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# Dose-dependent heart rate responses to drinking water: a randomized crossover study in young, non-obese males

**Erik Konrad Grasser**[1]

**Abstract**

**Purpose** The aim of the study was to explore a potential dose effect of water on heart rate responses and markers of vagal tone modulation.

**Methods** This was a randomized crossover study involving eight men whose heart rate and heart rate variability parameters were continuously measured following ingestion of different volumes of still mineral water (200, 400, 600, and 800 mL).

**Results** A significant volume by time effect for heart rate ($p < 0.005$) was observed. Ingestion of all volumes of drink water of more 200 mL significantly decreased the heart rate. Significant time effects for heart rate variability parameters were observed.

**Conclusion** Ingestion of a mineral water drink affected the heart rate in men in a time-dependent manner, possibly by changes in cardiac vagal modulation.

**Keywords** Cardiac · Heart rate variability · Natural product · Vagal modulation



## Introduction

Water drinking in humans results in a distinct activation of the autonomic nervous system that, in patients with severe autonomic neuropathies, leads to substantial increases in arterial blood pressure [1, 2]. Interestingly, such a blood pressure response is not seen in young, healthy humans, in whom a similar amount of water does not raise blood pressure values above baseline levels [2–6]. These observations suggest that a fully functional autonomic nervous system (i.e., sympathetic and parasympathetic branches) is necessary to balance the effect of water drinking on blood pressure. Indeed, evidence from studies on young, healthy subjects has shown that drinking water results in a concomitant activation of the parasympathetic system, by raising cardiac vagal modulation, and acutely induces a bradycardic response [3, 6]. However, research is lacking on the dose–response of drinking water on heart rate and autonomic markers representing cardiac vagal modulation. Hence, we chose a randomized crossover study design, in which the subjects did not know the order of the drinks, to investigate the effect of drink volume (200, 400, 600, and 800 mL) on both heart rate and the spectral autonomic markers suggestive of cardiac vagal modulation.

## Methods

### Study design

This study was conducted according to the guidelines laid down by the Helsinki Declaration of 1964, as revised in 2013, and all procedures involving human subjects were approved by the institute's ethical review board. Written informed consent was obtained from all subjects. Electronic Supplementary Material (ESM) Table 1 provides detailed information on the characteristics of the subjects.

All test sessions started between 0800 and 0830 hours in a quiet, air-conditioned (~22 °C) laboratory. Each subject participated in four separate test sessions (separated by at

**Electronic supplementary material**

✉ Erik Konrad Grasser
erikkonrad.grasser@unifr.ch

[1] Department of Endocrinology, Metabolism, and Cardiovascular System, Laboratory of Integrative Cardiovascular Physiology, University of Fribourg, 1700 Fribourg, Switzerland

least 2 days but by no more than 7 days) in a randomized crossover fashion. The session order was determined prior to the start of the study by using a random sequence generator (https://www.randomizer.org/), and heart rate and autonomic responses were monitored continuously for each experimental session. The drink water provided was a still mineral water (Evian, Danone S.A., Paris, France; composition: 360 mg $L^{-1}$ bicarbonate, 6.5 mg $L^{-1}$ sodium, 80 mg $L^{-1}$ calcium, 26 mg $L^{-1}$ magnesium, 1 mg $L^{-1}$ potassium, 3.8 mg $L^{-1}$ chloride, and 12.6 mg $L^{-1}$ sulfate), served at room temperature (~22 °C). Following ingestion of the prescribed volume (200, 400, 600, and 800 mL)—presented in a randomized order—responses of the subjects to the drink water were recorded. On arrival at the laboratory, subjects were asked to wear light trousers and a T-shirt, to empty their bladders if necessary, and to sit in a comfortable armchair. The study protocol sequence, as described below, is shown in ESM Fig. 1.

### Assessment of heart rate and heart rate variability parameters

A Task Force Monitor (CNSystems, Medizintechnik, Graz, Austria) was used to perform heart rate and autonomic recordings. Electrocardiography electrodes were positioned according to the manufacturer's guidelines in order to acquire high-resolution six-channel electrocardiograms, from which RR-intervals (RRI) were continuously extracted [5]. Very low-frequency (VLF_RRI: 0.003–0.04 Hz), low-frequency (LF_RRI: 0.04–0.15 Hz), high-frequency (HF_RRI: 0.15–0.40 Hz), and total power spectral density (PSD_RRI: 0.003–0.40 Hz) components were evaluated in absolute values ($ms^2$). These components were normalized (HF_RRI%) by the application of an autoregressive method to minimize disruptive effects of changes in total power [7], and they refer to autonomic modulation of the sinoatrial node and of vasomotion [7]. Although potential changes in the breathing pattern between intake of the four water drinks could alter heart rate variability [8], breathing was not controlled in the experiments in order to simulate a real-life scenario, which is characterized by spontaneous respiratory activity.

### Data and statistical analysis

Beat-to-beat values for heart rate, VLF_RRI, LF_RRI, HF_RRI, PSD_RRI, and HF_RRI_% were averaged for the last 10 min of a 30-min baseline period and over 10-min intervals within the 60-min post-drink period, respectively.

Statistical analyses were performed using GraphPad Prism version 6 statistical software (GraphPad Software, Inc., La Jolla, CA, USA). All values reported in this study are presented as means ± standard deviation. Testing for normal distribution was performed by using the D'Agostino and Pearson omnibus normality test. Natural logarithmic transformation was employed for absolute heart rate variability parameters (VLF_RRI_LN, LF_RRI_LN, HF_RRI_LN, and PSD_RRI_LN) due to their skewed distribution. Two-way repeated measures analysis of variance (ANOVA) was used with treatment and time as within-subject factors to determine a potential dose effect (treatment × time) for the applied drinks. Where time effects were observed in the two-way repeated measures ANOVA, one-way repeated measures ANOVA with Holm–Sidak's post-hoc testing was used to determine where these differences resided and to check for between-drink differences. For all tests, significance was set at $p \leq 0.05$.

## Results

At baseline, heart rate, heart rate variability parameters, and blood pressure were similar between the four drink conditions, and no adverse effects were observed in any subject during the observational period (ESM Table 1).

Two-way repeated measures ANOVA analysis revealed a significant treatment difference in average heart rate responses over the time intervals 0–30 min ($p < 0.005$), 0–60 min ($p < 0.005$), and 30–60 min ($p = 0.01$). Significant time-course changes were observed for water intake of 400, 600, and 800 mL, but not for 200 mL, where the heart rate initially decreased for at least 30 min after ingestion followed by a slow and gradual increase towards baseline thereafter. Heart rate responses to 800 mL only persisted significantly below baseline values until the end of the investigation (min 60: $-2.9 \pm 3.0$ beats $min^{-1}$; $p = 0.03$) (Fig. 1).

Two-way repeated measures ANOVA revealed no significant treatment difference in average heart rate variability responses over the post-drink time intervals 0–30 0–60, and 30–60 min (all $p > 0.05$). However, significant time-course effects, with increasing values compared to baseline levels, were observed for VHF_RRI_LN, LF_RRI_LN, HF_RRI_LN, and PSD_RRI_LN. Moreover, the majority of significant time effects and the peak values were seen within the first 30 min after water ingestion and mostly involved the high-frequency component of heart rate variability and the total power (ESM Table 2).

## Discussion

There is now compelling evidence that in young and healthy people a tap water drink exerts an acute bradycardic effect, which persists for up to 45 min after its ingestion [3–6]. The results presented here not only confirm a negative chronotropic effect in response to drinking

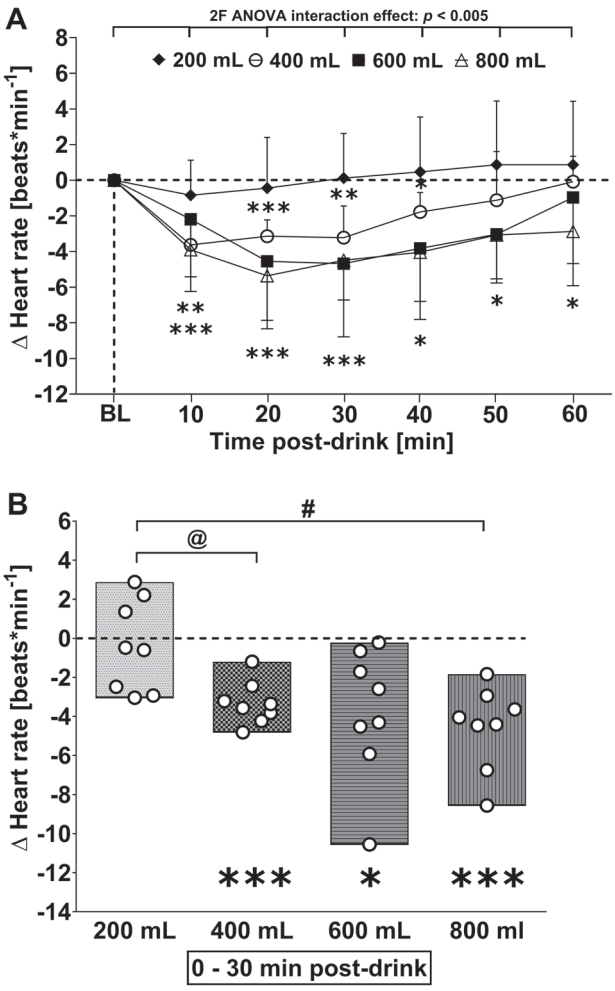


**Fig. 1** **a** Time course of changes in heart rate after ingestion of different volumes of water (200, 400, 600, and 800 mL) in young, non-obese subjects. Each mean value (i.e., 10-min blocks) corresponds to the average over the respective interval that was subtracted by its baseline (*BL*) values. Data are presented as means ± standard deviation. **b** Boxes (minimum to maximum values) represent individual mean responses (open circles) averaged over min 0–30 post-drink relative to baseline values and represented as a delta (Δ) for each drink volume, respectively. In both panels, asterisks show statistically significant differences over time from baseline values (both panels) at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.005$; in **b**, statistically significant overall drink volume effect indicated by hashtag for 200 vs. 800 mL ($^{\#}p < 0.005$) and by at sign for 200 vs. 400 mL ($^{@}p < 0.05$), as analyzed by one-way repeated measures analysis of variance with Holm–Sidak's post-hoc testing

water, but provide novel evidence that a drink volume of > 200 mL is necessary to achieve a significant reduction in heart rate that persists at least for 30 min. We did not observe significant differences in heart rate between the ingestion of 400, 600, and 800 mL drink volume, which indicates that a drink volume of 400 mL is sufficient to elicit significant decreases in resting heart rate. However, drink volumes that exceeded 400 mL, in particular 800 mL, tended to extend the bradycardic effect beyond 30 min after ingestion.

We are only aware of two studies that have compared heart rate responses to the ingestion of different volumes of water [3, 9]. In this context, and in opposition to our observations presented here and findings by Routledge and colleagues, who compared the ingestion of tap water at volumes varying from 20 to 500 mL [3], Boschmann and collegues failed to observe significant different heart rate responses to the ingestion of 50 and 500 mL of tap water [9]. However, these latter authors measured heart rate discontinuously every 5 min using a Dinamap blood pressure monitor. Consequently, it is possible that the electrographically and continuous measurements used by Routledge and colleagues [3] and our group were able to pick up subtle changes in heart rate not recorded by Boschmann et al. [9], thereby providing more accurate results.

Drinking water acutely perturbs gastrointestinal homeostasis by changing osmolality at or distal to the duodenum [10], thereby potentially activating the autonomic nervous system. Recent evidence suggests that a transient receptor potential, the vanilloid 4 channel (TRPV4), is an essential component in the autonomic response to water ingestion [10], thereby suggesting that water drinks differing in volume might elicit a dose-dependent activation of cardiac vagal fibers. Our spectral data provide evidence that modulated efferent cardiac vagal fibers could participate in the observed dose-dependent heart rate response. We observed significant bradycardic responses within the first 10 min after ingestion, which, and in consideration of the gastric emptying time of liquids (i.e., ~ 30 min for 500 mL water) [11], provide evidence that a drop in intragastric temperatures might contribute to our observed heart rate responses. This hypothesis is in line with recent findings from our laboratory [6].

In conclusion, this study provides evidence that the volume of a water drink significantly affects heart rate responses in young and non-obese males, which could be triggered by changes in cardiac vagal modulation. However, the low number of test subjects limits the conclusions which can be drawn from the study.

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## Compliance with ethical standards

**Conflict of interest** The author declare no potential conflicts of interest, financial or otherwise, with respect to this manuscript.

**Compliance with ethical standards** This study was conducted according to the guidelines laid down by the Helsinki Declaration of 1964, as revised in 2013, and all procedures involving human subjects were approved by the institute's ethical review board. Written informed consent was obtained from all subjects.

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