley et al., 2013a; Stanley et al., 2013b; Howland, 2014; Dong, 2016; Jungmann et al., 2018; Levin and Swoap, 2019; Laborde et al., 2022).

Another method used for stimulating the vagus nerve is the use of cold water. Previous researchers have used different methods to expose subjects to cold water to investigate its effects on HRV and have illustrated different methods. Cold water ingestion or immersion can modulate autonomic function and HRV. Shekarforoush and Fardaee (2020) observed that drinking 500 ml of water transiently increases sympathetic activity but also enhances cardiac vagal tone, which may result in a slight decrease or stabilization of heart rate despite the sympathetic stimulation. Pereira et al. used ingestion of 250 ml cold water at 3ºC and observed the shift in the autonomic balance towards parasympathetic dominance: demonstrated by increase in HF power and reduction in LF to HF ratio, alongside a modest reduction in heart rate (mean difference: -3.04 ± 3.94 bpm; $p=.001$) (Pereira et al., 2017). Chiang et al. reported that ice water ingestion increased high-frequency (HF) power and normalized HF values while reducing the low-frequency to high-frequency (LF/HF) ratio, suggesting enhanced vagal activity in healthy individuals (Chiang et al., 2010). Finally, a systematic review by Galvez-Rodriguez et al. (2025) concluded that cold water immersion (CWI) after exercise consistently promoted parasympathetic reactivation and improved HRV, suggesting its potential as an effective recovery strategy. Stakenborg et al. explain the cold-water intake as an easy way of stimulating the vagus nerve through the gastrointestinal system, which extends from the neck to the abdominal region and is intensely innervated through the vagus nerve. Thus, the relaxation mechanisms associated with the parasympathetic nervous system can be activated via vagus nerve stimulation with cold water (Stakenborg et al., 2013).

Although many studies showed that exposure to cold water affects HRV (Nuttall, 2015; Pereira et al., 2017; Shekarforoush and Fardaee, 2020; Ocalan et al., 2023), with current knowledge, none of them investigated the temporal change of the effects, how they change with the repetition of exposure, and how BMI contributes to this change. This study aims to close this gap in the literature.

2. Materials and methods

2.1. Subjects

This research included 18 healthy young adults (9 male, 9 female) with no medical history related to heart or other health conditions that might affect the measurements. The age of the subjects was between 18 and 25, and their anthropometric data are tabulated in Table 2.

Table 2
Anthropometric data of subjects.

	Mean (n=18)	SD
Age	21.3	1.6
Weight (kg)	71.1	14.1
Height (cm)	172.3	9.7
BMI (kg/m²)	24.1	3.8

The World Health Organization groups subjects as underweight, normal, or overweight based on their body mass index (BMI) (Nuttall, 2015). Subjects with a BMI index below

18.5 kg/m^2 were labeled as under-weighted (UW), above 30.0 kg/m^2 as obese (OW), and in this study, all other BMI scores were labeled as normal weight (NW).

2.2. Measurement setup

The electrocardiogram (ECG) measurement protocol was adjusted to avoid external and internal effects, especially digestive activities and participants' physical movements. To minimize internal physiological influences, participants were asked to abstain from food and drink for at least two hours before the measurement.

Subjects were seated in a comfortable chair in a controlled room temperature room set to 22°C, in their most comfortable posture, resting their arms on supportive stands. Subjects were instructed to remain stationary and silent during the entire measurement (Fig. 1).

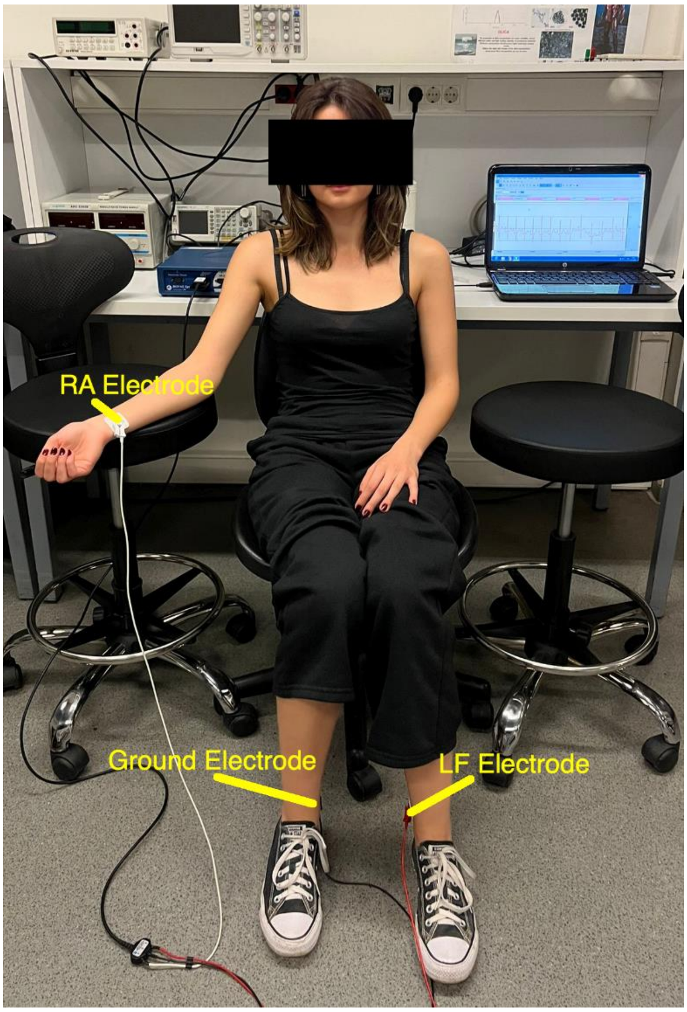

Fig. 1. Experimental setup.

2.3. Data acquisition

Biopac® MP36 Student Lab device was used for ECG data acquisition, and the signal sampling frequency was set as 1000Hz. Voltage sense electrodes were placed on the right wrist (RA) and left leg ankle (LL), the ground reference electrode was on the right leg ankle (RL); conforming to Einthoven's Lead II limb wire placement (Divyabharathi, 2019).

The data was collected for 90 minutes in three equal and distinct phases: the first period was named as the "Resting Phase (RP)", the second as the "First Stimulation Phase (FSP)" and the last period was the "Second Stimulation Phase (SSP)". The RP served as the reference HRV state with no external vagus stimulation to form the baseline measurements.

FSP started with stimulating the vagus nerve for the first time by drinking 500 ml of 7-8 °C cold water. Volume selected was in line with the previous studies (Shekarforoush and Fardaee, 2020), and temperature was selected arbitrarily. Subjects are asked to drink water in less than 60 seconds. SSP starts after drinking cold water for the second time, with the same duration, amount, and temperature. Subjects use a straw to drink cold water to ensure they do not move and to maintain their silent and stationary postures. ECG data are later exported to MATLAB file format to enable further analysis using MATLAB (MathWorks Inc., version: 23.2.0.2428915, 2023).

2.4. Data analysis

For data analysis, the intervals between the subsequent "R" waves of the ECG signal are first calculated as the RR interval, and later, the time difference of the RR intervals is calculated as the HRV of the subjects.

The spectral power distribution of the HRV time series is calculated using the Fourier Transform (FT). Using the Short-Time Fourier Transform (STFT) method, the temporal change in the HRV frequency spectrum was analyzed. The frequency range in both studies is selected as 0.04 to 0.5 Hz. Together with the classic power spectrum analysis in low- and high-frequency bands, the frequency range is divided into 100 equal intervals for finer analysis. Temporal frequency spectra are represented as spectrograms to visualize the change in power density over time.

FT is used to analyze the frequency components of HRV, ignoring changes in time, assuming the effect is stationary. STFT is used to analyze signal frequency components, preserving their temporal changes. A longer analysis window in STFT increases the frequency resolution, while a shorter window allows for a higher temporal resolution (Adamczyk and Polak, 2021). Considering this trade-off, to detect the early and late effects of vagus nerve stimulation, the STFT analysis window is set to a minimum suggested window length of 5 minutes, similar to traditional HRV studies (Hartikainen et al., 1998).

2.5. Statistical analysis

In this study, Student's paired t-test was used to infer the change in FSP and SSP concerning the RP baseline measurements. It is also used to analyze whether the change in HRV in SSP is statistically significant compared to FSP. Due to the limited number of underweight (2) and obese (3) subjects, this test was applied to normal BMI subjects only (13 subjects). The normality of the data was tested using the D'Agostino-Pearson test. The statistical significance level was set at p<0.05.

3. Results

Table 3 tabulates the baseline (RP) HRV frequency band distribution of different BMI groups. Table 4 shows how the frequency band powers change in FSP for the baseline (RP), and Table 5 shows how the change was from FSP to SSP.

For UW subjects, the changes in FSP compared to their baseline RP measurements were as follows: VLF band power density decreased by 3.0%, LF band increased by 0.1%, and HF increased by 2.9%, resulting in a net 1.5% decline in LF/HF ratio. For NW subjects, the change from RP to FSP in VLF, LF, and HF was a 1.9% increase, 2.6% decrease, and 0.6% increase, respectively, resulting in a net 5.2% reduction in LF/HF ratio. For OW subjects, the changes in VLF, LF, and HF were a 5.0% increase, a 4.3% decrease, and a 0.7% decrease, respectively, resulting in a net 7.1% reduction in the LF/HF ratio (Table 4). The changes in the LF band and LF/HF ratio for the NW subjects were statistically significant (p<0.05) (Table 6).

Table 3
Baseline (Resting phase) frequency band distributions of different BMI groups.

	VLF	LF	HF	LF/HF
	22.0%	20.3%	57.7%	35.2%
NW	21.7%	23.9%	54.3%	44.2%
OW	20.4%	24.4%	55.2%	43.5%
All	21.5%	23.6%	54.9%	43.0%

UW: underweight, NW: normal weight, OW: overweight subjects.

Table 4
Relative changes in frequency band power densities of different BMI groups, with respect to their baseline measurements.

	VLF	LF	HF	LF/HF
	Relative change after the first stimulation			
UW	-3.0%	0.1%	2.9%	-1.5%
NW	1.9%	-2.6%	0.6%	-5.2%
OW	5.0%	-4.3%	-0.7%	-7.1%
All	1.9%	-2.5%	0.7%	-5.1%
	Relative change after the second stimulation			
UW	-6.5%	4.1%	2.4%	5.4%
NW	0.2%	-2.0%	1.8%	-4.8%
OW	4.3%	-3.3%	-1.0%	-4.7%
All	0.1%	-1.5%	1.4%	-3.6%

UW: underweight, NW: normal weight, OW: overweight subjects.

Table 5
Relative changes in frequency band power densities of different BMI groups: from first stimulation to second stimulation.

	VLF	LF	HF	LF/HF
UW	-3.5%	3.9%	-0.5%	6.9%
NW	-1.8%	0.6%	1.2%	0.4%
OW	-0.6%	0.9%	-0.3%	2.5%
All	-1.8%	1.0%	0.7%	1.5%

UW: underweight, NW: normal weight, OW: overweight subjects.

Table 6
Statistical comparison of power densities for normal weighted subjects, p values.

P(T<=t) one-tail	VLF	LF	HF	LF/HF
FSP - RP	0.09	0.03	0.07	0.01
SSP - RP	0.46	0.15	0.02	0.09
SSP - FSP	0.08	0.33	0.01	0.43

RP: resting phase, FSP: first stimulation phase, SSP: second stimulation phase.

The change from RP to SSP was as follows: for UW subjects, VLF decreased by 6.5%, LF increased by 4.1%, and HF increased by 2.4%. Due to the greater increase in LF, the net LF/HF ratio increased by 5.4%. For NW subjects, VLF increased by 0.2%, LF decreased by 2.0%, and HF increased by 1.8%. Due to the decrease in LF, the LF/HF ratio decreased by 4.8%. For OW subjects, VLF increased by 4.3%, LF decreased by 3.3%, and HF decreased by 1.0%. Thus, the decrease in LF/HF ratio was 4.7%.

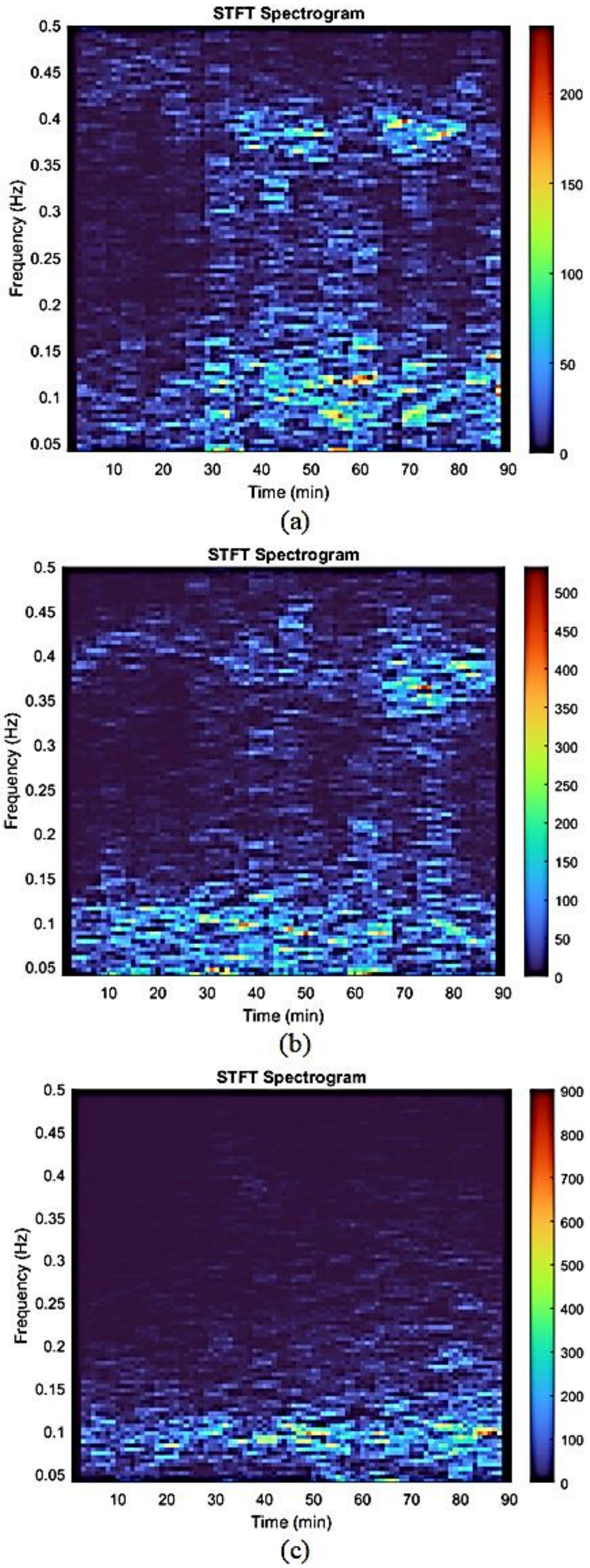

Fig. 2. Power spectrogram of HRV for (a) underweight, (b) normal weight, (c) overweight subject.

The change from FSP to SSP was as follows: for UW subjects, VLF decreased by 3.5%, LF increased by 3.9%, and HF increased by 0.5%. Due to the greater increase in LF, the net LF/HF ratio increased by 6.9%. For NW subjects, VLF decreased by 1.8%, LF increased by 2.0%, and HF increased by 1.8%. Due to the increase in LF, the LF/HF ratio increased by 0.4%. For OW subjects, VLF decreased by 0.6%, LF increased by 0.9%, and HF decreased by 0.3%. Thus, the LF/HF ratio increased by 2.5%.

Table 6 presents the Student's paired t-test results for the NW subject. The change in the LF band power density and LF/HF ratio from the baseline to the FSP is statistically significant (p=0.03 and p=0.01, respectively). For the SSP, the change in the HF band with respect to baseline and from the FSP to the SSP is statistically significant (p<0.05).

Spectrograms derived from the Short-Time Fourier Transform (STFT) highlight the typical spectral power distribution of each BMI group (Fig. 2). Specifically, UW or NW subjects demonstrated an initial increase in power across HF components, whereas, with OW subjects, a more muted response was observed in the SSP.

4. Discussion and conclusion

For UW subjects, VLF band power was shifted to higher frequency bands, and this change was more prominent after the second stimulation. There was an increase in the HF/LF ratio in SSP, but the change was due to a shift in power from VLF to LF (toward higher frequencies), suggesting an overall frequency shift to higher frequencies. Shifting to higher frequency components indicated that the parasympathetic nervous system was activated. The continued increase in HF in SSP suggested that this activation was prolonged. This finding suggests that being underweight leads to a naturally robust change in the high-frequency (HF) component of heart rate variability (HRV), supporting the literature on possible BMI-related variability in autonomic function during cold water stimulation (Triggiani et al., 2015).

For the NW subject, part of the LF power content was shifted to the VLF and HF bands; in FPS, it was mostly towards the VLF, and in SSP, it was mostly towards the HF. This adjusted power distribution after the SSP suggests that repeated stimulation enforced the parasympathetic activities. In the frequency spectrum analysis of HRV, the change in frequency power density compared to baseline measurements was statistically significant for normal-weight subjects (p<0.05). This finding supports the study conducted by Stanley et al. (2013b), who used cold water immersion as a form of stimulation. The results also support the studies undertaken by Pereira et al. (2017) and Shekarforoush and Fardaee (2020), who suggested that cold water can activate vagal pathways and change frequency components. The change in relative LF and HF power components indicates a shift towards sympathetic or parasympathetic dominance.

For the OW subject, LF power content was shifted to VLF and some HF band to LF, suggesting that the frequencies were shifted to lower frequencies. A relatively larger change in the LF band compared to the HF band caused a decrease in the LF/HF ratio, suggesting that deactivation in the sympathetic nervous system was greater than that in the parasympathetic system, resulting in relatively higher parasympathetic activity. The suppression with repeated stimulation was less, suggesting that repeated stimulation enforced parasympathetic activities. Yadav et al. (2017) suggest that increasing BMI can suppress HF band variability and could potentially explain these decreased responses in overweight subjects.

VLF band is linked to thermo-regulation, and the effect of cold-water ingestion is more pronounced in the low BMI group.

Although this study helped to understand how HRV frequency components change with repeated cold-water drinking and how they change with different BMI groups, this study has some limitations as well. With the limited number of UW and OW subjects, the changes in their HRV frequency bands could not be statistically verified and may require further studies to increase the number of subjects in all BMI groups. The effect of volume, temperature of water, or drinking duration on HRV is not clear: to better understand the complex nature of vagal nerve stimulation, future studies should examine the effects of these parameters. The effect of gender or age on results is unclear; a more comprehensive future study with different age and gender groups may be required.

Ethics approval: All measurements are performed at Bahcesehir University, in line with the Helsinki Declarations: all subjects signed and gave informed consent before participating in this study. This study is reviewed and approved by the Ethics Board of Bahcesehir University with the protocol number of 10.05.2024/E 85646034-604.01-81467.

Conflict of interest: The authors declare that they have no conflict of interests.

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