Complementary Therapies in Medicine 61 (2021) 102771

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

# Effects of yoga and mindfulness practices on the autonomous nervous system in primary school children: A non-randomised controlled study

Pune Ivaki [a,*], Steffen Schulz [a], Michael Jeitler [d,e], Christian S. Kessler [d,e], Andreas Michalsen [d,e], Farid I. Kandil [a,d], Saskia-Marie Nitzschke [d], Wiebke Stritter [a], Andreas Voss [b,1], Georg Seifert [a,c,*,1]

[a] *Department of Paediatrics, Division of Oncology and Haematology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany*
[b] *Institute of Innovative Health Technologies IGHT, Ernst-Abbe-Hochschule Jena, Jena, Germany*
[c] *Universidade de São Paulo, Faculdade de Medicina, Departamento de Pediatria, Instituto de Tratamento do Câncer Infantil (ITACI), São Paulo, São Paulo, Brazil*
[d] *Institute of Social Medicine, Epidemiology and Health Economics, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany*
[e] *Department of Internal and Integrative Medicine, Immanuel Hospital, Königstraße 63, 14109 Berlin, Germany*

ARTICLE INFO

*Keywords:*
Distress
Stress reduction
Children
School
Heart rate variability
Yoga

ABSTRACT

*Objectives:* The present study examined the effects of a yoga and mindfulness-based programme on the autonomic nervous system of primary school children by using heart rate variability parameters.
*Design:* A two-arm non-randomised controlled trial compared an integrated yoga and mindfulness-based programme (16 weeks) to conventional primary school lessons.
*Setting:* Primary school classrooms and conference rooms.
*Interventions:* Participants were allocated to a 16-week integrated yoga-based programme or conventional school lessons. A subgroup was randomised to receive 24h electrocardiogram-recordings.
*Main outcome measures:* Heart rate variability indices were measured, both linear (time and frequency domain) and non-linear (symbolic dynamics, compression entropy), calculated from 30-minute extracts of Holter-electrocardiogram-recordings. Assessments were conducted at baseline and at the end of intervention.
*Results:* 40 participants (42.5% female) were included into the analysis of HRV. No significant changes in heart rate variability parameters were observed between the groups after 16 weeks. In the intervention group, a trend towards increased parasympathetic activity could be seen over time, although not significantly enhanced compared to the control group.
*Conclusion:* Results obtained here do not clearly show that children in German primary school settings benefit from an integrated yoga-based intervention. However, exploratory post-hoc analyses point interestingly to an increased nocturnal parasympathetic activity in the intervention group. Further studies are required with high-quality study designs, larger sample sizes and longer-term follow-ups.

## 1. Introduction

Chronic stress is a growing public health concern in a globalised world and achievement-oriented societies. Schoolchildren are increasingly affected by this issue, leading to stress-related mental health problems (e.g., anxiety, depression) and various somatic complaints (e.g., headache, abdominal pain, false postures).[1] To prevent children from stress-associated somatic and mental diseases, this important topic needs greater recognition.[2] Given the amount of time spent in an educational setting, schools may play an important role in the recognition of this widespread problem. Growing evidence demonstrates that mental and physical health are fundamental requirements for academic and cognitive achievements.[3] Chronic stress leads to poorer school performances.[4] Schools should aim for a holistic approach to education, including cognitive, social-emotional and moral skills.[5] Integrating mind-body methods (MBM) - such as yoga and mindfulness - into

* Corresponding author at: Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany.
*E-mail addresses:* pune.ivaki@charite.de, pun_iva@posteo.de (P. Ivaki), georg.seifert@charite.de (G. Seifert).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ctim.2021.102771
Received 30 September 2020; Received in revised form 31 May 2021; Accepted 23 August 2021
Available online 24 August 2021

educational institutions may contribute to this issue and could potentially increase the physical and mental well-being.[6]

In Western medicine, yoga and mindfulness practices are categorised as MBM. Mindfulness practices can be performed on their own or they can be part of another MBM intervention (e.g., yoga, tai chi). In a recent meta-analysis by Carsley et al., specific effects of mindfulness interventions depend on programme characteristics and have the greatest effect when they are combined.[7] Combining physical and meditative yoga exercises may help individuals to reach a quiet mind and with it a peaceful state.[8] Thus, regular yoga practice has the potential to improve mindfulness and with it physical and mental health.[9,10] A quiet mind serves as a healthy foundation for a well-balanced reaction to stress. In a review on yoga in children, a reduction of stress-related symptoms such as headache, abdominal pain, anxiety and depression could be identified.[6] Schoolwork may be improved by promoting self-regulation, concentration and social behaviour. Yoga supports social-emotional learning and may assist as a coping strategy for stressors. In addition, yoga works on a physical level through static and dynamic postures. The physicality of yoga is crucial to yoga science for the verifiable antidepressant and anxiolytic effects of physical exercise it provides.[11]

Currently, various challenges in assessing stress remain.[12] Numerous studies have been carried out using mainly psychometric data and biological markers (e.g., cortisol, amylase). The role of heart rate variability (HRV) as a parameter of stress has gained traction recently. For instance, HRV has proven useful in predicting common mental (e.g., stress, depression, anxiety) and physical disorders (e.g., inflammation, chronic pain, diabetes, concussion, asthma, insomnia, fatigue), which increase sympathetic activity and create a self-sustaining cycle to produce autonomic imbalance and greater allostatic stress.[13]

Yet, most studies have only been applied to adults. HRV has seldom been used for the evaluation of yoga interventions in paediatrics, especially in schools,[14] and data remains sparse. HRV is an objective, non-invasive tool that is applied to describe the influence of the autonomic nervous system (ANS) on heart activity. HRV is found to be a recognised, valuable parameter for psychological stress and health in adults.[15] However, Kim et al. state that further assessment of HRV as a stress indicator is still needed to fully explore the potential of its future clinical use.[15]

The dually innervated heart is mainly under the control of the ANS and its two branches, the sympathetic (SNS) and the parasympathetic nervous system (PNS). The SNS is primarily associated with energy mobilisation while the PNS is associated with vegetative and restorative functions, maintaining homeostasis and physiological stability.[16] Except for few conditions during which both branches may be activated or inhibited (e.g., sexual arousal, anaesthesia), the SNS and PNS have antagonistic effects on organ function.[17] Although often described as antagonistic, the activity of these branches normally maintains a dynamic balance. However, their activity can rapidly change and adapt to environmental demands, both physical and mental.[16] In the presence of chronic physical or psychological stress, PNS activity typically decreases. Chronic stress can additionally lead to an increase of SNS activity. These changes of ANS regulation are considered an indicator for reduced autonomic health and may be a risk factor for various diseases as well as an indicator for chronic stress.[16,18]

One way to identify adaptive abilities and resulting stability of the ANS is to measure the variability and complexity of heart rate fluctuations. More complex heart rate fluctuations indicate a better adaptability to endogenous and exogenous stressors. High HRV measures are associated with the ANS' ability to regulate and adapt and are seen as a health preserving factor.[19] Standard parameters of HRV, both linear (time and frequency domain) and non-linear (symbolic dynamics, compression entropy) contain information related to the functioning of the ANS.[20] According to the parameter, parasympathetic and/ or sympathetic modulations can be derived. For instance, rmssd, pNN50, HF (and sdNN) are related to parasympathetic activity whereas LF/HF is related to both. In different studies, it could be shown that HRV measures (linear, non-linear) changed in response to stress. The most frequently reported factor associated with alterations in HRV measures was low parasympathetic activity, which is characterised by a decrease in the HF band and an increase in the LF band.[15] Moreover, findings of Luque-Casado et al. indicate that HRV is very sensitive to the overall demands of sustained attention over and above the influence of other cognitive processes (HRV varied as a function of task demands, HRV significantly decreases as a function of time on task).[21]

Several physiological factors (e.g., age, gender, circadian rhythm, respiration and body position) may influence HRV.[22] However, it has been shown that influencing effects on HRV measures, such as gender, seem to differentiate from an age of 30 onwards. This said, significant differences in HRV according to gender disappeared from an age of 55 onwards and age dependencies disappeared from an age of 65 onwards.[23–25] In this study, we investigated very young and healthy participants from age 8–12. Age and gender dependencies can probably be excluded due to the very young age and the small variability of age and gender within the group. Thus, we decided to treat all participants as one group.

Little data exists for paediatrics given the necessary raw data required to calculate the variability and complexity of HRV measures obtained from short-term recordings (30 min). In this study, we aim to close this gap by using well established linear and nonlinear HRV measures that have been shown to provide reliable results in patients with various cardiovascular diseases even with short recording times.[25–29]

This study investigated a well-established, yoga-based programme (YoBEKA, an acronym for the German terms Yoga, Bewegung, Entspannung, Konzentration and Achtsamkeit - yoga, movement, relaxation, concentration and mindfulness). YoBEKA is an educational concept created by German pedagogues and yoga teachers to introduce and establish MBM in schools. This study assessed stress in children using HRV in addition to psychometric and qualitative data. This paper reports on the HRV indices that were applied to evaluate the effects of YoBEKA on the ANS in children. Psychometric and qualitative data, the primary outcome measures for the investigation of stress and stress management, will be presented in a separate article. This article examined linear and nonlinear analytical methods to investigate potential changes in the regulation of the ANS and the physiology of stress management.

## 2. Methods

### 2.1. Study design

This trial is a two-arm non-randomised controlled study (NCT) with a control group (CON) given the usual treatment: conventional primary school lessons. The study protocol was approved by the ethics committee of the Charité - Universitätsmedizin Berlin and was carried out in accordance with the Declaration of Helsinki.[30] The study was registered at ClinicalTrials.gov on 2nd January 2019 (Clinical trial registration number: NCT03796754). All participating children's guardians gave written informed consent. Three primary schools were recruited in spring 2018 via invitation by mail and approved to take part. School administration allocated school classes either to the YoBEKA ($k = 11$) or control group ($k = 8$). This step was undertaken by school administration due to their dependency on teachers' initiative and willingness to participate. Thus, the study team was not able to randomise. The primary outcome of the present study assessed stress in children by means of a questionnaire (SSKJ 3-8 R). Secondary outcomes measured quality of life (Kidscreen-27) and somatoform disorders (SOMS-E). Moreover, qualitive focus group interviews were conducted. The psychometric and qualitative data will be published elsewhere. A subgroup of 54 children was asked and consented to participate in 24-h ECG monitoring. This subgroup was randomly selected from the participating classes for electrocardiogram-recordings (ECG-recordings) and subsequent HRV analysis. Data on primary and secondary outcomes including psychometric questionnaires, qualitative interviews and ECG-recordings were assessed at baseline (January 2019), post intervention (June 2019) and at 12 months follow-up (January 2020) (Fig. 2).

### 2.2. Sampling

Participants were included in the study if they fulfilled both eligibility criteria: they were primary school students and provided informed consent from their parents. Participants were excluded if they 1) suffered from a severe acute or chronic disease, 2) had an immobility or serious restriction for gymnastic exercises due to orthopaedic, neurological or other medical reasons or 3) were simultaneously participating in any other trial. Each participant received a unique anonymous identification number. A total of 453 children between the ages of 8 and 12 (3rd-5th grade) was screened for eligibility. Of these, 262 children were included after written informed consent from their parents was obtained. From the randomised ECG subgroup (n = 54), 40 were included in the HRV analysis (Fig. 1).

### 2.3. Interventions

#### 2.3.1. YoBEKA group

The YoBEKA concept was especially created for educational institutions (www.yobee-active.de). YoBEKA claims to be ideologically neutral, secularised intervention and all exercises are simplified and age appropriate. I.e., traditional mantras and philosophical yoga theories are left out of the programme.

YoBEKA combines yoga postures, mindfulness, positive affirmations, songs, movements (e.g., body tapping, mindful lifting and moving of chairs, rubbing palms together), rest and silence exercises (e.g., minute of stillness). All exercises are easy to perform and appropriate to school settings. Over 16 weeks during the intervention period, YoBEKA was established in the participating schools by qualified trainers (at least 10 years of experience) of the YoBEKA institute (Fig. 2). Class teachers continued to independently perform exercises at least 5 min per day during and after the implementation of the programme to achieve long-term integration. The participating teachers received an initial two-hour introductory course. Then, teachers participated in a YoBEKA training course for 1–2 h every two weeks. During the intervention time period, teachers and children of YoBEKA classes were guided in YoBEKA practice once a week for 45 min by a YoBEKA trainer.

YoBEKA exercises offer high variability within the programme. Short exercises (2–3 min) can be used during lessons by teachers, e.g., to calm turmoil and re-establish lesson attentiveness. These were intended to raise concentration levels and were designed to be used by teachers in the long term within the classroom. Long sessions with various exercises (45–90 min) were undertaken when YoBEKA trainers attended school lessons. These long sessions helped to train and acclimate class teachers and children to the exercises (Fig. 3).

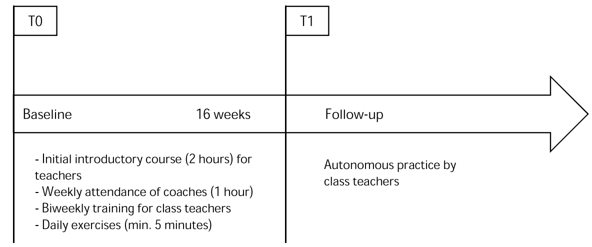


**Fig. 2.** Study design. Timelines: T0 – start of the study and first collection of data, T1 – post-intervention and second collection of data.
Data collection at each point includes psychometric data and ECG-recordings.

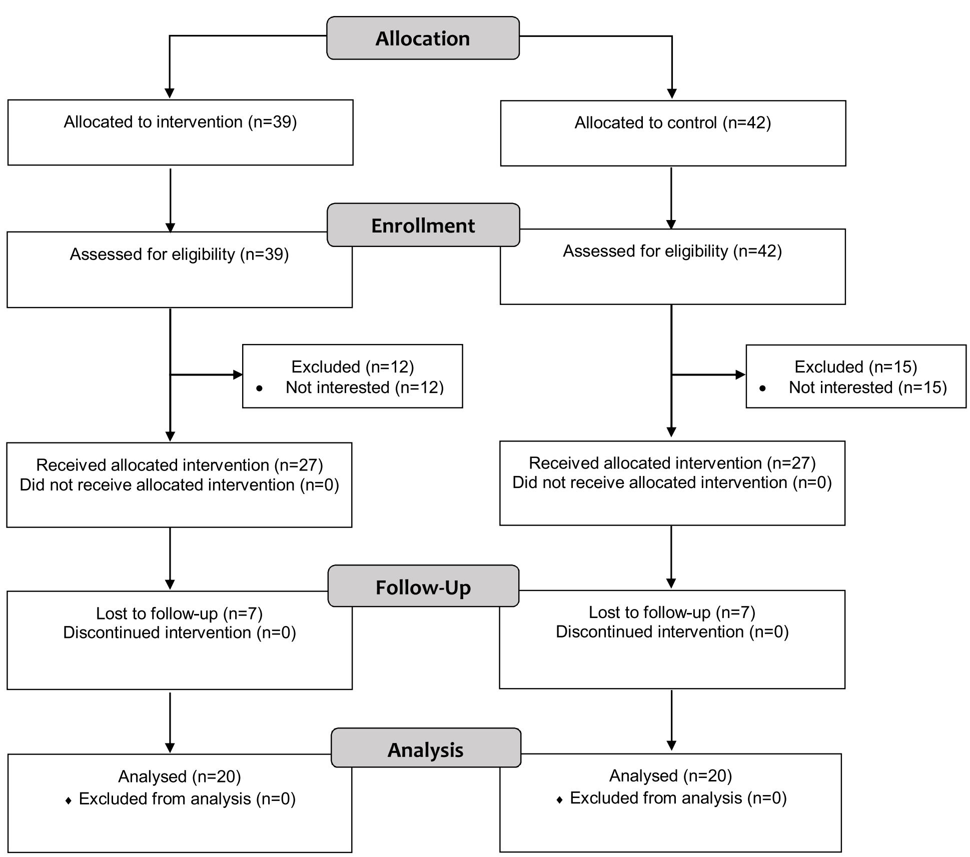


**Fig. 1.** Flow Chart.

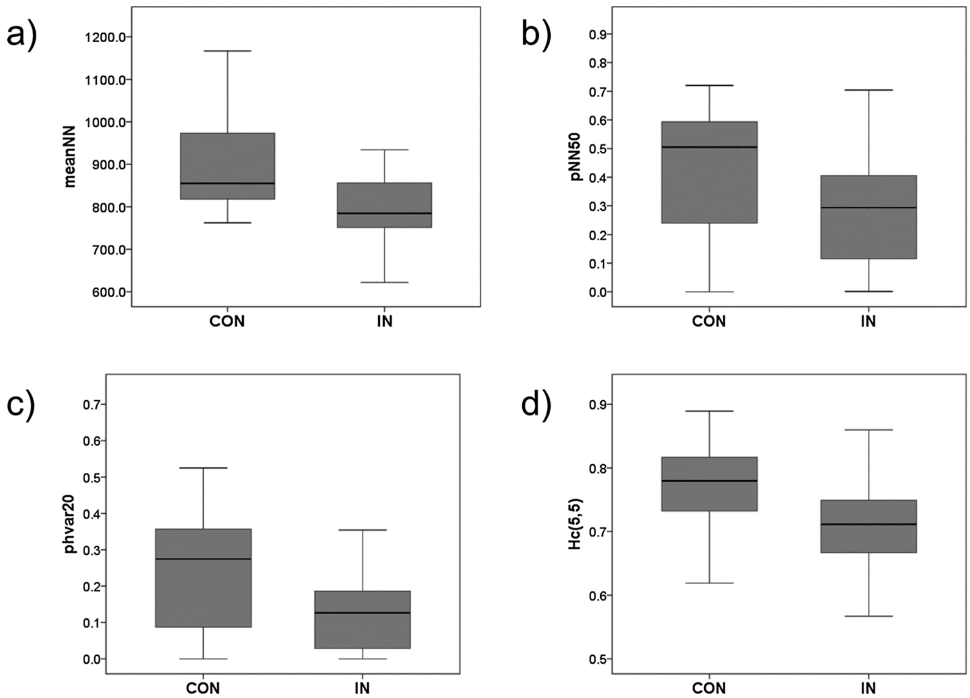


**Fig. 3.** Box plots of HRV indices for the control group (CON) and the intervention group (IN) at time points (T0) for night-time: a) *meanNN* as the mean value of the NN-intervals; b) *pNN50* as the proportion derived by dividing the number of interval differences of successive NN-intervals greater than 50 ms by the total number of NN-intervals; c) *phvar20* as a nonlinear symbolic dynamics index describing sequences containing six consecutive '1' symbols indicate reduced high variability, and d) $H_{C(5,5)}$ as the compression entropy describing the complexity of the NN-intervals using buffer size $b = 5$ and window length $w = 5$.

#### 2.3.2. Control group

In the CON, children were taught by their regular teachers in accordance with the standard curriculum. Following the study, CON received the same YoBEKA training.

### 2.4. Data collection

Data was collected in two rounds. The first collection of data was surveyed at baseline (T0), prior to intervention (January 2019), and the second data collection took place after the intervention (T1) had been completed (June 2019). Portable ECG devices (Faros 180, sampling rate: 500 Hz) were used for the 24 -h-recordings. Of these 54 recordings, a subgroup of 40 (42.5 % female) was used for HRV analysis: 20 controls (CON), 40 % female and 20 intervention (IN), 45 % female. The remaining 14 recordings could not be analysed due to poor quality or recording disturbance. Due to a high number of dropouts in the ECG-subgroup (change of school, illness, unwillingness to participate) the third data collection T2 at 12 months follow-up could not be included in the analysis.

Psychometric questionnaires concerning stress as primary outcome and quality of life were filled out by all children, parents and some teachers. Parents' questionnaires concerned quality of life and somatoform disorders while teachers of participating classes were asked to fill out questionnaires about stress, anxiety and their perception of YoBEKA.

### 2.5. Data analysis

#### 2.5.1. Data recording and data pre-processing

Time series of 4 h length for day (D) and 4 h for night-time (N) were visually scanned and extracted from the ECG raw data, using the "EDF browser" (European Data Format browser). The EDF browser was used to scan ECG's visually and to pre-select 4 h segments, ideally with a minimum of artefacts (i.e., caused by movement, detachment of electrodes etc.). Afterwards, from these 4 h data recordings, heart rate time series consisting of successive beat-to-beat intervals (BBI) were extracted automatically (programming environment MATLAB® R2011b). For the HRV analyses, adequate simultaneous ECG signals with sufficient signal quality and without artefacts were necessary for further processing. From the 4 h BBI segments, segments of 30 min length were selected that fulfilled the criterion of stationarity (HRV analyses in the frequency domain analysis need stationary conditions).

Stationarity requires that statistical properties such as mean and standard deviation of the investigated time series remain the same throughout the investigated epoch. Stationarity does not exclude variability; however, it sets up boundaries (limitations) for variability analyses such that variability does not change with time or duration of measurement. If the stationarity needs are not given, as is the case with most complex physiological signals (not random), then an impact of trends on the change of the mean of the time series has to be considered in the interpretation of the results.[25,31] By using stationary time segments, a possibility of introducing bias on our final analysis should be excluded and minimised. Therefore, we applied a test where the extracted 30 min time segments fulfilled our pre-selection criteria of stationarity in all participants. The exact description of the algorithm is described elsewhere.[32]

Thereafter, all-time series were visually inspected and edited if necessary. Afterwards, all-time series were filtered by applying an adaptive variance estimation algorithm to remove and interpolate ventricular premature beats and artefacts in order to ensure normal-to-normal beat time series (NN).[33] Subsequently, the dynamic behaviour at baseline (T0) and after follow-up (T1) of the intervention was analysed to quantify any changes in autonomic regulation.

#### 2.5.2. Analysing autonomic regulation by heart rate variability in the time and frequency domains

Heart rate variability (HRV) was quantified by well-established indices of time and frequency domains and nonlinear dynamics to assess autonomic modulation (sympathetic, vagal).[25,34,35]

In the time domain (TD), the following standard indices were calculated:

*meanNN:*The mean value of the NN-intervals of BBI [ms];

*sdNN:*Standard deviation of the NN-intervals of BBI [ms];

*rmssd:*Square root of the mean squared differences of successive NN intervals [ms];

*pNN50:*Proportion derived by dividing the number of interval differences of successive NN intervals greater than 50 ms by the total number of NN intervals [%];

*renyi4:* Rényi entropy as a complexity measure of the BBI estimated from $\alpha$-weighted probabilities distributions ($\alpha = 4$) [bit].

In the frequency domain (FD), the following standard indices were calculated:

*LF/HF:* The ratio between the low (*LF*: 0.04–0.15 Hz) and high (*HF*: 0.15–0.4 Hz) frequency power of the estimated spectrum [a.u.];

*LFn:* Normalised low-frequency power (0.04–0.15 Hz) of BBI [a.u.];

*HFn:* Normalised high-frequency power (0.15–0.4 Hz) of BBI [a.u.].

The power spectra of equidistant linear interpolated (10 Hz) NN interval time series (resampled to 2 Hz) were obtained by applying the Fast Fourier Transformation. To avoid leakage effects a Blackman Harris window function was applied.

### 2.5.3. Analysing autonomic regulation by heart rate variability with nonlinear dynamics

*2.5.3.1. Symbolic dynamics.* The analysis of symbolic dynamics has been proven to be sufficient for the investigation of complex systems and describes nonlinear aspects within time series (BBI).[36] Therefore, BBI were transformed into a symbol sequence of four symbols with a given alphabet.

We used an alphabet consisting only of symbols ‘0’ or ‘1’ and calculated the number of words with 6 successive equal symbols to detect epochs of low and high variability.[37] Here, the symbol ‘1’ represents those cases where the difference between two successive NN-intervals exceeds this special limit (20 ms – *phvar*). In this way, words with the symbol sequence ‘111111’ characterise high variability epochs. The following indices from this probability distribution were estimated:

*plvar20:* Sequences containing six consecutive ‘0’ indicate reduced low variability, and

*phvar20:* Sequences containing six consecutive ‘1’ indicate reduced high variability.

### 2.5.4. Compression entropy

Methods based on entropies have the common feature that they analyse a putative information transfer between time series and either address the uncertainty or predictability of time series. Complexity analysis can be performed by evaluating the entropy and entropy rate. Entropy (e.g., Shannon) calculates the degree of complexity of a signal’s sample distribution. The largest entropy is obtained when the distribution is flat (i.e., the samples are identically distributed). On the contrary, if some values are more alike (e.g., the sample distribution is Gaussian), the entropy decreases.[38,39]

In 1977, Ziv and Lempel introduced a universal algorithm for lossless data compression (LZ77) using string-matching on a sliding window (i.e. GIF image, WinZip®).[40] The compression entropy ($H_{C(b,w)}$) algorithm was later on introduced in the field of cardiology and quantifies the complexity of a time series.[29] $H_C$ indicates in which extent time series can be compressed using the detection of repetitive sequences. Here, we analysed the NN-intervals from BBI using buffer size $b = 5$ and window length $w = 5$ ($H_{C(5,5)}$).

### 2.5.5. Statistics

A multivariate analysis (MANOVA) with pair-wise comparisons as post-hoc analyses was conducted for all HRV indices together to investigate the global effects of the factors GROUP, TIME and the interaction TIME*GROUP. Main analyses and all exploratory post-hoc tests were made using SPSS (v 21.0) and results are presented as mean ± standard deviation. A Bonferroni-corrected alpha* = 0.05 / 6 = 0.00833 was used for testing the six primary endpoints, i.e., the two main factors and the one interaction of each of the MANOVAs for day and night-time data (Tables 1 and 2).

**Table 2**
The results of the post-hoc analyses between the participants (CON vs. IN for T0 & T1) and within the participants (T0→T1 for CON & IN) for heart rate variability indices expressed as *F* (partial $\eta^2$).

| Domain | Index | day-time | | night-time | |
|---|---|---|---|---|---|
| | | CON vs. IN (T0 & T1) | T0→T1 (CON & IN) | CON vs. IN (T0 & T1) | T0→T1 (CON & IN) |
| **TD** | *meanNN* | 1.1E-04 (2.8E-06) | 2.480 (0.061) | 3.318 (0.080) | 2.582 (0.064) |
| | *sdNN* | 0.902 (0.023) | 3.375 (0.082) | 2.147 (0.053) | 0.247 (0.006) |
| | *rmssd* | 0.131 (0.003) | 5.010 (0.116) | 2.020 (0.050) | 0.001 (1.4E-05) |
| | *pNN50* | 0.079 (0.002) | 3.754 (0.09) | 2.542 (0.063) | 0.184 (0.005) |
| | *renyi4* | 1.980 (0.050) | 3.472 (0.084) | 2.837 (0.069) | 0.173 (0.005) |
| **FD** | *LF/HF* | 0.395 (0.010) | 1.716 (0.043) | 0.091 (0.002) | 0.772 (0.020) |
| | *LFn* | 0.215 (0.006) | 1.251 (0.032) | 0.177 (0.005) | 0.224 (0.006) |
| | *HFn* | 0.215 (0.006) | 1.251 (0.032) | 0.177 (0.005) | 0.224 (0.006) |
| **SD** | *phvar20* | 0.135 (0.004) | 1.461 (0.037) | 2.980 (0.073) | 0.080 (0.002) |
| **$H_C$** | *$H_{C(5,5)}$* | 0.001 (3.8E-05) | 12.41 (0.246) | 3.023 (0.074) | 0.012 (3.1E-04) |

*F*: indicates that the test procedure used an F statistic, which is based on an F distribution; $\eta^2$: the partial eta squared, a measure of effect size and variance explanations. Higher values indicate that the observed group differences are due to the independent variable, $\eta^2$ = 0.01, 0.06, 0.14 indicate small, medium and large effects.

**Table 1**
Descriptive statistics of heart rate variability indices at the time points (T0, T1) for the control group (CON) and the intervention group (IN) for daytime and night-time represented as mean ± standard deviation.

| Domain | Index | *daytime* | | | | *night-time* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CON_T0 | CON_T1 | IN_T0 | IN_T1 | CON_T0 | CON_T1 | IN_T0 | IN_T1 |
| **TD** | *meanNN* | 615.91 ± 46.31 | 611.84 ± 98.82 | 633.91 ± 98.17 | 593.38 ± 78.69 | 910.67 ± 152.64 | 913.35 ± 154.33 | 813.54 ± 121.61 | 865.37 ± 114.25 |
| | *sdNN* | 58.75 ± 14.75 | 53.95 ± 17.67 | 64.11 ± 19.17 | 57.11 ± 17.93 | 71.17 ± 34.33 | 65.41 ± 35.11 | 55.13 ± 23.71 | 56.71 ± 24.14 |
| | *rmssd* | 34.26 ± 12.27 | 29.60 ± 17.93 | 38.03 ± 21.63 | 28.95 ± 13.27 | 70.01 ± 35.51 | 64.61 ± 31.10 | 52.25 ± 26.56 | 57.48 ± 25.33 |
| | *pNN50* | 0.14 ± 0.09 | 0.11 ± 0.14 | 0.17 ± 0.17 | 0.10 ± 0.12 | 0.43 ± 0.22 | 0.40 ± 0.21 | 0.29 ± 0.20 | 0.35 ± 0.20 |
| | *renyi4* | 4.27 ± 0.47 | 4.14 ± 0.50 | 4.47 ± 0.45 | 4.29 ± 0.46 | 4.49 ± 0.62 | 4.30 ± 0.59 | 4.07 ± 0.60 | 4.18 ± 0.57 |
| **FD** | *LF/HF* | 2.73 ± 1.22 | 4.06 ± 5.79 | 2.78 ± 1.68 | 3.00 ± 1.46 | 1.03 ± 1.20 | 1.08 ± 1.48 | 0.88 ± 0.56 | 1.05 ± 0.69 |
| | *LFn* | 0.70 ± 0.10 | 0.72 ± 0.10 | 0.68 ± 0.14 | 0.71 ± 0.12 | 0.43 ± 0.17 | 0.42 ± 0.18 | 0.42 ± 0.17 | 0.46 ± 0.17 |
| | *HFn* | 0.30 ± 0.10 | 0.28 ± 0.10 | 0.32 ± 0.14 | 0.29 ± 0.12 | 0.57 ± 0.17 | 0.58 ± 0.18 | 0.58 ± 0.17 | 0.54 ± 0.17 |
| **SD** | *phvar20* | 0.04 ± 0.04 | 0.04 ± 0.08 | 0.07 ± 0.10 | 0.03 ± 0.06 | 0.24 ± 0.17 | 0.21 ± 0.14 | 0.13 ± 0.13 | 0.18 ± 0.13 |
| **$H_C$** | *$H_{C(5,5)}$* | 0.69 ± 0.07 | 0.64 ± 0.08 | 0.69 ± 0.07 | 0.64 ± 0.08 | 0.76 ± 0.09 | 0.75 ± 0.08 | 0.71 ± 0.09 | 0.72 ± 0.09 |

## 3. Results

### 3.1. Multivariate analysis of HRV parameters for daytime (D)

The multivariate MANOVA found no statistically significant differences, neither for the TIME x GROUP interaction ($F$(9, 30) = 1.222, $p$ = 0.319, partial $\eta^2$ = 0.268), nor for the two factors GROUP ($F$ (9, 30) = 0.984, $p$ = 0.473, partial $\eta^2$ = 0.228) or TIME ($F$(9, 30) = 1.879, $p$ = 0.094, partial $\eta^2$ = 0.360

This holds true also for all post-hoc tests, which were conducted on an exploratory level, the comparisons between the two groups at baseline (T0; $F$(9, 30) = 1.141, $p$ = 0.366, partial $\eta^2$ = 0.255) and follow-up visit (T1; $F$(9, 30) = 0.533, $p$ = 0.839, partial $\eta^2$ = 0.138), as well as the comparisons between the timepoints T0 and T1 for the control group (CON; $F$(9, 30) = 2.042, $p$ = 0.069, partial $\eta^2$ = 0.380) and the intervention group (IN; $F$(9, 30) = 1.059, $p$ = 0.419, partial $\eta^2$ = 0.241)

As a reference for further studies, additional analyses for all eleven single indices are listed in Table S1.

### 3.2. Multivariate analysis of HRV parameters for night-time (N)

A similar picture was obtained for the night-time data. Multivariate MANOVA revealed no statistically significant differences in the compound dependent variable, neither for the TIME*GROUP interaction ($F$ (9, 30) = 0.683, $p$ = 0.718, partial $\eta^2$ = 0.170), nor for the factors GROUP ($F$(9, 30) = 1.781, $p$ = 0.114, partial $\eta^2$ = 0.348) or TIME ($F$(9, 30) = 1.441, $p$ = 0.215, partial $\eta^2$ = 0.302) (Table S2).

Post-hoc analyses of the compound dependent variable were again conducted on an exploratory level: for the factor GROUP (Intervention vs Control) at T0 ($F$(9, 30) = 2.119, $p$ = 0.06, partial $\eta^2$ = 0.389) and T1 ($F$(9, 30) = 0.683, $p$ = 0.718, partial $\eta^2$ = 0.170) as well as for the factor TIME for the Control ($F$(9, 30) = 1.283, $p$ = 0.287, partial $\eta^2$ = 0.278) and the intervention group ($F$(9, 30) = 0.841, $p$ = 0.583, partial $\eta^2$ = 0.202). The results obtained for the eleven single indices can be found in Table S2.

#### 3.2.1. Underpowering

Based on the combination of high effect sizes for the mainly descriptively noteworthy MANOVA interactions in both; day and night-time data ($\eta^2$ of 0.268 and 0.170, respectively) and high counteracting number of dependent single indices that form the compound dependent variable, the number of participants should have been approximately 140, or rather 150 in order to render the effects significant.

## 4. Discussion

The two MANOVAs employed here for the day and night-time data did not show any significant difference in the combined heart-variability measured between the yoga and mindfulness-trained experimental group and the control group receiving the standard school curriculum. Neither the mainly descriptive specific change in HRV over time in the experimental group vs. the control group (interaction), nor the comparisons within the groups over time or at the two timepoints between groups (main factors) showed significance.

Subsequential exploratory post-hoc analyses were conducted with regard to the effect sizes, $\eta^2$, in order to determine which factors would be worthwhile to investigate in a new full experiment. For this, we relied here on the assessment that $\eta^2$ = 0.01, 0.06, 0.14 to indicate small, medium and large effects[41] recognising that only medium and large effects ($\eta^2 >= 0.05$, corresponding approximately to a Cohen's d $>=$ 0.40) are of clinical importance.[42]

In the IN, markedly altered HRV indices were primarily observed at night, while only marginal changes were seen during daytime. Although IN showed somewhat lower HRV indices at baseline as compared to the CON, a tendency toward increased vagal activity can be seen over time. The intervention group HRV were lower at baseline for unknown reasons. Possible reasons may either be coincidental or caused by the lack of randomisation at the allocation of classes. Nevertheless, it can be assumed that the intervention had a recovering effect in the IN group, tending towards HRV values of CON. If this subtle effect was to continue beyond the course of the trial it could be considered beneficial. But long-term effects remain unclear due to the limitations of the study design. It should also be taken into account that these effects had non-specific causes due to the study design and a multitude of other potential factors in a child's life.

At T0 and T1, no significant differences in HRV indices were found between the two groups during daytime. However, CON showed a promising decrease in HRV complexity ($H_{C(5,5)}$) over time, indicating an increased regularity of HRV. For IN, these differences were more obvious. A further presumed notable decrease of *meanNN* (corresponding to an increased heart rate), a decrease in vagal tone (*rmssd*) and a decrease in HRV complexity ($H_{C(5,5)}$) (increased regularity of HRV) can be found. Overall, IN showed a decrease in vagal activity and an increase in sympathetic activity over time (T0→T1) (tendency shown by increased *LFn*). The intervention appears to lead to changes in sympathovagal tone towards reduced vagal activity that should be explored in further research. In contrast, night phases revealed differences between the two groups at baseline (T0) but no differences to T1 were found. In particular, IN at T0 showed an interesting decrease of *meanNN* (= increase in heart rate), a decrease in HRV (*phvar20*), in vagal activity (*pNN50*) and in HRV complexity (*renyi4*, $H_{C(5,5)}$) as well as an increased regularity of HRV, suggesting that IN was more stressed at time T0 (increase in sympathetic activity and decrease in vagal activity). Though not to significant levels, this higher "stress level" during T0 (night-time) balanced out over time (T1, long-term effect over months). This was most clearly demonstrated over time T0→T1, as IN showed a possibly interesting increase in *meanNN* (decrease in heart rate) at time point T1 in comparison to time point T0, indicating a recovery towards the HRV values of the CON and suggesting exploratively a potential long-term benefit. Therefore, initially lower HRV might have been balanced out during intervention period. Whether this effect was caused primarily by the intervention or by other factors, remains unclear.

Healthy heart rate fluctuations exhibit a complex type of variability that embeds fractal self-similar fluctuations on time scales from seconds to hours, thus generating far-reaching power-law correlations.[43] Results from several studies suggest that greater complexity (irregularity) occurs in healthy cardio-vascular systems. The common hypothesis is that the organism is a highly complex adaptive system and that its complexity provides the broadest range of adaptive responses due to different input levels on the physiological spectrum.[25] Several studies have used psychometric data and biomarkers to determine beneficial effects of MBM, but our study uniquely applied HRV to demonstrate how variation in heart rate correlates with MBM effects in paediatrics.

A pilot study in 2015 examined salivary cortisol concentrations to document the effects of a classroom-based yoga intervention.[44] Although results of this uncontrolled study must be interpreted with caution as well, a significant decrease of cortisol concentrations was seen in one of the two subgroups. A recent study conducted by Frank et al. used HRV parameters as an outcome measure in older participants (17–25 y) using yoga to replace physical education.[14] Significant increases of multiple HRV variables were demonstrated, suggesting improved vagal activity.[14] Likewise, a meta-analysis by Kim et al. revealed that various HRV methods indicate changes in sympathovagal balance in response to stress.[15] The most commonly reported factor associated with variation in HRV indices was low parasympathetic activity, characterised by a decrease in the high frequency band and an increase in the low frequency band. Stressful situations often contribute to a momentary reduction in vagal influence as part of a stress response, corresponding to a change in HRV. However, in our study, which may be viewed as a baseline, snapshot measurements rather than chronic responses were considered. Although HRV is impacted by stress, these findings remain unanswered whether HRV is an inappropriate assessment measure of psychological health and stress in MBM. Furthermore, it should be recognised that more vagal activity is not always indicative of better outcomes (i.e., vagal withdrawal during stressful situations is considered part of a healthy stress response). The general consensus is that higher HRV indicates better capacity to cope with stress since it entails a flexible autonomic nervous system. There is also evidence that HRV is chronically lowered under circumstances of higher stress.

In a broader sense, HRV is an index of ANS flexibility which is correlated with many promising health outcomes. By targeting the modulation of self-regulatory mechanisms, yoga and mindfulness practices may be expected to increase HRV measures.

Strengths of this study are its unique setting in primary schools combined with the application of HRV to young children. Prior studies have primarily examined the benefits of MBM in adults. Chronic stress is a growing public health issue and rising cause for absenteeism and health issues, early counteraction in childhood is essential. Another major strength is the diversity in the study population. The three participating schools are located in fundamentally differing neighbourhoods in Berlin, Germany.

Several limitations should be noted and may need to be addressed in future research. One major limitation of this study is the lack of randomisation at the allocation of classes. School administration allocated classes to the study after consultation with the class teachers as they depended on their teachers' initiative and willingness to participate. Teachers with challenging class dynamics might have been more motivated to take part in the programme. This may explain the higher stress levels of the intervention group at baseline (T0) (see Fig. 1). However, performance anxiety could also explain the higher stress levels of the intervention group at baseline (T0). Among authors of similar studies, individuals have been described to need time to familiarise with any form of yoga or mindfulness practice.[45] Therefore, adding MBM to a curriculum for children, might induce more temporary stress in early stages. A daily target of only 5 min was set for YoBEKA practice to provide balance between the challenging daily routine of class teachers and the motivation to integrate school-based yoga. Stronger teacher support might have strengthened long-term effects of the programme and should be considered in the planning of similar future studies. While we aimed to have teachers conducted a 5-minute minimum of yoga a day, we were unable to gather data on the extent of the teacher's ability to adhere to this guideline. Therefore, potential adherence unknown fluctuations in the intervention groups may have had an effect on the study outcome and can be seen as a limitation.

The benefits of a 15–30-minute yoga practice was shown in a systematic review of yoga-based school interventions to be particularly beneficial for children's attention span.[45] Thus, daily YoBEKA exercises may have been too short to yield a crucial effect. In future studies, sub-analyses should be conducted with a larger data pool in order to better delineate and evaluate the effect of individual teachers, individual schools and individual class compositions on the effectiveness of the intervention.

Apart from these points raised above, post-hoc analysis of the effects size of the interaction terms in the two MANOVAs revealed that the study was underpowered. In order to obtain significant results and thus be able to investigate the effects of yoga and mindfulness effects on the HRV further, a sample of 150 enrolled participants was necessary for the study.

YoBEKA is a flexible and sustainable concept, applicable for educators from various school backgrounds, pupil populations and geographical regions. A rising number of kindergartens, primary and secondary schools have integrated YoBEKA over the past 10 years in Berlin and Brandenburg, attesting for its growing acceptance. Fundamentally, yoga is highly accessible, and the practice is not dependent on skill. An individual approach to yoga has potential to create joy and ease – especially in an educational system. Furthermore, early exposure to yoga practice may provide a positive foundation for mental health in adulthood by raising chances of continued practice later on. School-based yoga programmes may therefore have a long-term and immeasurable effect. While evidence for the benefits of yoga in children remains insufficiently studied, evidence for health benefits in adults is significant.[46–48] Thus, further research in the paediatrics - and longitudinal studies in particular - are necessary to establish lifetime health benefits and to evaluate long-term effects.

## 5. Conclusion

The present trial did not reveal evidence for an effect of a yoga and mindfulness-based programme on the autonomic nervous system of primary school children in measures of heart rate variability. However, exploratory post-hoc analyses point to interesting trends in cardiac autonomic modulation at night towards parasympathetic activity after a 16-week intervention of yoga-based intervention in primary schools. Indicated by parameters of the autonomic nervous system, an enhanced self-regulation might be one of the reasons for improvements in the yoga group over time. However, due to baseline differences and limitations of the study design, this interpretation must be considered with caution. This trial may serve as a template for further studies in the field of yoga-based interventions since deepening research is required considering high quality study designs, larger sample sizes and long-term follow-ups.

## Author statement

**Pune Ivaki:** Investigation, Writing – Original Draft, Visualisation **Steffen Schulz:** Methodology, Formal analysis, Writing – Original Draft, Visualisation **Michael Jeitler:** Conceptualisation, Investigation, Writing – Editing, Project administration **Christian S. Kessler:** Conceptualisation, Writing – Editing **Andreas Michalsen:** Conceptualisation **Farid I. Kandil:** Methodology, Formal analysis, Writing – Editing **Saskia-Marie Nitzschke:** Investigation **Wiebke Stritter:** Conceptualisation, Project administration **Andreas Voss:** Methodology, Formal analysis **Georg Seifert:** Conceptualisation, Writing – Editing, Project administration, Funding acquisition

## Funding

Software AG Stiftung, Damus-Donata e.V., Koenig-Stiftung. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgements

We would like to thank all the participating schools, their staff and pupils, as well as the *yobee-active* institute, especially Ms. Ilona Holterdorf, and Sarah Blakeslee for language editing.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ctim.2021.102771.

## References

1 Ryan-Wenger NA, Sharrer VW, Campbell KK. Changes in children's stressors over the past 30 years. *Pediatr Nurs*. 2005;31(4).
2 White KS, Farrell AD. Anxiety and psychosocial stress as predictors of headache and abdominal pain in urban early adolescents. *J Pediatr Psychol*. 2006;31(6):582–596.

3 DeSocio J, Hootman J. Children's mental health and school success. *J Sch Nurs*. 2004;20(4):189–196.
4 Natvig GK, Albrektsen G, Anderssen N, Qvarnstrøm U. School-related stress and psychosomatic symptoms among school adolescents. *J Sch Health*. 1999;69(9):362–368.
5 Bhardwaj A. Importance of education in human life: a holistic approach. *Int J Sci Consc*. 2016;2(2):23–28.
6 Nanthakumar C. The benefits of yoga in children. *J Integr Med*. 2018;16(1):14–19.
7 Carsley D, Khoury B, Heath NL. Effectiveness of mindfulness interventions for mental health in schools: a comprehensive meta-analysis. *Mindfulness*. 2018;9(3):693–707.
8 Hartranft C. *The Yoga-Sutra of Patanjali: A new translation with commentary*. Shambhala Publications; 2003.
9 Ross A, Thomas S. The health benefits of yoga and exercise: a review of comparison studies. *J Altern Complement Med*. 2010;16(1):3–12.
10 Hariharananda SA. *Yoga philosophy of Patanjali: Containing his yoga aphorisms with Vyasa's commentary in Sanskrit and a translation with annotations including many suggestions for the practice of yoga*. SUNY Press; 1983.
11 Salmon P. Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory. *Clin Psychol Rev*. 2001;21(1):33–61.
12 Thayer JF, Åhs F, Fredrikson M, Sollers IIIJJ, Wager TD. A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci Biobehav Rev*. 2012;36(2):747–756.
13 Shaffer F, Ginsberg J. An overview of heart rate variability metrics and norms. *Front Public Health*. 2017;5:258.
14 Frank J, Seifert G, Schroeder R, et al. Yoga in school sports improves functioning of autonomic nervous system in young adults: a non-randomized controlled pilot study. *PLoS One*. 2020;15(4), e0231299.
15 Kim H-G, Cheon E-J, Bai D-S, Lee YH, Koo B-H. Stress and heart rate variability: a meta-analysis and review of the literature. *Psychiatry Investig*. 2018;15(3):235.
16 Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int J Cardiol*. 2010;141(2):122–131.
17 Porges SW. Vagal tone: a physiologic marker of stress vulnerability. *Pediatrics*. 1992;90(3 Pt 2):498–504.
18 Thayer JF, Lane RD. The role of vagal function in the risk for cardiovascular disease and mortality. *Biol Psychol*. 2007;74(2):224–242.
19 Kemp AH, Quintana DS. The relationship between mental and physical health: insights from the study of heart rate variability. *Int J Psychophysiol*. 2013;89(3):288–296.
20 Seifert G, Calaminus G, Wiener A, Cysarz D. Heart rate variability reflects the natural history of physiological development in healthy children and is not associated with quality of life. *PLoS One*. 2014;9(3), e91036.
21 Luque-Casado A, Perales JC, Cárdenas D, Sanabria D. Heart rate variability and cognitive processing: the autonomic response to task demands. *Biol Psychol*. 2016;113:83–90.
22 Sztajzel J. Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. *Swiss Med Wkly*. 2004;134(35–36):514–522.
23 Voss A, Heitmann A, Schroeder R, Peters A, Perz S. Short-term heart rate variability—Age dependence in healthy subjects. *Physiol Meas*. 2012;33(8):1289.
24 Voss A, Schroeder R, Heitmann A, Peters A, Perz S. Short-term heart rate variability—Influence of gender and age in healthy subjects. *PLoS One*. 2015;10(3), e0118308.
25 Voss A, Schulz S, Schroeder R, Baumert M, Caminal P. Methods derived from nonlinear dynamics for analysing heart rate variability. *Philos Trans Math Phys Eng Sci*. 2009;367(1887):277–296.
26 Mäkikallio TH, Høiber S, Køber L, et al. Fractal analysis of heart rate dynamics as a predictor of mortality in patients with depressed left ventricular function after acute myocardial infarction. *Am J Cardiol*. 1999;83(6):836–839.
27 Tapanainen JM, Thomsen PEB, Køber L, et al. Fractal analysis of heart rate variability and mortality after an acute myocardial infarction. *Am J Cardiol*. 2002;90(4):347–352.
28 Voss A, Hnatkova K, Wessel N, et al. Multiparametric analysis of heart rate variability used for risk stratification among survivors of acute myocardial infarction. *Pacing Clin Electrophysiol*. 1998;21(1):186–196.
29 Baumert M, Baier V, Haueisen J, et al. Forecasting of life threatening arrhythmias using the compression entropy of heart rate. *Methods Inf Med*. 2004;43(02):202–206.
30 Association WM. Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Jahrbuch Für Wissenschaft Und Ethik*. 2009;14(1):233–238.
31 Seely AJ, Macklem PT. Complex systems and the technology of variability analysis. *Crit Care*. 2004;8(6):1–18.
32 Schulz S, Reulecke S, Eiselt M, et al. Quantification of compensatory processes of postnatal hypoxia in newborn piglets applying short-term nonlinear dynamics analysis. *Biomed Eng Online*. 2011;10(1):1–16.
33 Wessel N, Voss A, Malberg H, et al. Nonlinear analysis of complex phenomena in cardiological data. *Herzschrittmachertherapie und Elektrophysiologie*. 2000;11(3):159–173.
34 Schulz S, Voss A. *Symbolic dynamics, Poincaré plot analysis and compression entropy estimate complexity in biological time series. Complexity and Nonlinearity in Cardiovascular Signals*. Springer; 2017:45–85.
35 Rawenwaaij-Arts C, Kallee L, Hopman J. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiologic interpretation, and clinical use. *Circulation*. 1996;93:1043–1065. Intern Med 1993;118: 436-447.
36 Voss A, Dietz R, Fiehring H, et al. High resolution ECG, heart rate variability and nonlinear dynamics: tools for high risk stratification. In: *Proceedings of Computers in Cardiology Conference*. 1993:261–264.
37 Voss A, Kurths J, Kleiner H, et al. The application of methods of non-linear dynamics for the improved and predictive recognition of patients threatened by sudden cardiac death. *Cardiovasc Res*. 1996;31(3):419–433.
38 Porta A, Guzzetti S, Montano N, et al. Entropy, entropy rate, and pattern classification as tools to typify complexity in short heart period variability series. *IEEE Trans Biomed Eng*. 2001;48(11):1282–1291.
39 Schulz S, Bär K-J, Voss A. Analyses of heart rate, respiration and cardiorespiratory coupling in patients with schizophrenia. *Entropy*. 2015;17(2):483–501.
40 Ziv J, Lempel A. A universal algorithm for sequential data compression. *IEEE Trans Inf Theory*. 1977;23(3):337–343.
41 Smith IL. The eta coefficient in MANOVA. *Multivariate Behav Res*. 1972;7(3):361–372.
42 Engel L, Beaton DE, Touma Z. Minimal clinically important difference: a review of outcome measure score interpretation. *Rheumatic Dis Clin*. 2018;44(2):177–188.
43 Goldberger AL, Amaral LA, Hausdorff JM, Ivanov PC, Peng C-K, Stanley HE. Fractal dynamics in physiology: alterations with disease and aging. *Proc Natl Acad Sci*. 2002;99(suppl 1):2466–2472.
44 Butzer B, Day D, Potts A, et al. Effects of a classroom-based yoga intervention on cortisol and behavior in second-and third-grade students: a pilot study. *J Evid Based Complementary Altern Med*. 2015;20(1):41–49.
45 Ferreira-Vorkapic C, Feitoza J, Marchioro M, Simões J, Kozasa E, Telles S. Are there benefits from teaching yoga at schools? A systematic review of randomized control trials of yoga-based interventions. *Evid Based Complement Altern Med*. 2015;2015.
46 Balasubramaniam M, Telles S, Doraiswamy PM. Yoga on our minds: a systematic review of yoga for neuropsychiatric disorders. *Front Psychiatry*. 2013;3:117.
47 Hendriks T, de Jong J, Cramer H. The effects of yoga on positive mental health among healthy adults: a systematic review and meta-analysis. *J Altern Complement Med*. 2017;23(7):505–517.
48 Innes KE, Selfe TK. Yoga for adults with type 2 diabetes: a systematic review of controlled trials. *J Diabetes Res*. 2016;2016.