Improved stress coping using the Wim Hof breathing method, a clinical trial

Author: Jorick Bakker
Student number: S4524349
Date: 5-6-2023
Research course: BSc Research Projects Behaviour and Neurosciences
Supervisors: Anton Scheurink, Cato Drion, Jocelien Olivier

Abstract

The Wim Hof method was assessed for its claim to be able to lower anxiety and stress. The Wim Hof method, created by the Dutch athlete Wim Hof, is a technique based on three pillars that claims to improve overall health. The three pillars are breathing, cold therapy and commitment. Here specifically the Wim Hof breathing method (WHBM) was examined. A group following the WHBM and a control group were tested over the duration of 2 weeks. In total 12 people participated. Stress was measured examining heart rate during an ice shock experiment. This ice shock experiment showed how well participants were able to deal with psychological and physical stress. It was expected that the WHBM group would improve in stress coping over the two weeks compared to the control group. However looking at the heart rate and heart rate variability, the WHBM group did not significantly increase their level of stress coping. This suggests that the WHBM on its own does not help with stress coping and might need to be combined with the other pillars to do so. However these findings are limited by the small sample size and future research is required.

Introduction

Mental health disorders are very present in our current society, with 1 in 8 people suffering from a mental disorder in 2019. Anxiety and depressive disorders are the most prevalent among these (WHO, 2022). These disorders are associated with high societal costs (Davis et al, 2002), and in general high levels of stress are associated with bad health. As such the Wim Hof method (WHM), which claims to reduce stress and anxiety among other health benefits (Hof, 2019), would have great societal benefit.

The WHM is a technique created by the dutch athlete Wim Hof, also known as the “iceman”, this because of his ability to withstand freezing temperatures. He has multiple world records under his name, such as running half a marathon above the arctic circle barefoot and being covered with ice for over 112 minutes (Hof, 2019). He claims these feats are possible due to the method he has created. This method is based on three pillars: breathing, cold exposure and commitment. Breathing refers to the Wim Hof breathing method (WHBM) which consists of 30-40 deep breaths, followed by a deep breath in and out into a breath hold, again followed by a recovery breath that is held for 15 seconds. Typically this is done for 3-4 rounds, building up the duration of the breath hold (Hof, 2019). Cold exposure speaks for itself, but commitment refers to body awareness, equivalent to mindfulness.

There is a degree of scientific backing of the WHM, it was shown that using the WHM some level of control could be enacted over the autonomic nervous system (Kox et al, 2014). And it is shown that following the WHM participants are able to voluntarily influence the psychological stress response (Van Middendorp et al, 2015). It is also shown that the Wim Hof method significantly reduced perceived stress (Kopplin & Rosenthal, 2022). However, despite this, research is still limited and incomplete. It is also not clear exactly how the WHM would induce these changes.

Looking at the breathing pillar, it is found that respiration and emotions are involved in a complex feedback loop, this suggesting that breathing exercises like the WHBM could be promising in lowering stress levels (Jerath et al, 2015). In fact it is shown that yogic breathing alone has lowered perceived stress in subjects (Kanchibhotla et al, 2021). The WHBM is similar to yogic breathing and they both appear to induce similar results (Kiecolt-Glaser et al, 2010). Yogic breathing and WHBM both result in the activation of the sympathetic nervous system (Zwaag et al, 2022). An important concept in understanding how this is beneficial for lowering stress levels, is the concept of hormesis. While high levels of stress are considered to be a marker for bad health, a moderate amount of stress is related to a positive effect on the immune system and adaptation (Mattson, 2008). These moderate levels of stress allow for adaptation and through this better coping with stress in the future (Le Bourg, 2020). Similar to the WHBM, the cold exposure also acts as a moderate stressor, given that the exposure is not too intense or long (Gálvez et al, 2018). However cold exposure is for most people more unpleasant than breathing exercises such as the WHBM. As such it is of interest to analyze the sole effect of the WHBM, without cold exposure, on stress levels and stress coping.

For this, unlike earlier studies such as Kopplin & Rosenthal, 2022 and Petraskova Touskova et al, 2022 which both looked at perceived stress, here physiological readouts are used to give more objective data. Looking at heart rate during stressful events can tell about the level of stress experienced. Stress causes the heart rate (HR) to increase and stabilize (Kim et al, 2018). Aside from being able to observe the extent of this increase in HR, the stabilization of the HR will cause a decrease in heart rate variability (HRV), which can be calculated using the HR. HRV is the deviation in length of heart beats between heart beat intervals (Kim et al, 2018). An increase in stress is associated with a decrease in HRV (Sloan et al, 1994). As such HRV can be used as an indicator of stress levels, though autonomic context should be considered (Kim et al, 2018).

Seeing as the WHBM should cause an adaptation, increasing stress coping (Le Bourg, 2020), HR and HRV should indicate lower stress following WHBM practice. To analyze this HR and HRV were measured here during an ice shock experiment. In this experiment from the polygraph manual of Noordhuis & Siertsema, 2021, where one hand is put into ice, the WHBM should lower the stress response. The ice shock experiment was used because putting a hand in ice should act as a physical stressor (pain) while the anticipatory period just before should act as a psychological stressor (expecting pain). However, comparing a group following the WHBM daily for two weeks with a control, they did show significant increases in coping over the two weeks. This is neither for the physical (hand in ice), nor the psychological (anticipatory period) stressors. This indicates that the WHBM on its own, at least, does not improve stress coping.

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 mV and Channel 41 showed the heart rate in 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. 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.

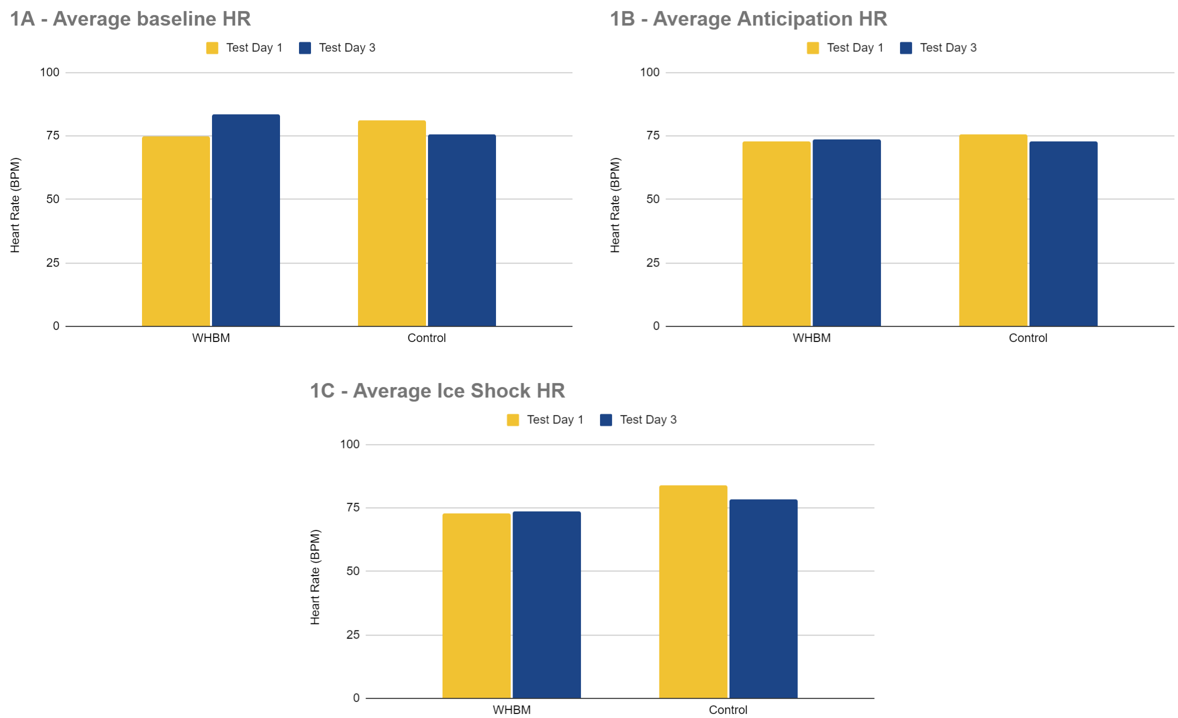


Figure 1 - Bar charts showing the heart rates (HR) in both experimental groups on Test Day 1 (TD1 or Day 0) and Test Day 3 (TD3 or Day 13/14/15). ***1A)*** *The change in average HR during Baseline (WHBM from 75.02 BPM on TD1 to 83.66 BPM on TD3, p>0,05; control from 81.18 BPM on TD1 to 75.61 BPM on TD3, p>0,05).* ***1B)*** *The change in average HR during Anticipation (WHBM from 72.94 BPM on TD1 to 73.50 BPM on TD3, p>0,05; control from 75.71 BPM on TD1 to 72.89 BPM on TD3, p>0,05).* ***1C)*** *The change in average HR during Ice shock (WHBM from 72.67 BPM on TD1 to 73.53 BPM on TD3, p>0,05; control from 83.92 BPM on TD1 to 78.56 BPM on TD3, p>0,05).*

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).

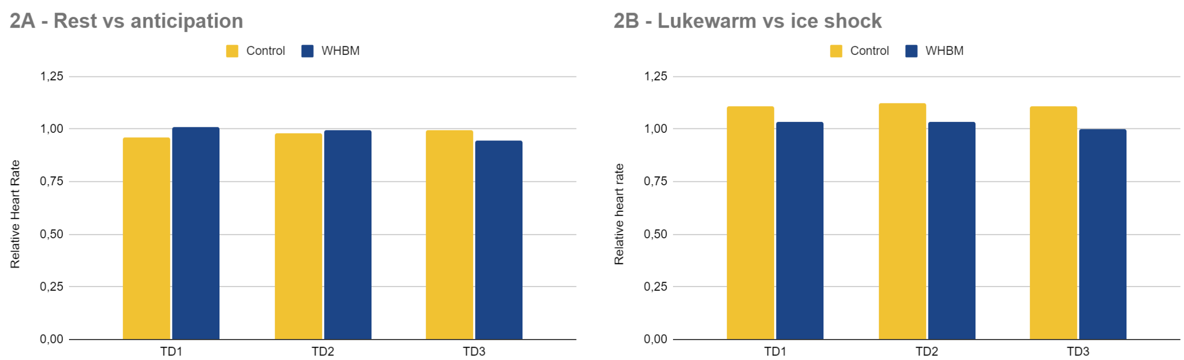


Figure 2 - Bar charts showing the relative heart rates (HR) in both experimental groups on Test Day 1 (TD1 or Day 0), Test Day 2 (TD2 or Day 6/7/8) and Test Day 3 (TD3 or Day 13/14/15). ***2A)*** *HR during Anticipation expressed as a decimal fraction of HR during Rest Ice (WHBM from 1.01 on TD1, 0.99 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 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 hypothesis that HR during Anticipation and Ice shock decreases over two weeks of following the WHBM. However to determine the significance of the relative decrease in HR shown in figures 2A and 2B, we looked at individual changes within both experimental groups and performed the Mann-Whitney test. This showed that the decrease in relative HR was not 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 sampled 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).

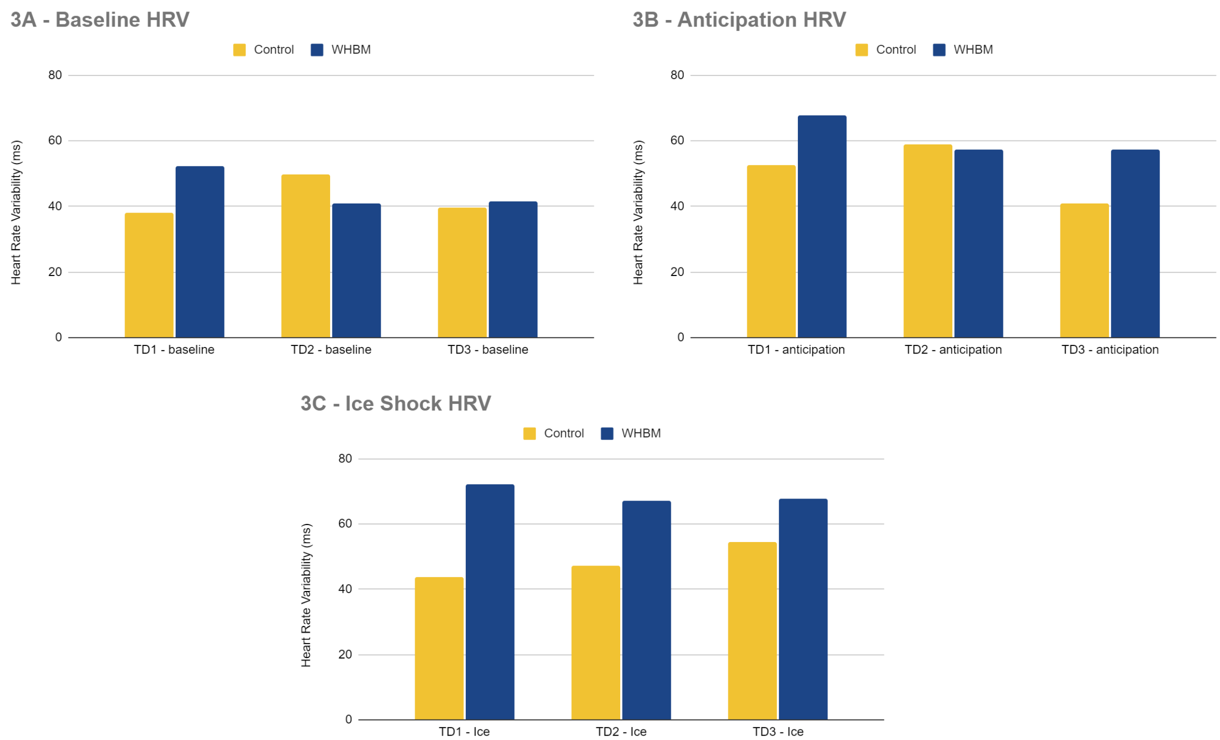


*Figure 3 - Bar charts showing the heart rate variability (HRV) in ms for both experimental groups on Test Day 1 (TD1 or Day 0), Test Day 2 (TD2 or Day 6/7/8) and Test Day 3 (TD3 or Day 13/14/15). **3A)** The average HRV during Baseline (WHBM 52.16 on TD1, 41.04 on TD2 to 41.49 on TD3; control from 38.07 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).*

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).

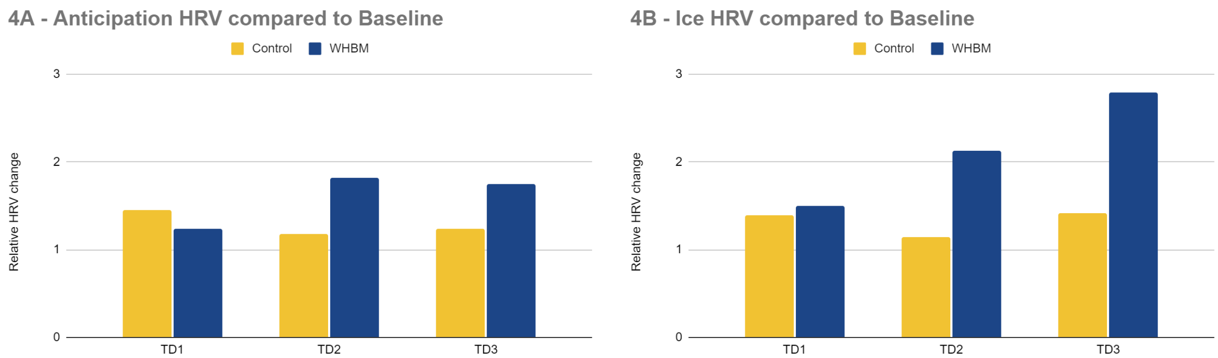
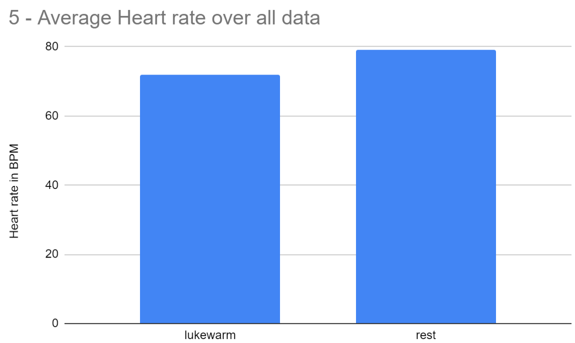


Figure 4 - Bar charts showing the relative heart rate variability (HRV) in both experimental groups on Test Day 1 (TD1 or Day 0), Test Day 2 (TD2 or Day 6/7/8) and Test Day 3 (TD3 or Day 13/14/15). ***4A)*** *HRV during Anticipation expressed as a decimal fraction of HRV during Baseline (WHBM from 1.24 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).*

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 test was performed. For this, the individual data was analyzed and showed that the increases were not statistically significant ($p>0,05$).

It is of note that as seen in figure 4, HRV was higher during ice shock and ice anticipation than during warm water and rest respectively. For the ice anticipation period this might be due to the effect of the psychological stress being relatively small, being overshadowed by a possible relaxation from the 2 minutes of lukewarm water between the rest and anticipation period. As shown by figure 5, the average HR during the lukewarm period was lower than during rest suggesting this possibly relaxed the participants.
To help understand why the HRV was so high during the ice shock it is important to look at the overall HR pattern during the Iceshock period. As shown by figure 6, the iceshock period on average started with a high HR which decreased over time. This causes a relatively high HRV due to this large drop in HR.



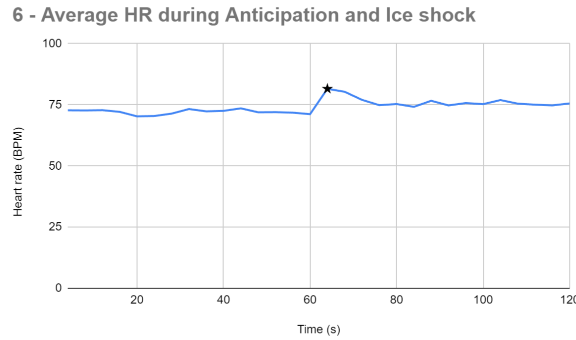


Figure 5 & 6, Fig. 5 - bar chart showing the average heart rate over all data of the lukewarm period and rest period in BPM. The Lukewarm period showed an average HR of 71,80 BPM while the rest period showed an average HR of 79,01 BPM. Fig. 6 - Line chart showing the average HR of all participants over the Ice anticipation + Iceshock period, with the star indicating the start of the Ice shock period.

Discussion

Heart rate

The WHBM is claimed to improve overall health and lower anxiety and depression (Hof, 2019). As such it was expected that after two weeks of following the WHBM, heart rate would decrease since this is associated with better health and lower levels of stress (Kim et al, 2018). However the WHBM group showed the opposite, an increase in HR over all periods (baseline, anticipation and ice shock) as shown in figure 1. This while the control group showed a decrease in HR over all periods. As such this indicates that the WHBM did not improve overall stress and stress coping. However this is based on an average HR which does not take into account individual and temporal differences. Since the experiments were not always conducted at the same time of day and with individuals of varying fitness levels and overall health, large variations in HR could be observed between individuals and between test days.

To compensate for this, relative HR on test days was calculated. This way the iceshock would show how this period affects the HR in comparison to a similar state, the lukewarm water, and give a more accurate measure of stress increase. The same principle is applied for the ice anticipation period, where it is compared to the period of rest at the beginning of the ice shock experiment. As shown in figure 2, for both the iceshock and the anticipation, relative HR goes down over the 2 week period for the WHBM group while the control group's relative HR increased for the anticipation period and remained the same for ice shock over the two week period. This suggests that the WHBM group did improve in stress coping. However, this improvement was not statistically significant. This might be due to the small sample size of n=6 for both the WHBM and control group, with a greater sample size possibly showing significant results in future research.

HRV

Heart rate is however not the most accurate measure of stress. HRV is more often used as a measure of stress, as an increase in stress is well associated with a decrease in HRV (kim et al, 2018). As such an increase in HRV was expected for the WHBM group over the 2 week period. However as seen in figure 3, HRV actually went down in all three periods (baseline, anticipation and iceshock). This suggests that stress levels actually went up over the two week period instead of going down for the WHBM group. However here again it is important to note that this is the average HRV and it does not compensate for individual and temporal variation.

To do this the relative HRV was calculated, in the same way as it was calculated for the HR. As seen in figure 4, the WHBM group showed an increase in relative HRV in both the ice shock and the ice anticipation period. This would suggest that the WHBM is effective in improving stress coping. However, again this data was not statistically significant. Again this may be due to the small sample size, with a larger sample size possibly being able to show statistically significant data.

It is also of note that the HRV data was not completely in line with the expectations based on known literature. As seen in figure 4, HRV was higher during ice shock and ice anticipation than during warm water and rest respectively. These periods should be more stressful and as such have a lower HRV. For the ice anticipation period this might be due to the effect of the psychological stress being relatively small, possibly being overshadowed due to relaxation during the 2 minutes of lukewarm water between the rest and anticipation period. As shown by figure 5, the average HR during the lukewarm period was lower than during rest suggesting participants got into a more relaxed state during this period. For future research a better psychological stressor should be used. A VR horror experiment could be a possible solution, though the induced stress from this might vary greatly between participants.

To help understand why the HRV was so high during the ice shock it is important to look at the overall HR pattern during the Iceshock period. As shown by figure 6, the iceshock period on average started with a high HR which decreased over time. This could be due to relaxation and a hypothermic response to the cold of the ice. This sharp decrease in HR is likely why the HRV is relatively high. However this decrease should be more pronounced in individuals who come to relax earlier, as such HRV should still make a good indicator of reduced stress. For future research, a positive control for HRV during ice shock could clarify the relationship between HRV and stress levels during the ice shock period.

Lastly, the HRV was calculated using the method seen in appendix 1. This was because the equipment used (Biopac lesson 9) could only be readout in BPM. This method is less accurate and it would be better for future research to use an ECG readout for calculating the HRV.

In all, the findings showed no statistically significant difference between the WHBM group and the control group. This would suggest that the WHBM does not increase coping with stress. However due to the small sample size (n=6 for both WHBM and control) only very strong correlations could show statistically significant data. When looking at the specifics, relative HR and relative HRV did suggest improvements following the WHBM though not significant. It is important to note that the WHBM is, however, only one of three pillars of the WHM and in the paper by Kopplin & Rosenthal, 2022, though they also did not show improvements in perceived stress following the WHBM alone, they did when combined with the ice therapy. In conclusion more research into the WHM is needed to prove its validity and the interplay between the WHBM and ice therapy is an interesting subject for future study.

References

Davis, K. L., & Neuropsychopharmacology, A. C. of. (2002). Neuropsychopharmacology: The Fifth Generation of Progress : an Official Publication of the American College of Neuropsychopharmacology. In *Google Books*. Lippincott Williams & Wilkins. https://books.google.nl/books?hl=nl&lr=&id=BKwkonZwZD0C&oi=fnd&pg=PA9&ots=ccLS_2XwAP&sig=BWFwFR4PJIXBPxTvpYOTfsKkOtY&redir_esc=y#v=onepage&q&f=false

Gálvez, I., Torres-Piles, S., & Ortega-Rincón, E. (2018). Balneotherapy, Immune System, and Stress Response: A Hormetic Strategy? *International Journal of Molecular Sciences*, *19*(6). https://doi.org/10.3390/ijms19061687

Hof, W. (2019). *Welcome to the Official Wim Hof Method Website*. Wimhofmethod.com. https://www.wimhofmethod.com/

Jerath, R., Crawford, M. W., Barnes, V. A., & Harden, K. (2015). Self-Regulation of Breathing as a Primary Treatment for Anxiety. *Applied Psychophysiology and Biofeedback*, *40*(2), 107–115. https://doi.org/10.1007/s10484-015-9279-8

Kanchibhotla, D., Subramanian, S., & Kaushik, B. (2021). Association of yogic breathing with perceived stress and conception of strengths and difficulties in teenagers. *Clinical Child Psychology and Psychiatry*, *26*(2), 406–417. https://doi.org/10.1177/1359104521994633

Kiecolt-Glaser, J. K., Christian, L., Preston, H., Houts, C. R., Malarkey, W. B., Emery, C. F., & Glaser, R. (2010). Stress, Inflammation, and Yoga Practice.

Psychosomatic Medicine, *72*(2), 113–121. https://doi.org/10.1097/psy.0b013e3181cb9377

Kim, H.-G., Cheon, E.-J., Bai, D.-S., Lee, Y. H., & Koo, B.-H. (2018). Stress and Heart Rate Variability: A Meta-Analysis and Review of the Literature. *Psychiatry Investigation*, *15*(3), 235–245. https://doi.org/10.30773/pi.2017.08.17

Kopplin, C. S., & Rosenthal, L. (2022). The positive effects of combined breathing techniques and cold exposure on perceived stress: a randomised trial. *Current Psychology*. https://doi.org/10.1007/s12144-022-03739-y

Kox, M., van Eijk, L. T., Zwaag, J., van den Wildenberg, J., Sweep, F. C. G. J., van der Hoeven, J. G., & Pickkers, P. (2014). Voluntary activation of the sympathetic nervous system and attenuation of the innate immune response in humans. *Proceedings of the National Academy of Sciences*, *111*(20), 7379–7384. https://doi.org/10.1073/pnas.1322174111

Le Bourg, É. (2020). Characterisation of the positive effects of mild stress on ageing and resistance to stress. *Biogerontology*. https://doi.org/10.1007/s10522-020-09870-2

Mattson, M. P. (2008). Hormesis defined. *Ageing Research Reviews*, *7*(1), 1–7. https://doi.org/10.1016/j.arr.2007.08.007

Noordhuis, R. J., & Siertsema, M. (2021). *Polygraph Practical - Instructor's Manual*. Rijksuniversiteit Groningen.

Petraskova Touskova, T., Bob, P., Bares, Z., Vanickova, Z., Nyvlt, D., & Raboch, J. (2022). A novel Wim Hof psychophysiological training program to reduce stress responses during an Antarctic expedition. *Journal of International Medical Research*, *50*(4), 030006052210898. https://doi.org/10.1177/03000605221089883

Sloan, R. P., Shapiro, P. A., Bagiella, E., Boni, S. M., Paik, M., Bigger, J. T., Steinman, R. C., & Gorman, J. M. (1994). Effect of mental stress throughout the day on cardiac autonomic control. *Biological Psychology*, *37*(2), 89–99. https://doi.org/10.1016/0301-0511(94)90024-8

van Middendorp, H., Kox, M., Pickkers, P., & Evers, A. W. M. (2015). The role of outcome expectancies for a training program consisting of meditation, breathing exercises, and cold exposure on the response to endotoxin administration: a proof-of-principle study. *Clinical Rheumatology*, *35*(4), 1081–1085. https://doi.org/10.1007/s10067-015-3009-8

World Health Organization. (2022, June 8). *Mental disorders*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/mental-disorders

Zwaag, J., Naaktgeboren, R., van Herwaarden, A. E., Pickkers, P., & Kox, M. (2022). The Effects of Cold Exposure Training and a Breathing Exercise on the Inflammatory Response in Humans: A Pilot Study. *Psychosomatic Medicine*, *84*(4), 457–467. https://doi.org/10.1097/psy.0000000000001065

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