Does two weeks of following the Wim Hof Breathing Method improve coping with physiological and psychological stressors?

A bachelor research report by: Nick Veenhof

Student number: S3906515

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Supervisors: Anton Scheurink, Cato Drion, Jocelien Olivier

Abstract
Stress has a big impact on several aspects of health. It is therefore important to find clinical ways to reduce stress levels. Stress can be measured by looking at heart rate variability (HRV), which is the variability in time interval between heartbeats. Breathing techniques can be used to improve heart rate variability (HRV) and reduce stress, but little research has been conducted on the Wim Hof Breathing Method (WHBM). The WHBM is one of the three pillars of the Wim Hof Method, designed by 'The Iceman' Wim Hof. There are claims that his method has several health benefits, but more research needs to be done to confirm these claims. The current experiment aims to investigate if two weeks of practicing the WHBM can improve stress-coping during both physiological and psychological stressors. Results did not show any significant improvements in the way the participants were able to cope with stress. This means that this current experiment failed to show that the WHBM is a valid way to reduce stress. Nevertheless, recent research has shown that there are preliminary indications that the WHBM, possibly in combination with the other pillars of the WHM, could work stress-relieving.

Introduction
Stress, both physiological and psychological, is an ever present problem in our current society. Both types of stress can have a variety of impacts on health and disease (Birdee et al., 2023). Despite this, there is not one clear consensus on what stress exactly is and how to accurately measure it. One way to measure stress in a non-invasive manner is by looking at heart rate variability (HRV). The link between HRV and stress has been looked into with increasing frequency over the past decade. But what exactly is HRV, and what does it tell us about stress? HRV is the variability or fluctuation in the time interval between adjacent heartbeats. A common and simple way to calculate and express HRV is by measuring the normal-to-normal (NN) interval between heartbeats for a certain period and taking the standard deviation (SDNN, Malik et al., 1996).
HRV is susceptible to changes in the activity of the autonomic nervous system (ANS). The ANS consists of two branches: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The interplay and dynamics between these two branches determines how people physiologically respond to stress. Increased activity of the SNS during a stressful situation leads to the familiar 'fight-or-flight' response, which among other things increases and stabilizes heart rate (Kim et al., 2018). When heart rate is stable, the HRV logically decreases, as the time between heartbeats becomes very similar. Therefore, low HRV can be seen as a marker for higher stress levels, as well as a marker for disease in general (Thayer et al., 2012).

To try and reduce stress, breathing techniques can be used and are already being applied in psychotherapy (Steffen et al., 2021). There is data showing that certain breathing techniques might help in lowering both physiological and psychological stress levels in PTSD patients (Fonkoue et al., 2020) and healthy adults (Hopper et al., 2019). One breathing technique that hasn't yet been widely used in psychotherapy is the Wim Hof Breathing Method (WHBM), part of the Wim Hof Method (WHM). Wim Hof, often coined as 'The Iceman', has broken multiple world records related to cold exposure, such as standing in a container filled with ice cubes for almost two hours (Wim Hof Method, z.d.-a). To be able to achieve this, he developed his own method based on three fundamental pillars: breathing, cold therapy and mindfulness or meditation. Wim states that following his method will result in a variety of health benefits such as an improved immune system, better sleep and reduced stress (Wim Hof Method, z.d.-b). Over the years, several case studies have been performed on Wim, trying to explain his ability to withstand extreme cold (Kox et al., 2012; Muzik et al., 2018). The study by Kox et. al (2012) looked at the effects the WHM has on the activity of the autonomic nervous system (ANS) and its relation with the innate immune response. From their data, it seemed the WHM allowed Wim to evoke a controlled stress response, leading to SNS activation. The following increase in cortisol attenuated the immune response (Kox et al., 2012). They followed this study up two years later, testing if the same effect could be found in a group of healthy subjects (Kox et al., 2014). The results indeed showed that practicing the WHM could suppress the immune response, which has implications for the treatment of autoimmune diseases (Kox et al., 2014). Both these studies, as well as the other case study by Muzik et al. (2018), suggest that the WHM allows voluntary control over the ANS which was previously thought to not be possible. Since the ANS is one of the major players in the stress response, these findings give an interesting insight into how the WHBM could be used in treatment to help with reducing stress levels.

So the research that has been done on the WHM is mainly focused on the immune system and has shown some interesting results, but claims that the WHBM has positive effects on stress still require a lot more scientific validation, with the current literature on the specific topic still being in its infancy. One article that does look into the effects that the WHM has on the stress response is an article by Touskova et al. (2022). In their experiment, people that were part of an Antarctic expedition underwent eight weeks of Wim Hof training to see if certain neuroendocrine measures (cortisol and melatonin) and psychometric measures (depression and trauma) of stress would be significantly reduced at the end of the training. The study provides preliminary results that would suggest a positive effect of the WHM on certain measures of stress (Touskova et al., 2022). However, the researchers did mention they had to work with a small and selective sample. Therefore, further research is needed in healthy subjects under non-extreme conditions to validate this type of training as a way to reduce stress.

That is why this current experiment aims to investigate the possible positive effects of following the Wim Hof Breathing Method (WHBM) on coping with physiological and psychological stressors. We hypothesize that, by looking at heart rate and HRV, two

weeks of WHBM training will show a significant improvement to the physiological and psychological stress-coping ability of the WHBM participants. We expect to see that baseline heart rate decreases and baseline HRV increases after 2 weeks of WHBM. Additionally, we expect HRV to decrease less in the WHBM participants during the stress-inducing experimental manipulations when compared to their baseline HRV.
Results showed that heart rate did not decrease and HRV did not increase after two weeks of WHBM training. Additionally, non-significant changes in relative HRV were observed. These results show that in this current setup, the WHBM did not improve coping with physiological and psychological stress, although this doesn't rule out the possible positive effects the training might have on reducing stress.

Methods

Participants

Participants were recruited and divided into two experimental groups: an intervention group (WHBM group) and a control group. Upon intake, participants were asked if they were willing to be part of the intervention group, since they would have to follow the WHBM for the duration of the experiment (two weeks). The age and sex of all participants was noted at the start of the first test day. At the end of the final test day a short questionnaire had to be filled in by all participants, assessing their general fitness level and subjective change in stress, happiness and mental tiredness over the two week experimental period. Additionally, all participants were asked if they could cope better or worse with the Ice shock on the final test day.

Experimental Setup

Experimental groups

Both groups were subjected to a total of 3 test days: one at the beginning of the experiment, one after one week and one after two weeks (±1 day). Since not all participants could be tested on the same day, the start and ending dates for participants differed. For the WHBM group, participants were asked to watch a 10-minute video every day, in which a version of the Wim Hof Breathing Method was shown for them to follow: https://www.youtube.com/watch?v=0BNejY1e9ik.
The participants in the control group had no obligations outside of the test days.

Test Day Structure

To perform the measurements, the following components of the BIOPAC system were used: the respiration transducer, ECG and GSR transducer. With this equipment the respiratory rate, heart rate and skin conductance were recorded. At the start of the test day, participants were seated comfortably and connected to the BIOPAC system. Electrodes were attached to the right wrist and both ankles of the participant to connect the ECG cables with. The participants were also fitted with a respiratory transducer belt and the GSR transducer (Noordhuis & Siertsema, 2021). Instead of using electrode gel, two additional electrodes were attached to the index and middle finger of the subject's left hand to improve the readouts of the GSR transducer. After participants were all set up and in a comfortable seating position again, participants were instructed to relax and close their eyes. A baseline measurement was then taken for two minutes (referred to as 'Baseline' from now on).

After that, participants in the WHBM group watched the first part of the WHBM video (see link above), while the control group watched a nature video for the same amount of time (3 minutes, https://www.youtube.com/watch?v=eNUpTV9BGac). Both groups then continued with the ice shock and physical exercise experiments, which are explained in further detail below.

Ice shock

A resting measurement, similar to Baseline, was taken for 60 seconds prior to continuing with the ice shock experiment. This resting measurement will be referred to as 'Rest Ice'. After 60 seconds, a tub with lukewarm water was placed next to the participant's right hand. Upon instruction, the participants put their right hand fully into the lukewarm water. Measurements were taken for 120 seconds, which will be referred to as 'Lukewarm'. After 120 seconds, participants were instructed to move their hand out of the tub again. Next, a container with ice was placed next to the participant. A 60-75 second measurement was done before the participants were instructed to put their hand into the ice, to measure their anticipation of the upcoming ice shock. This measurement will be referred to as 'Anticipation'. After the Anticipation the participants were instructed to put their hand into the ice. When the participant had their hand completely immersed in the ice and stopped moving, measurements were taken for 120 seconds. Participants were allowed to take their hand out if they felt they couldn't tolerate the ice any longer. After a maximum of 120 seconds, participants were instructed to take their hand out of the ice again. This two minute measurement will be referred to as 'Ice shock'. After the Ice shock the participants moved on to the physical exercise experiment. This protocol has been adapted from the "ice shock" experiment described in the Instructor's Manual for the Polygraph Practical (Noordhuis & Siertsema, 2021).

Physical exercise

Just as in the ice shock experiment, a resting measurement was taken for 60 seconds prior to the physical exercise experiment. This measurement will be referred to as 'Rest Cycling'. Participants then had to take place on a Tunturi hometrainer and were instructed to cycle for two minutes at a moderate intensity. This meant cycling at a predetermined pace of 60 RPM (rounds per minute) with a resistance of 6 Nm. Measurements were taken during the 120 seconds of cycling, and will be referred to as 'Cycling'. After 120 seconds, participants sat down again and a final recovery measurement was taken for 120 seconds. This measurement will be referred to as

'Recovery'. After recovering, the participants left and their test day was over. This protocol has been adapted from the "physical exercise" experiment described in the Instructor's Manual for the Polygraph Practical (Noordhuis & Siertsema, 2021).

Data acquisition and analysis

The BIOPAC system

For this experiment the BIOPAC MP35 unit was used in combination with the three components mentioned before (ECG, respiratory transducer and GSR transducer). The transducers were plugged into the correct channels of the BIOPAC unit at the start of the experimental phase. Within the BIOPAC software, 'Lesson 9 - EDA and Polygraph' was used throughout the experiment. At the start of each test day, the correct channels were selected within Lesson 9 to allow for data analysis: Channel 3 displayed electrodermal activity (EDA) in delta microsiemens, Channel 40 showed the respiration in millivolt (mV) and Channel 41 showed the heart rate in beats per minute (BPM). For each experimental condition, the mean values were taken for skin conductance, respiration and heart rate, as well as the standard deviation of the heart rate (Noordhuis & Siertsema, 2021).

Heart Rate Variability analysis

The programme within the BIOPAC software that was used in this experiment did not include an ECG readout, and instead showed the heart rate in BPM across time. This meant that HRV calculations had to be performed in a suboptimal way. Instead of being able to accurately measure the time interval between every adjacent heartbeat, the average heart rate was taken every 4 seconds. This average was then converted from BPM to beats per second (BPS), from which the seconds per heartbeat was calculated by taking the inverse of the BPS. The last step then was to convert the seconds per heartbeat to milliseconds per heartbeat. This approach allowed for the, albeit less than optimal, calculation of a HRV that approximates a value resembling the SDNN. A calculation example is available in Appendix 1.

Results

A total of 12 participants (11 males, 1 female) were recruited for this experiment and were divided equally between both experimental groups: the WHBM, or invention group (n = 6, 5 males and 1 female) and the control group (n = 6, all 6 males). The average age in the WHBM group was 26.8 years (SD = 13.4 years) and the average age in the control group was 20 years (SD = 1.9 years). For the intervention group, participants had an average WHBM video watching rate of 88.1%, which means they on average failed to watch the video on approximately 2 out of 14 days.

Heart rate

To assess stress levels, the average heart rates (HR) were calculated for the Baseline, Anticipation and Ice shock conditions. From the Baseline measurement (2 min rest) the average HR was sampled from t = 30s until t = 90s. Figure 1A shows that the WHBM group on average had an increase (from 75.02 to 83.66 BPM) in Baseline HR over the two weeks, while the control group showed a decrease in HR over the same period (81.18 to 75.61 BPM). The average HR of the Anticipation was taken from the 60s prior to the Ice shock. As seen in figure 1B, the WHBM group shows an increase in average Anticipation HR (from 72.94 to 73.50 BPM), while the control group shows a decrease (75.71 to 72.89 BPM). During the Ice shock, the average HR was taken from t = 0s (hand fully into the ice) until t = 60s. As shown by Figure 1C, the average Ice shock HR of the WHBM increased over the two weeks (from 72.67 to 73.53 BPM), while the average HR of the control group decreased (from 83.92 to 78.56 BPM).
A higher heart rate is a general marker for elevated stress levels. The expectation therefore was to observe a decrease in HR after two weeks of following the WHBM, which would be an indication of a lower stress response. However, in all 3 situations (Baseline, Anticipation and Ice shock) an increase in HR was observed in the WHBM group when comparing TD1 and TD3. This means the hypothesis that two weeks of following the WHBM lowers HR during stress is not supported by the current data.

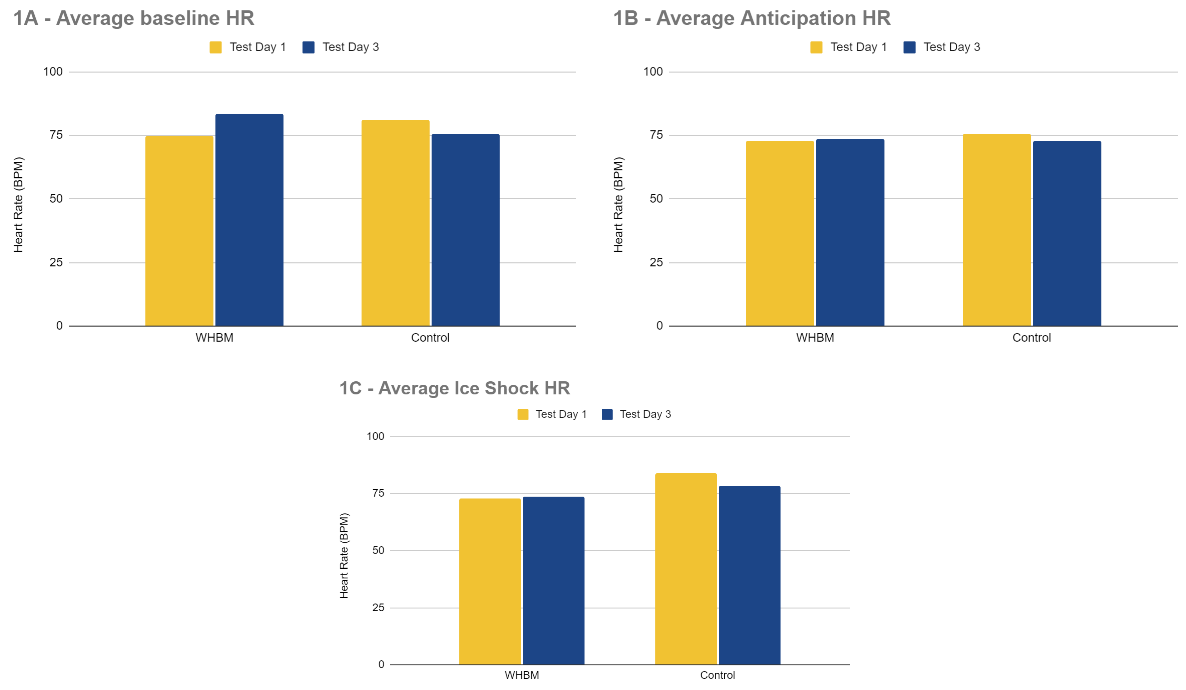

Figure 1 - Bar charts showing the heart rates (HR) in both experimental groups on Test Day 1 (TD1 or Day 0) and Test Day 3 (TD3 or Day 13/14/15). ***1A)*** *The change in average HR during Baseline (WHBM from 75.02 BPM on TD1 to 83.66 BPM on TD3; control from 81.18 BPM on TD1 to 75.61 BPM on TD3).* ***1B)*** *The change in average HR during Anticipation (WHBM from 72.94 BPM on TD1 to 73.50 BPM on TD3; control from 75.71 BPM on TD1 to 72.89 BPM on TD3).* ***1C)*** *The change in average HR during Ice shock (WHBM from 72.67 BPM on TD1 to 73.53 BPM on TD3; control from 83.92 BPM on TD1 to 78.56 BPM on TD3).*

Relative heart rate

Compensating for the individual variation in baseline HR, the relative heart rate is calculated. In Figure 2A, the average HR during Anticipation is expressed as a decimal fraction of the HR during Rest Ice. If the HR during Anticipation is equal to the Rest Ice HR, this results in a relative HR of 1. A value greater or lower than 1 therefore means a respective increase or decrease in HR during Anticipation compared to Rest Ice HR. In Figure 2B the same is done, now expressing the HR during the Ice shock as a decimal fraction of the HR during Lukewarm. Figure 2A shows a decrease in relative HR over the two weeks in the WHBM group (from 1.01 on TD1 to 0.95 on TD3), while the control group shows an increase over this period (from 0.96 on TD1 to 1.00 on TD3). In Figure 2B a decrease in relative HR over the two weeks in the WHBM group is seen as well (from 1.03 on TD1 to 1.00 on TD3), with the control group showing no change (1.11 on TD1 and TD3).

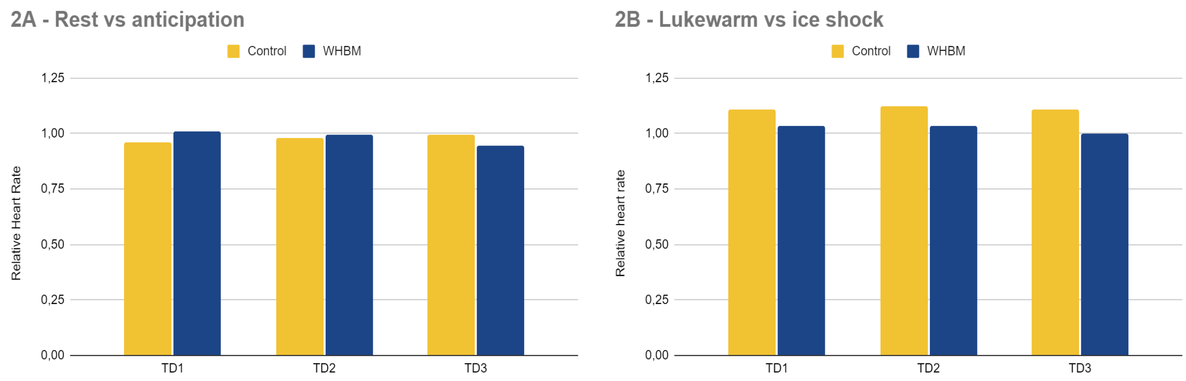

Figure 2 - Bar charts showing the relative heart rates (HR) in both experimental groups on Test Day 1 (TD1 or Day 0), Test Day 2 (TD2 or Day 6/7/8) and Test Day 3 (TD3 or Day 13/14/15). ***2A)*** *HR during Anticipation expressed as a decimal fraction of HR during Rest Ice (WHBM from 1.01 on TD1, 0.99 on TD2 to 0.95 on TD3; control from 0.96 on TD1, 0.98 on TD2 to 1.00 on TD3).* ***2B)*** *HR during Ice shock expressed as a decimal fraction of HR during Lukewarm (WHBM from 1.03 at TD1, 1.03 on TD2 to 1.00 on TD3; control from 1.11 on TD1, 1.13 on TD2 to 1.11 on TD3).*

Relative HR was looked at across all three test days in both groups. By comparing a resting state (Rest Ice or Lukewarm) with a supposedly stressful state (Anticipation or Ice shock), the expectation would be that the relative HR would decrease over the two weeks in the WHBM group due to a better ability to cope with stress. For both comparisons a decrease was observed in the WHBM group. This decrease would indicate that HR during the stressful states becomes more similar to the resting state HR over two weeks. This data supports the idea that the WHBM helps with stress coping, seeing as the HR decreases over the two weeks in both experiments. To statistically examine if these decreases were significant, the Mann-Whitney U test was performed. For this, the individual data was analyzed and showed that the decreases were not statistically significant ($p>0,05$).

Heart Rate Variability

The heart rate variability (HRV) was also calculated, using the example calculation shown in Appendix 1, in order to assess stress levels during Baseline, Anticipation and Ice shock. From the Baseline measurement (2 min rest), the SDNN was calculated from t = 30s until t = 90s. For both Anticipation and Ice shock the first 60 seconds were used to obtain the SDNN. Figure 3A shows that the HRV decreased in the WHBM over the two weeks (from 52.16 ms on TD1 to 41.49 ms on TD3). The control group showed an increase in HRV over the two weeks (from 38.07 ms on TD1 to 39.49 ms on TD3). In Figure 3B the HRV during Anticipation is shown for both groups. In the WHBM group the Anticipation HRV decreases over the two weeks (from 67.79 ms on TD1 to 57.39 ms on TD3), with the control group also showing a decrease over this period (from 52.63 ms on TD1 to 40.76 ms on TD3). Figure 3C shows the Ice shock HRV decreasing over two weeks in the WHBM group (from 72.10 ms on TD1 to 67.61 ms on TD3), while the control group shows an increase (from 43.77 ms on TD1 to 54.60 ms on TD3).

A better ability to cope with stress is normally associated with higher HRV. The WHBM group showed a decrease in HRV between TD1 and TD3 in all tests, therefore not supporting the hypothesis that two weeks of WHBM training improves stress coping.

Relative HRV

Similar to HR analysis, to compensate for individual and temporal variation in HRV, the relative HRV on each day is calculated. Figure 4A shows the HRV during Anticipation expressed as a decimal fraction of the HRV during the Baseline. Figure 4A shows an increase in relative heart rate for the WHBM group (from 1.24 on TD1 to 1.74 on TD3). The control group showed a decrease in relative HRV (from 1.45 on TD1 to 1.24 on TD3). Figure 4B shows the HRV during Ice shock expressed as a decimal fraction of the HRV during the Baseline. Figure 4B shows an increase in relative heart rate for the WHBM group (from 1.50 on TD1 to 2.79 on TD3). The control group also showed an increase in relative HRV (from 1.39 on TD1 to 1.42 on TD3).

These averages support WHBM as helping with stress coping, seeing as the HRV increases over the two weeks in both experiments. To statistically examine if these increases were significant, the Mann-Whitney U test was performed. For this, the individual data was analyzed and showed that the increases were not statistically significant ($p>0,05$).

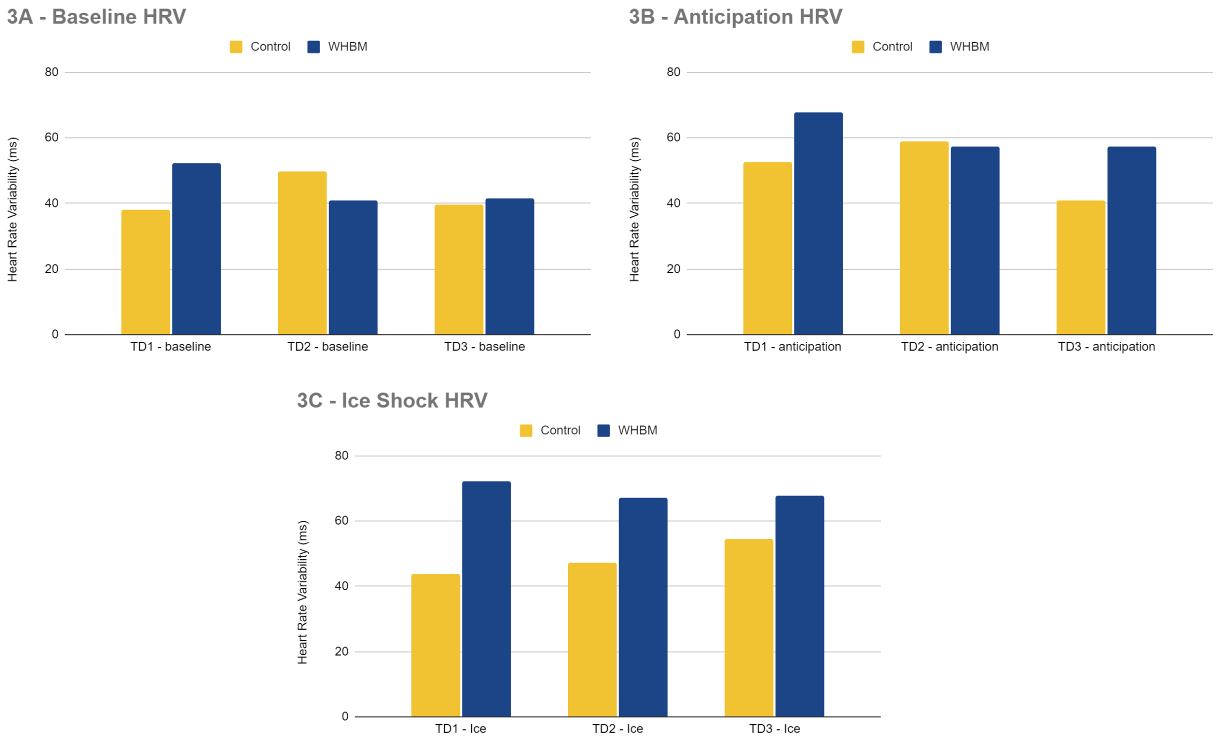

Figure 3 - Bar charts showing the heart rate variability (HRV) in ms for both experimental groups on Test Day 1 (TD1 or Day 0), Test Day 2 (TD2 or Day 6/7/8) and Test Day 3 (TD3 or Day 13/14/15). ***3A)*** *The average HRV during Baseline (WHBM 52.16 on TD1, 41.04 on TD2 to 41.49 on TD3; control from 38.07 on TD1, 49.79 on TD2 to 39.49 on TD3).* ***3B)*** *The average HRV during Anticipation (WHBM 67.79 at TD1, 57.28 on TD2 to 57.39 on TD3; control from 52.63 on TD1, 59.01 on TD2 to 40.76 on TD3).* ***3C)*** *The average HRV during Ice shock (WHBM 72.10 at TD1, 66.98 on TD2 to 67.61 on TD3; control from 43.77 on TD1, 47.20 on TD2 to 54.60 on TD3).*

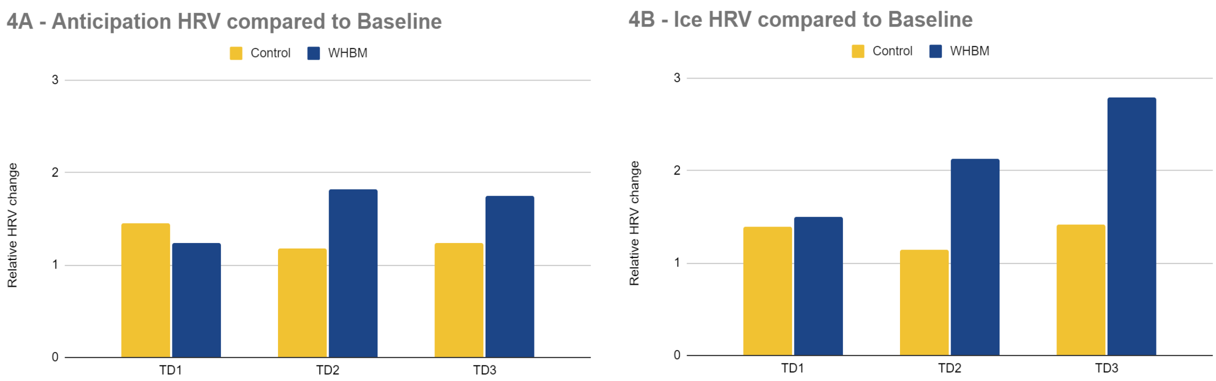

Figure 4 - Bar charts showing the relative heart rate variability (HRV) in both experimental groups on Test Day 1 (TD1 or Day 0), Test Day 2 (TD2 or Day 6/7/8) and Test Day 3 (TD3 or Day 13/14/15). ***4A)*** *HRV during Anticipation expressed as a decimal fraction of HRV during Baseline (WHBM from 1.24 on TD1, 1.81 on TD2 to 1.74 on TD3; control from 1.45 on TD1, 1.18 on TD2 to 1.24 on TD3).* ***4B)*** *HRV during Ice shock expressed as a decimal fraction of HRV during Baseline (WHBM from 1.50 on TD1, 2.13 on TD2 to 2.79 on TD3; control from 1.39 on TD1, 1.14 on TD2 to 1.42 on TD3).*

Discussion

The main outcome of this experiment is that, based on the data presented, two weeks of the WHBM does not significantly improve coping with physiological and psychological stress. While the expectation was that heart rate would go down and HRV would go up in the WHBM group between the first and last test day, the opposite was observed. This effect was then corrected for by looking at relative heart rate and HRV for each test day, but the outcome was still not significant. Individual variation combined with the small sample size heavily contributed to this, meaning the results do not per definition rule out a possible positive effect of the WHBM on stress coping.
The results in Figure 4 seem the most promising at first glance, with an increase in HRV across the two weeks of WHBM training for both the Anticipation and Ice shock conditions, which would indicate improved stress-coping. However, what wasn't expected is the fact the HRV would be higher than the Baseline condition. The fact this is observed not only on all test days in the WHBM group, but in the controls as well is an interesting finding. This would mean that during the Baseline condition, which was designed to be a measure of the participants resting state, people are more stressed than during a supposedly stressful situation. This is possibly down to flaws in the experimental setup. It is very well possible that when participants came in for their test day, they had just been active, cycling to the test location and/or walking up the steps in the building. Not allowing for a suitable amount of time for them to fully relax and instead starting the test day rather quickly might have contributed to these results. Since the participants had just been active, their SNS activation was higher, contributing to a lower HRV than what they might have had when completely relaxed.

The results focus on the heart rate and HRV during the ice shock experiment, but do not include the data from the physical exercise experiment. The respiratory and GSR data were also excluded from the final results. This decision was mainly based on the fact a lot of that data turned out to be inaccurate and in some cases even unusable. Unfortunately, we experienced major difficulties with the heart rate readouts when participants were performing the physical exercise experiment. This meant we observed irregular and unexplainable peaks and valleys in the heart rate, sometimes ranging between 20 and 160 BPM, with the heart rate even dropping down all the way to 0 BPM on some occasions. A possible explanation for this is that the movement of the pedals of the Tunturi bike were interfering with the electrical signal that the ECG cables are supposed to pick up from the points on the body where it is attached. Additionally, the cables were moving and hitting the bike during the experiment, which might have contributed to the obscure data we observed. Individual HRV data during Cycling is presented in Appendix 2. A similar trend was seen for the GSR data. The transducers seemed to pick up every slight finger movement of the participants, meaning the readouts most likely were a representation of this finger movement instead of sweat production, which is what the transducer is meant for. Therefore this data was deemed too inaccurate to use for interpretation of the participants' stress levels. Lastly, there was too little time available to perform a thorough analysis of respiratory patterns, which would have had to be done manually because of the limitations of the BIOPAC system.

The decision to only focus on a single pillar of the WHM (breathing) in this experiment was made to find out if the WHBM alone was capable of reducing stress levels. Additionally, to increase the chance of a higher number of willing participants we aimed for the tests and experimental manipulation to be as non-invasive as possible. The inconclusive results obtained in the current experiment could indicate that the WHBM is not able to improve stress-coping on its own, and instead needs the other pillar(s). A study by Kopplin and Rosenthal (2022) used questionnaires to evaluate the stress-reducing effects of two of the pillars: breathing and cold exposure. They looked at the pillars separately as well as combining them. Their subjects reported lower stress levels in all groups after two weeks, but showed the biggest difference when breathing and cold exposure were combined (Kopplin & Rosenthal, 2022). Appendix 3 shows some of the data obtained from the questionnaire carried out in the current experiment.

An interesting angle of future research to consider is to see if the WH(B)M can improve athletic performance. A pilot study done by Citherlet et al. (2021) looked at the effect on anaerobic performance of a single WHBM session before a Repeated Ability Sprint Test (RAST). While they found that this single session did not enhance the performance of the athletes, perhaps following the WHBM for multiple weeks could still have positive effects. Adding to this is the study by Krentzman (2021), who states that the cyclic hyperventilation in the WHBM increases oxygen availability for mitochondria. This in turn might have an effect on the ATP production by mitochondria (Krentzman, 2021). Higher ATP production could improve certain aspects of athletic performance, although it is unlikely it would benefit anaerobic sprinting performance. In the current setup this theory could be incorporated and tested, by letting participants exercise (e.g. cycling) to their maximum ability and seeing if WHBM training over a certain period of time improves their performance.
The study by Kopplin and Rosenthal (2022) also provides a starting point for future research. Their findings are an early indication that the WHBM, but even more so a combination of multiple pillars of the WHM can decrease stress levels. Despite the fact they only show a correlation between subjective stress and following the treatment, it still has implications for the future research on the stress-relieving ability of the WHM. Tests like those performed in the current experiment, although in need of further improvement, could provide tangible, physiological readouts of stress (e.g. HRV) and give a more detailed answer to the question if the WHBM, in combination with cold exposure, is a feasible way to reduce stress.

Like every experiment, this one had several limitations. Firstly and most notable was the small sample size, meaning it was very hard to find significant differences within an experimental group. Secondly, the timeframe in which the experiments could be conducted was relatively short. A setup where subjects serve as their own control both before and after WHBM training would have been interesting, to examine the possible lasting effects of the WHBM, but unfortunately this was not achievable. Furthermore, we would have preferred the incorporation of an additional type of psychological stress into the current setup, for example a VR horror movie. In future experiments this other way of applying psychological stress could generate more accurate data on the possible stress-relieving effects of the WHBM. Another limitation of this experiment was the way the HRV was calculated. As mentioned, the programme within the BIOPAC software didn't allow for an accurate way to determine the exact time interval between heartbeats, which means the SDNN values in our results don't perfectly represent the HRV. Another aspect to consider is the period that was used to obtain the HRV measurements, which was either 60 or 120 seconds in the current setup. A usual time interval to measure the SDNN is either 24 hours or 5 minutes (Thayer et al., 2012). This could imply that the intervals used in the current experiment weren't long enough to provide us with an accurate representation of a person's HRV during the different conditions.

In conclusion, while the data obtained in this experiment is inconclusive, recent research has shown that there are positive effects of the WHBM on several physiological and psychological markers of health (Kox et al., 2014; Krentzen, 2021; Kopplin & Rosenthal, 2022; Touskova et al., 2022; Zwaag et al., 2022). However, there is more research needed on the exact mechanisms through which the WHBM and the other pillars of the WHM can influence and affect these markers, before we can start to confidently say that the Wim Hof Method is as beneficial as claimed.

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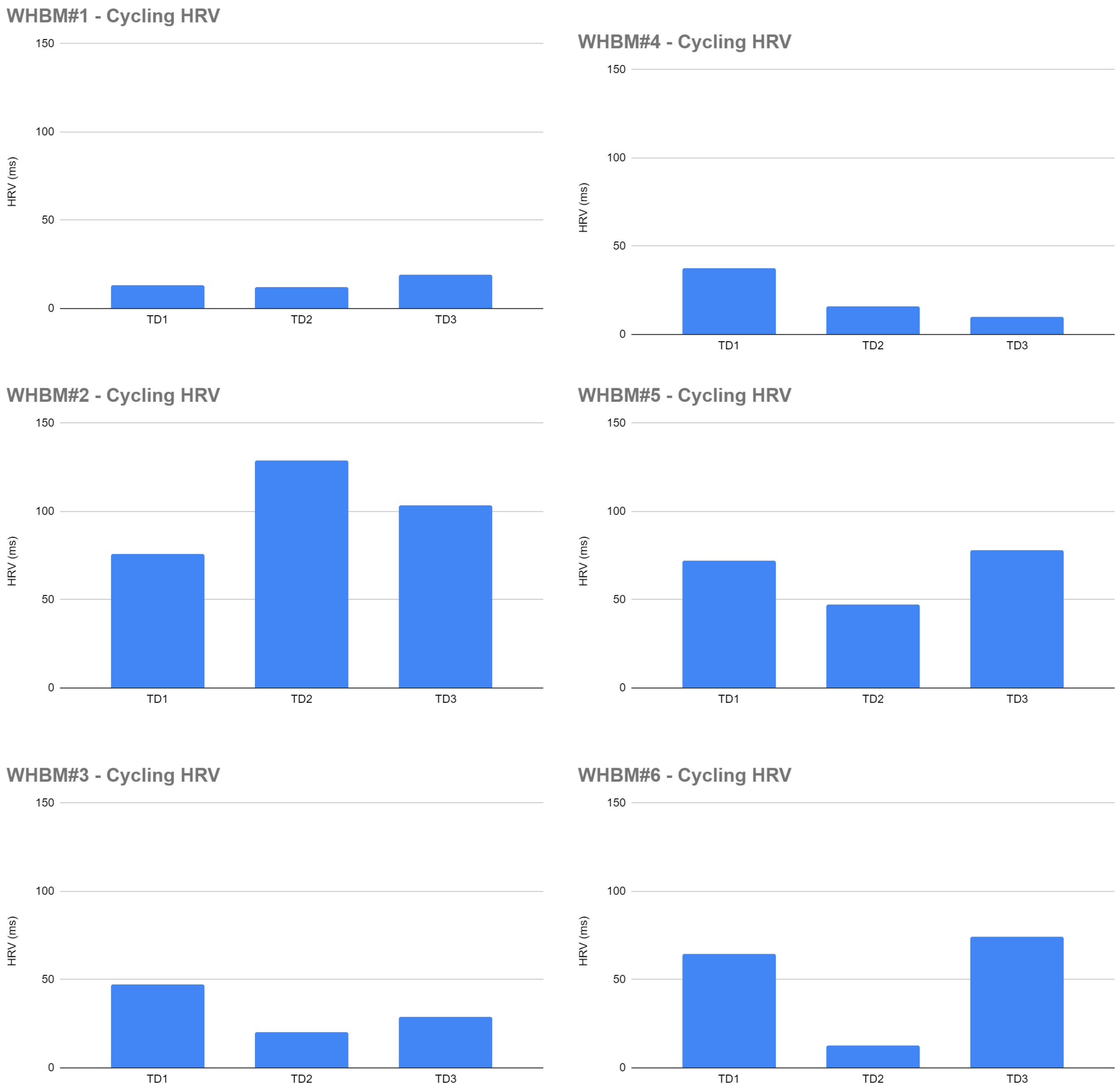
Appendix 1

Heart Rate every 4s (BPM)	BPS	Seconds per heartbeat	Milliseconds per heartbeat	SDNN (ms)
70,57359	1,1762265	0,8501763903	850,1763903	33,06193156
72,85003	1,214167167	0,8236098187	823,6098187	
73,32973	1,222162167	0,8182220226	818,2220226	
71,94616	1,199102667	0,8339569478	833,9569478	
68,3871	1,139785	0,8773584492	877,3584492	
67,97419	1,132903167	0,8826879732	882,6879732	
64,7084	1,078473333	0,9272366493	927,2366493	
66,33012	1,105502	0,9045664323	904,5664323	
66,5613	1,109355	0,9014247017	901,4247017	
66,38542	1,106423667	0,9038129155	903,8129155	
69,99717	1,1666195	0,8571775116	857,1775116	
70,85051	1,180841833	0,8468534665	846,8534665	
71,31372	1,188562	0,841352828	841,352828	
70,7598	1,17933	0,8479390841	847,9390841	
70,64475	1,1774125	0,8493200132	849,3200132	

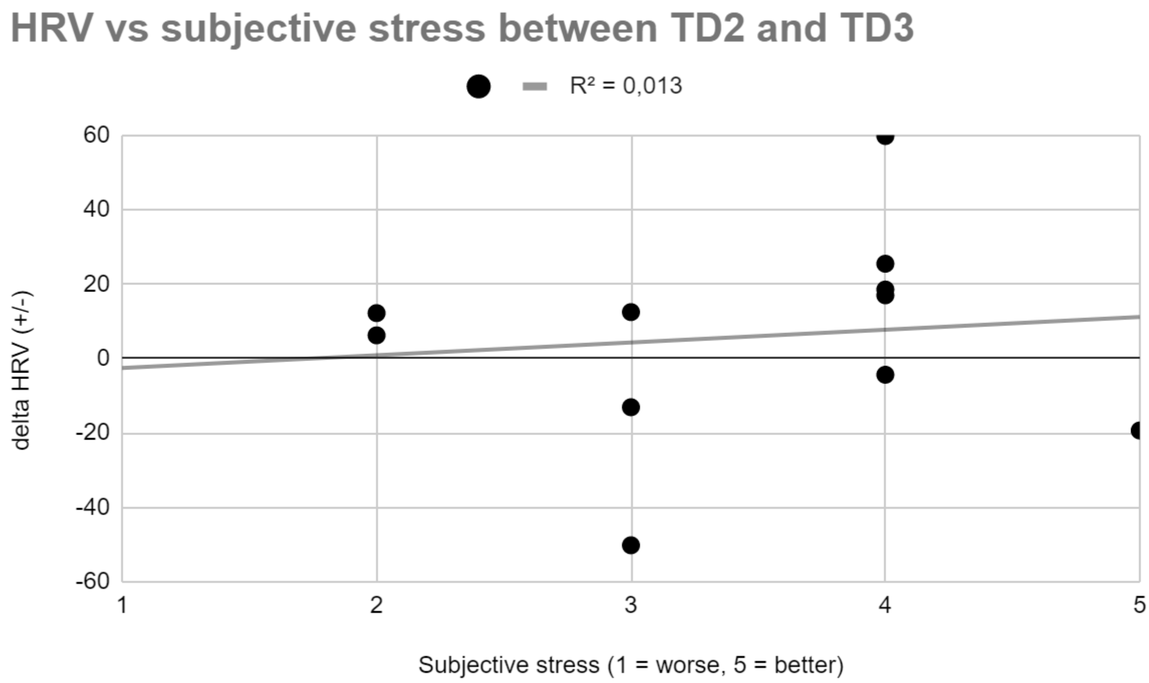
Example calculation of the HRV: In the image above the calculation that has been used to obtain the SDNN during a 60 second measurement. Every 4 seconds the average heart rate was obtained, resulting in 15 measurements for one condition (e.g. Baseline or Anticipation). Explained per column:
"Heart Rate every 4s (BPM)": Here, the average heart rate from a 4 second segment is shown
"BPS": The values from the previous column are divided by 60 to obtain the beats per second
"Seconds per heartbeat": The inverse from the BPS is taken to obtain the seconds per heartbeat
"Milliseconds per heartbeat": Dividing the previous column by 1000, obtaining the time in milliseconds between each heartbeat
"SDNN (ms)": Taking the standard deviation from the previous column results in the SDNN in milliseconds (ms), a measure of the HRV

Appendix 2



The individual heart rate variability (HRV) in the WHBM group during the Cycling experiment across the 3 test days. It is visible that there is high variation both between and within individuals, which is expected to be down to the interference of the Tunturi bike with the ECG signal. Upon critical evaluation of the individual heart rate data during Cycling, only WHBM#1 showed no irregular/inaccurate data across all test days.

Appendix 3



Correlational data between the participants' change in subjective stress and the change in their heart rate variability (HRV) during the Ice Shock between Test Day 2 (TD2) and Test Day 3 (TD3) (R^2 = 0.013). An increase in HRV from TD2 and TD3 is an indication of better stress-coping. The subjective stress is represented by a 5-point scale, with a reported score of 1 representing worse ice coping, 3 being equal and 5 being better coping. The graph shows a very slight positive correlation between the reported change in stress and the actual physiological response (HRV) of the participants.