



# Measuring Heart Rate Variability Responses to Slow-Paced Breathing in Sedentary Young Adults: A Proposed Laboratory Protocol

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## Abstract

Slow-paced breathing at about six breaths per minute produces large oscillations in heart rate, and it is widely used in teaching laboratories and in biofeedback practice. Studies of the response in sedentary young adults are hard to compare, because they differ in how inactivity is defined, how breathing is paced, how long recordings last and which indices of heart rate variability are reported. This article proposes a laboratory protocol intended to remove the avoidable differences. It specifies the screening of volunteers and the classification of sedentary status, preparation before the visit, a within-subject crossover of slow-paced breathing against a paced control at the habitual rate, electrocardiographic and respiratory recording, artefact handling, and a short list of outcome measures with a declared primary index. Particular attention is given to a known difficulty: during slow breathing, respiratory sinus arrhythmia moves into the low-frequency band, so that conventional spectral indices no longer mean what they mean at rest. The protocol is assembled from published standards and recommendations. It has not been applied or validated here, and the article sets out the pilot work and the reliability checks that would be needed before it is adopted.

**Keywords:** Heart rate variability, Slow-paced breathing, Respiratory sinus arrhythmia, Sedentary behaviour, Baroreflex, Study protocol

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## 1. Introduction

When a healthy person breathes slowly and regularly, heart rate rises with each inspiration and falls with each expiration, and the swing grows as the breathing rate comes down towards six breaths per minute. The effect is easy to demonstrate with an electrocardiograph and a metronome, which is one reason it appears in so many undergraduate physiology practicals. It also underlies heart rate variability biofeedback, in which people are trained to breathe at the rate that gives them the largest oscillation (Lehrer et al., 2000).

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Sedentary young adults are an obvious group in which to study the response. They are free of the cardiovascular disease and medication that complicate work in older people, yet low habitual activity is associated with lower resting vagal indices than are seen in trained peers, so there is room for change. They are also the people most often recruited, as students, into laboratory studies. The literature on voluntary slow breathing is now large enough to have been pooled ([Laborde et al., 2022](#)), and reviews agree that the acute rise in variability is robust while its interpretation, and its persistence after the breathing stops, are less certain ([Zaccaro et al., 2018](#)).

Much of that uncertainty is methodological. This article therefore proposes a protocol for measuring the acute response in a consistent way. It is a design on paper, built from existing standards, and no recordings made with it are reported.

## 2. Physiological Background

Two mechanisms combine during slow breathing. The first is respiratory sinus arrhythmia, the vagally mediated fluctuation of heart rate at the breathing frequency, which arises from central coupling of respiratory and cardiac vagal neurones and from reflexes driven by lung inflation and by changes in arterial pressure ([Berntson et al., 1997](#)). The second is the baroreflex. The closed loop formed by heart rate, arterial pressure and the baroreceptors has a natural period of roughly ten seconds, so breathing near 0.1 Hz drives it at resonance and the oscillations in heart rate become very large ([Vaschillo et al., 2006](#); [Lehrer and Gevirtz, 2014](#)). Slow breathing has also been shown to raise measured baroreflex sensitivity ([Bernardi et al., 2001](#)), and it alters tidal volume, gas exchange and venous return as well.

The measurement problem follows directly. By long-standing convention the high-frequency band of the heart rate spectrum runs from 0.15 to 0.40 Hz and the low-frequency band from 0.04 to 0.15 Hz ([Task Force, 1996](#)). At six breaths per minute the respiratory peak sits at 0.1 Hz, inside the low-frequency band. Low-frequency power then rises steeply and high-frequency power may fall, although nothing resembling sympathetic activation or vagal withdrawal has taken place. The ratio of the two bands, already a doubtful index of autonomic balance at rest ([Billman, 2013](#)), becomes uninterpretable. Nor can a larger respiratory sinus arrhythmia be read simply as greater vagal tone when breathing rate and depth have changed ([Grossman and Taylor, 2007](#)). Any protocol has to be built around this.

## 3. Design Principles

Four principles shape the proposal. Each participant serves as their own control, because differences between people in resting variability are large compared with the effect of any brief manoeuvre. The comparison condition is paced breathing at the participant's habitual rate, not spontaneous breathing, so that the act of following a pacer is common to both. Respiration is recorded and not assumed. And the outcome measures are chosen in advance, with one primary index, in line with published reporting guidance ([Laborde et al., 2017](#); [Quintana et al., 2016](#)). The protocol should be written up in full and registered before the first participant is seen, so that later choices cannot be shaped by the recordings.

## 4. The Proposed Protocol

The protocol has six parts, described below in the order in which they would be carried out. Table 1 lists the main design choices with their reasons, and Figure 1 shows the sequence of a single recording visit.

### 4.1. Participants and screening

Volunteers would be adults at the younger end of working age, for example students and staff aged eighteen to thirty, without known cardiovascular, respiratory, metabolic or neurological disease and taking no medication that affects heart rate. Smoking status, usual caffeine intake and, for women, phase of the menstrual cycle or use of hormonal contraception are recorded, since each is a recognised influence on variability ([Laborde et al., 2017](#)). The study follows the Declaration of Helsinki, with approval from the local ethics committee and written consent before any procedure ([World Medical Association, 2013](#)).

The word sedentary needs care. The consensus definition refers to waking behaviour at very low

energy expenditure in a sitting, reclining or lying posture, which is distinct from being physically inactive in the sense of not meeting activity guidelines (Tremblay et al., 2017). A person can sit all day and still run every evening. The protocol therefore classifies participants on both counts: not meeting the World Health Organization recommendation for weekly aerobic activity (Bull et al., 2020) and reporting prolonged daily sitting. The International Physical Activity Questionnaire provides both items in a validated form (Craig et al., 2003), and a week of waist-worn or thigh-worn accelerometry is recommended as confirmation wherever the equipment exists.

**Table 1: Main design choices in the proposed protocol and the reason for each**

| Element           | Proposed choice                                                               | Reason                                         |
|-------------------|-------------------------------------------------------------------------------|------------------------------------------------|
| Sedentary status  | Questionnaire screening, confirmed by a week of accelerometry where available | Self-report alone misclassifies activity       |
| Design            | Within-subject crossover, order counterbalanced                               | Controls large differences between people      |
| Control condition | Paced breathing at the habitual rate                                          | Separates slow rate from the task of pacing    |
| Slow rate         | Six breaths per minute, fixed                                                 | Near baroreflex resonance; simple to reproduce |
| Recording         | ECG at 500 Hz or more, with a respiration belt                                | Accurate R-wave timing; verifies adherence     |
| Epoch length      | Five minutes per condition                                                    | Matches the short-term recording standard      |
| Primary outcome   | RMSSD, with peak-to-trough heart rate change                                  | Less dependent on spectral band limits         |

#### 4.2. Preparation before the visit

Participants are asked to avoid vigorous exercise and alcohol for a day beforehand, to avoid caffeine and a heavy meal for some hours beforehand, to sleep as usual and to empty the bladder on arrival. All visits take place at the same time of day in a quiet room at a comfortable temperature. Recordings are made seated, with back support and both feet on the floor, because this is the posture in which paced breathing is ordinarily practised; body position is kept constant throughout, as it changes vagal indices on its own.

#### 4.3. Breathing conditions

After a period of seated rest, a five-minute baseline with spontaneous breathing is recorded, and breathing rate during it is taken as the habitual rate. Two paced conditions follow in counterbalanced order, separated by unpaced rest: slow breathing at six breaths per minute, and control breathing at the habitual rate. A visual pacer, such as a bar that rises and falls, is preferred to spoken instructions because it does not add speech to the recording. Participants breathe through the nose, comfortably and without straining for depth, and are told to stop if they feel light-headed. The ratio of inspiration to expiration is fixed and reported; a somewhat longer expiration is common in practice, and the protocol adopts it for both paced conditions.

A single fixed slow rate is proposed in preference to finding each person's resonance frequency. Individual resonance rates do differ slightly, and biofeedback protocols determine them by stepping through several rates (Lehrer et al., 2000), but the procedure adds time and a further source of variation between laboratories. A laboratory with the time to do so can add the stepped assessment as a separate visit.

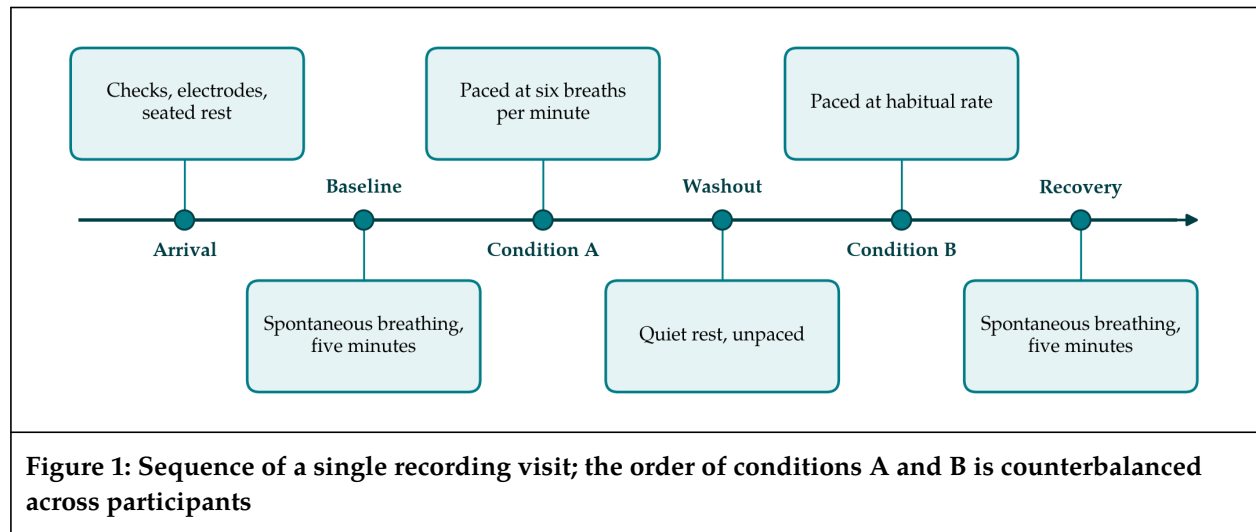
#### 4.4. Recording

A chest-lead electrocardiogram is sampled at 500 Hz or faster so that R-wave peaks are located precisely. Respiration is recorded with a strain-gauge or inductance belt throughout. The respiratory trace has two uses: it confirms that the participant kept to the pacer, and it gives the actual breathing frequency against

which the heart rate spectrum is read. Where a continuous finger-cuff pressure monitor is available, beat-to-beat arterial pressure allows baroreflex gain to be estimated, but this is an optional extension.

#### 4.5. Signal processing and outcome measures

Interbeat intervals are extracted with an established package such as Kubios HRV (Tarvainen *et al.*, 2014), and every record is inspected by eye. Ectopic beats and artefacts are corrected by interpolation, the proportion of corrected beats is reported, and a record that exceeds a threshold fixed in advance is excluded. The first part of each paced epoch can be discarded to allow the pattern to settle, provided the same rule is applied to every condition.



The primary outcome proposed is the root mean square of successive differences, RMSSD, supplemented by the mean peak-to-trough difference in heart rate across breaths. Both are time-domain measures and do not rely on band limits, although RMSSD is not wholly independent of breathing rate and the report should say so (Shaffer and Ginsberg, 2017). The standard deviation of normal intervals, mean heart rate and spectral power in the conventional bands are secondary. Power at the measured breathing frequency is reported alongside the band values, and the ratio of low to high frequency power is not interpreted for the slow condition.

#### 4.6. Planned analysis

The contrast of interest is slow against control pacing within the same person, with order and baseline value as covariates. Skewed indices are log-transformed before analysis. The number of participants is fixed beforehand from a power calculation that uses an effect size taken from the pooled literature, with allowance for excluded recordings. Stopping rules, exclusions and the handling of missing epochs are written into the registered plan.

### 5. Fitting the Protocol to Teaching and Research Laboratories

The protocol was drafted with ordinary physiology departments in mind. Everything in the core version can be done with a teaching-grade bioamplifier, a respiration belt and free or low-cost software. Consumer chest-strap monitors that export interbeat intervals are a tempting substitute for the electrocardiogram. They may be adequate for time-domain indices at rest, but they give no waveform to inspect, and a laboratory that uses one should first compare it against an electrocardiogram on its own participants under slow breathing.

A single visit of under an hour is enough for the acute response. Laboratories interested in training effects would need a different design, with repeated sessions and a follow-up recording at rest, and the present protocol would serve only as its measurement component. For class practicals the crossover can be kept and the registration and power calculation dropped, as long as the results are presented as a

demonstration and not as a study.

## 6. Limitations and What Remains to Be Shown

The protocol is proposed and has not been validated here. Three questions would need answers before it could be recommended as a standard. One is feasibility: whether untrained sedentary volunteers can follow a pacer at six breaths per minute for five minutes without discomfort or overbreathing. Slow, deep breathing can lower carbon dioxide, which itself affects heart rate and may cause dizziness, and the core protocol does not measure end-tidal carbon dioxide. A pilot with capnography would show whether this matters. The second is reliability: whether the primary outcome is repeatable when the same person is tested on two days. The third is whether the control condition does its job, or whether pacing at the habitual rate itself changes variability compared with spontaneous breathing.

There are also limits that no amount of piloting removes. A fixed rate will be slightly off resonance for some participants and will understate their largest possible response. The protocol measures a reflex response lasting minutes and says nothing about health outcomes; a large oscillation during slow breathing is not evidence of benefit, and the article makes no claim that slow breathing treats any condition. Finally, sedentary status defined by questionnaire remains imprecise even with accelerometry as a check.

## 7. Conclusion

The acute heart rate variability response to slow-paced breathing is easy to elicit and hard to measure in a way that others can reproduce. The protocol proposed here fixes the choices that most often differ between studies: who counts as sedentary, what the slow condition is compared with, how breathing is verified, and which index is primary once respiration has moved into the low-frequency band. It is offered as a starting point for laboratories to pilot, criticise and refine, and its worth will depend on reliability data that have yet to be collected.

## Conflict of interest

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