Variability and similarity of inter-beat intervals of the heart as markers of perceived stress and behavioral regulation

Elisabet Kvadsheim

Thesis for the degree of Philosophiae Doctor (PhD)
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Name: Elisabet Kvadsheim

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Scientific environment

I have certainly enjoyed my employment at the Department of Clinical Medicine (K1) at the University of Bergen (UiB). Furthermore, a substantial amount of work leading up to the current dissertation was performed when I took part in the Medical Student Research Programme at the UiB from 2016 to 2020. During these years, I appreciated being a part of the Emotion and Cognition Group, and also working with other employees, at the Department of Biological and Medical Psychology (IBMP). Moreover, I valued partaking in arrangements by the KG Jebsen Centre for Neuropsychiatric Disorders and the Norwegian Research School in Neuroscience. In addition, I was privileged to be supported with two summer grants from the Regional Centre for Child and Youth Mental Health and Child Welfare (RKBU Vest).

Three of my brilliant supervisors are affiliated with the University of Bergen: Prof. Ole Bernt Fasmer at the Department of Clinical Medicine (K1), Prof. Lin Sørensen at the IBMP, and Prof. Jan Haavik at the Department of Biomedicine. Prof. Fasmer and Prof. Haavik are also affiliated with the Division of Psychiatry at Haukeland University Hospital. My last — but in no way least — supervisor, Prof. Julian Koenig, is currently affiliated with the Faculty of Medicine at the University of Cologne, and the Department of Child and Adolescents Psychiatry, Psychosomatics and Psychotherapy at the University hospital of Cologne in Germany.

I have had the pleasure to work with several other great researchers as co-authors of the papers in the current dissertation. These include Assoc. Prof. Berge Osnes, Dr. Steinunn Adolfsdottir and Dr. Heike Eichele, affiliated with the IBMP. Dr. Adolfsdottir is also affiliated with the Statped West - National service for special needs education in Bergen. Moreover, I have enjoyed the competence of Prof. Kerstin Jessica von Plessen from the Department of Psychiatry at the University of Lausanne and Lausanne University hospital in Switzerland. I have also been privileged to cooperate with Prof. Julian F. Thayer and Asst. Prof. DeWayne P. Williams at the Department of Psychological Science, University of California, Irvine, United States. I have also received generous help from Erlend E. Fasmer, an independent researcher situated in Harstad, and Dr. Erik R. Hauge at the Division of Psychiatry at Haukeland University Hospital in Bergen.

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Introduction

Inter-beat intervals (IBIs) are defined as the time between consecutive heart beats of an electrocardiogram (ECG) recording (5). Analyses of IBIs provide information about autonomic nervous system (ANS) regulation on the heart, and the indices of cardiac vagal activity (CVA; also known as vagally mediated heart rate variability) have been of particular interest (6). CVA is considered to reflect vagus nerve modulation on the heart (6). Importantly, the vagus nerve is indirectly connected with the prefrontal cortex and amygdala — brain areas that are considered crucial for self-regulation (7). CVA has consequently been suggested as a biomarker of self-regulation (see 8, 9-11), both of inner processes such as perceived stress (see 12, 13, 14), and processes leading to externally observable outcomes such as behavior (see 15). However, CVA might predominantly reflect certain core aspects of self-regulation (8, 9), for instance functions in which deficits lead to typically internalizing symptoms (in contrast to externalizing symptoms). One candidate for such a core function is the internal regulation of perceived stress.

The current dissertation aims to investigate which self-regulatory functions CVA and other IBI indices of vagal activity — such as those deducted from graph theory-based analyses — marks, and how this marker is related to contextual factors. This was investigated in a sample from the general population, as well as in a patient population with a psychiatric disorder, exemplified by adolescents with attention-deficit hyperactivity disorder (ADHD). Based on previous studies and theoretical frameworks, there are reasons to believe that CVA might be applied as a marker of perceived stress regulation. However, several aspects require further elucidation before it can be established as a valid biomarker. Therefore, we investigated A) How CVA and perceived stress is related to contextual factors, such as perceived social support and sex, in healthy adults B) CVA as a marker of emotion regulation — an important process for the regulation of perceived stress — compared to as a marker of behavioral regulation in a sample of adolescents with Attention-Deficit/Hyperactivity Disorder (ADHD); A population which typically shows both internalizing and externalizing symptoms. and C) The hypothesis that the current methods for CVA analysis are not sensitive enough to mark all aspects of self-regulation, explaining why CVA might associate more strongly with some aspects of self-regulation compared to others. Therefore, we investigated whether a novel, nonlinear method for IBI analysis based on graph theory could differentiate adolescents with ADHD — showing deficits in behavioral regulation — and controls. This method might increase the sensitivity and specificity of IBI indices as markers of general self-regulation.

As deficits in the regulation of stress might underlie the symptoms of several psychiatric disorders (16-18), the current dissertation can hopefully provide a useful theoretical foundation for the development of designs and hypotheses for future clinical studies. Thus, it might also contribute towards the goal of developing effective diagnostics, monitoring, prevention and treatment for psychiatric disorders. Although considerably more research is needed before CVA can be generally applied in clinical settings as a biomarker, it has been described as a "certainly viable candidate" (7). The fact that IBI analyses are inexpensive, non-invasive and demand relatively few resources further supports its clinical utility.

The dissertation is a synthesis of the three papers on which it is based. For additional details, figures and tables, readers are referred to the attached reproductions of these papers. The last literature search for relevant studies to include in the current dissertation was performed in late January 2022.

Abstract

The current dissertation investigated inter-beat interval (IBI) indices of variability and similarity, reflecting autonomic nervous system (ANS) modulation on heart rate. IBI indices of cardiac vagal activity (CVA) are further considered to reflect activity in brain areas involved in self-regulation. Yet, it is unclear which specific aspect(s) of self-regulation such IBI indices load most highly on, and their relation to contextual factors. Thus, in a sample of college students (n = 143) in paper I, we investigated how CVA and perceived stress associated with contextual factors of perceived social support and sex. Moreover, we expected indices to load highly on the internal regulation of perceived stress, compared to the external regulation of behavior. This was examined in adolescents with attention-deficit/hyperactivity disorder (ADHD) and controls (n = 67) in paper II. In paper III, we investigated the use of a nonlinear, graph theory-based method for illustrating IBI differences in adolescents with ADHD and controls (n = 73). In all studies, IBI indices were derived from short-term resting electrocardiogram (ECG) recordings, with high frequency-heart rate variability (HF-HRV) as the applied measure of CVA. Self-report questionnaires assessed emotion regulation difficulties (the Difficulties in Emotion Regulation Scale) perceived stress (the Perceived Stress Scale), and perceived social support (The Medical Outcomes Study Social Support Survey). In the moderation analysis of paper I, CVA associated positively with perceived social support in females with intermediate and high, compared to low, perceived stress levels, but not in males. Linear regression analyses in paper II showed that CVA associated negatively with access to emotion regulation strategies in adolescents with ADHD and controls. In paper III, independent samples t-test showed that the similarity graph algorithm illustrated IBI differences between the ADHD and control groups which traditional CVA analyses did not. In sum, the studies suggest that CVA might mark perceived stress regulation, and emphasize the consideration of contextual factors such as perceived social support and sex in the interpretation of this marker. Furthermore, the similarity graph algorithm might increase the sensitivity of IBI markers, possibly also indexing behavioral regulation. Although further research is required, IBI markers might have potential clinical use in the diagnosis, monitoring and treatment of psychiatric disorders.

List of Publications

Kvadsheim, E., Sørensen, L., Fasmer, O.B., Osnes, B., Haavik, J., Williams, D.P., Thayer, J.F., & Koenig, J. Vagally mediated heart rate variability, stress and perceived social support: a focus on sex differences. Stress. 2022; 25(1): 113-121. DOI: 10.1080/10253890.2022.2043271

Kvadsheim, E., Fasmer, O.B., Osnes, B., Koenig, J., Adolfsdottir, S., Eichele, H., Plessen, K.J., Sørensen, L. Lower Cardiac Vagal Activity Predicts Self-Reported Difficulties with Emotion Regulation in Adolescents with ADHD. Front. Psychiatry. 2020; 11(244): 1-8. DOI: 10.3389/fpsyt.2020.00244

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Contents

Abbreviations

ADHD	Attention-Deficit/Hyperactivity Disorder
ANS	Autonomic Nervous System
CAN	Central Autonomic Network
CNS	Central Nervous System
CVA	Cardiac Vagal Activity
DERS	Difficulties in Emotion Regulation Scale
HF	High Frequency
HR	Heart Rate
HRV	Heart Rate Variability
IBI	Inter-Beat Interval
LF	Low Frequency
MOS	Medical Outcomes Study Social Support Survey
PNS	Parasympathetic Nervous System
PSS	Perceived Stress Scale
RMSSD	Root mean square of successive RR interval differences
RQA	Recurrence Quantification Analyses
SD	Standard Deviation
SDNN	Standard deviation of normal-to-normal (i.e. normal R-R) intervals
SNS	Sympathetic Nervous System

1. Introduction

1.1 Self-regulation

There are numerous definitions of self-regulation, suggesting that it is challenging to agree on a theoretical characterization of this construct (19). One definition, which aligns well with the application of the term in the current dissertation, is "the processes by which people initiate, maintain, and control their own thoughts, behaviors, or emotions, with the intention of producing a desired outcome or avoiding an undesired outcome" (16). Importantly, self-regulation requires the capability to set aside impulses to respond in a given way — based on habits or previous learning experiences — and respond in an alternative manner (18). This self-regulation might occur deliberately and consciously, or as an automated process (see 16, 20).

The purpose of self-regulation is to ensure goal-directed behavior — including the achievement of short-term and long-term goals — for the individual, and balance effects of this behavior with environmental demands (19). As personal and environmental contexts frequently change, self-regulation requires flexible adaptation to shifting demands. Individuals tend to differ in such adaptivity, and thus their general capacity of self-regulation (18). These differences could be due to genetics, physiology, cognition, motivation, emotions, or social systems (16). Importantly, self-regulation is often considered as a depletable, albeit renewable, resource (18). For instance, during high perceived stress, self-regulatory resources might be limited (18). As a result, a given individual might self-regulate effectively during one condition but not another — although individuals still tend to differ consistently in self-regulation (18).

Deficits in self-regulation might underlie the symptoms of several psychiatric disorders, including depressive disorders, anxiety disorders, addictive disorders, schizophrenia, autism (16) and ADHD (21). Moreover, such deficits not only contribute to the symptoms of these disorders, but the disorders also tend to influence self-regulation in a negative manner (16).

Factors operating at any levels, ranging from genetic to social systems, can impede an individual's self-regulation (16). At the physiological level, central nervous system (CNS) and ANS functioning is crucial for effective self-regulation (20). In fact, current theoretical frameworks suggest that psychiatric disorders mainly occur due to malfunctions of neural systems, such as the frontal and prefrontal cortical areas, the amygdala, hypothalamus, anterior cingulate cortex and brainstem centers (20). Consequently, a biomarker reflecting CNS and ANS functioning might have clinical implications for the psychiatric field. One such promising marker is CVA, which can be deducted from analyses of IBIs.

1.2 Cardiac vagal activity

1.2.1 Inter-beat intervals

ECG recordings display IBIs, and from these one can deduct the variability in heart rate (HR): heart rate variability (HRV; 6) (See figure 1.1). Such HRV occurs in large part due to activity of the two branches of ANS — the sympathetic (SNS) and the parasympathetic nervous system (PNS) — on the heart (6). These branches interplay constantly in order to maintain homeostasis: The SNS increases HR when the body is in need for mobilization ("fight or flight"; 22), and oppositely the PNS decreases HR when the body conserves energy ("rest and digest"; 22).

Figure 1.1. Illustration of an ECG recording. The deflections of the ECG are named P, Q, R, S and T, respectively. The distance between two R-deflections is called an R-R-interval, and make up an IBI. Varying IBIs, due to activity of the ANS, results in HRV.

The most effective way to change HR and thus increase HRV is by stimulation or withdrawal of the vagus nerve of the PNS (23): It exerts its effect in milliseconds (23), whereas the SNS has a peak effect after about five seconds (24). Normally, most

HRV therefore results from stimulation or withdrawal of the vagus nerve, rather than changes in SNS activity (6). Modulation of the vagus nerve on the heart (i.e. vagally mediated HRV) is described as CVA. Consequently, a higher CVA corresponds to rapid adaptation of the heart rate to bodily and environmental demands (25).

Notably, also organic disease, for instance atrial fibrillation, might lead to higher HRV (see 26). Therefore, the ECG from which IBIs are deducted always has to be inspected for signs of arrythmias or organic disease. In the following sections, the term "higher CVA" will refer to a relatively high CVA as deducted from an ECG recording with normal sinus rhythm in an individual without known cardiac disease.

1.2.2 Analysis of cardiac vagal activity

The first observation of HRV might have been performed already in 1733 (27). However, its relationship to psychological factors was not investigated until the 1960s (see (23) for a historical review). In this regard, there has been a particular interest in the vagus nerve and its adaptivity, as reflected in CVA (23).

In short, there have been applied two main methods for deducting CVA: Time domain- and frequency domain analyses (5). These analyses are based on linear models for IBI analysis, assuming a linear relation between consequent IBIs (28). Time domain analyses calculate the amount of variability based on IBI lengths in milliseconds (6). On the other hand, frequency domain analyses calculate the distribution of IBIs in specific frequency bands, analogous to a prism that refracts light into different wavelengths (28). The low frequency (LF: .04–.15 Hz) and the high frequency (HF: .15–.40 Hz) bands are commonly used (6; See Figure 1.2), and referred to as indices of LF-HRV and HF-HRV, respectively. LF-HRV likely represents both sympathetic and parasympathetic influences (6, 23). In contrast, HF-HRV is often used as a "pure" index of CVA (6), as the vagus nerve is the only ANS component that

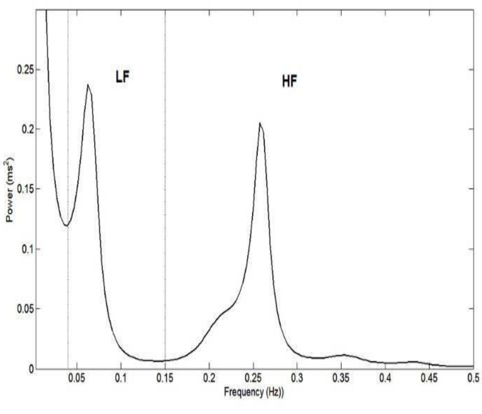

Figure 1.2. *An example of a distribution of IBIs into frequency bands. Figure retrieved from (1).*

exerts its effect rapidly enough to result in such high frequency activity. Moreover, the ratio between the two bands, the LF/HF ratio, has often been used to index the relative activity of the ANS branches ("sympatho-vagal balance") on the heart (5). Although this interpretation has been debated (see 28), a low LF/HF ratio has often been interpreted as vagal dominance of the ANS (5).

As previously noted, HF-HRV is a commonly applied index of CVA. Notably, the use of the term CVA for describing HF power is not perfect. This is because vagal influence on the sinoatrial node (the "pacemaker") of the heart — i.e. cardiac vagal activity — not necessarily affects the HF-HRV band in an exact one-to-one correspondence, as the SNS and PNS interact in a complex manner (28). Therefore, some prefer the term "vagally mediated heart rate variability". However, the choice of term is likely more academically interesting than consequential for the interpretation of the index. In the current dissertation the applied term is "CVA", which might be more intuitively understandable for those not familiar with the concept of HRV.

In CVA studies, there are generally two conditions for collection of ECGs: A resting and a reactive (i.e., phasic) condition (see 29), which provide complementing perspectives on CVA. During a reactive condition, the ECG is recorded during a task — for instance a stress task — and during recovery from the task (29). Higher CVA reactivity might be interpreted as adaptive vagal responding (29). As CVA changes deducted from reactive conditions index a "state" vagal response, it is considered as a "within-subject" measure (29). On the other hand, CVA indices from resting ECG recordings have shown excellent test-retest reliability (30, 31), and are thus often considered as "between-subjects" (29) measures of "trait" responses. A resting condition is consequently often used in studies of factors that are considered to vary in a trait-like manner — such as self-regulation. Analyses of resting ECGs are therefore relevant for the use of CVA as a marker of self-regulation.

1.3 Cardiac vagal activity as a marker of self-regulation

1.3.1 The nature of a marker of psychological processes

One definition of a "marker" or "biomarker" is "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" (32). In other words, it is an objectively measured, quantifiable representation of a biological process (33). There are various categories of such markers, based on their clinical applications: Diagnostic, monitoring, response, predictive, prognostic, safety, or susceptibility/risk markers (34).

In the psychiatric field, the current diagnostic systems are based on observable or experienced symptom levels (35, 36). However, the use of biological, objective markers could improve our understanding of how biological processes are related to clinical outcomes and as such contribute to the management of psychiatric disorders (34). A successful marker for such clinical use would have to be reproducible, accessible, and easily detectable (34). Most importantly, it must be modifiable in a dynamic and reliable manner in line with clinical progression (34). CVA could meet this criterion, and this notion is supported by neuroanatomical foundations for the relation between CVA and self-regulation.

1.3.2 Neuroanatomical basis

The Neurovisceral integration model provides considerable neuroanatomical evidence of CVA as a marker of "trait" self-regulation (7, 11, 37). In short, the theory suggests that CVA reflects activity in a brain network termed the "central autonomic network" (CAN), which is crucial for self-regulation (11). Two important components of the CAN are the prefrontal cortex and the amygdala. In situations with efficient self-regulation, the prefrontal cortex exerts an inhibitory effect on the amygdala (7). This inhibitory activity is considered to be reflected in a higher CVA, as the brain areas have neural connections with brainstem nuclei involved in ANS regulation — including vagal activity (7). Interestingly, although not the main focus of the current dissertation, vagal activity also affects the same brain networks by reciprocal connections (14, 28, 37).

Importantly, the CAN is considered to be organized hierarchically (37). As such, the Neurovisceral integration model suggests that self-regulation might occur in a graded and flexible fashion, which is reflected in various levels of CVA (37). These hierarchical relations are believed to occur because the various neural levels serve to accommodate needs relevant for different situations (37). Whereas operations at lower levels of the hierarchy serve basic metabolic needs, higher levels allow for cognitive, emotional and social goal-directed behavior (37). As such, brain activity corresponding to higher levels of self-regulation would according to the model be reflected in a higher CVA compared to lower levels of self-regulation (37).

According to the Neurovisceral integration model, stress is a factor that might explain why some individuals tend to operate at lower rather than higher levels of this neural hierarchy (37). Stress is considered to reduce the influence of higher levels of the neural systems, resulting in less goal-directed behavior (37). Instead, learned or habituated behavior is promoted (37). Interestingly, this model aligns well with theories viewing self-regulation as a depletable resource, for instance in relation to stress.

1.3.3 Self-regulatory functions associated with cardiac vagal activity

Although the Neurovisceral integration model has increased the understanding of CVA and self-regulation, a limitation of the model is that it provides little specificity with regards to which aspects of self-regulation CVA marks. In the model, CVA has consistently been described as a marker of regulation of emotion, cognition and autonomic physiology (7, 11, 37). In the same model, CVA has also been suggested to reflect the regulation of stress, attention, inhibition, working memory, behavior, executive functions, social responses, affect and motivation (7, 11, 37). According to the authors, these processes might have a common basis, and therefore CVA could associate with all of them (7). Although this notion has been supported (10), other authors have noted that CVA seems to "mark one or more core self-regulatory functions that are disrupted across diverse forms of psychopathology" (8, 9). Thus, one might ask whether CVA loads more highly on certain aspects self-regulation, for instance related to internalizing or externalizing symptoms, than others. Such a clarification might increase our understanding of psychophysiology, and thus contribute to targeted applications of CVA in clinical psychiatry.

Lower CVA has been found in numerous psychiatric disorders where deficits in self-regulation might be considered to underlie the symptoms: Both internalizing (e.g. anxiety and depressive disorders; 38, 39), externalizing (e.g. substance abuse; 40) and thought disorders (e.g. schizophrenia; 41). Individuals with these disorders often have complex symptoms (see 34). For instance, internalizing symptoms often occur in individuals with disorders that are typically considered externalizing — such as anxiety in individuals with substance abuse (42). Therefore, the fact that CVA alterations have been detected in a sample with a given psychiatric disorder does not necessarily mean that these alterations are related to the core symptoms of the diagnosis.

One of the self-regulatory functions that have been proposed to be marked by CVA are executive functions: The cognitive aspect of self-regulation, including inhibitory control, working memory and task–set switching (43). Deficits in these functions often lead to externalizing symptoms (see 44), but can also lead to internalizing symptoms such as anxiety and depression (see 45). The notion that CVA reflects executive functions has been supported in several studies (8-10). However, there are some populations in which it is not supported — for instance in ADHD. Although ADHD is a disorder characterized by deficits in everyday behaviorally observed executive functions, a meta-analytic study has shown that resting CVA is not significantly altered in this patient population compared to controls (46). Consequently, there might be other aspects of self-regulation that CVA loads more highly on than executive functioning. For instance, CVA has regularly been suggested as a marker of emotion regulation (10, 11, 29). Notably, there is a substantial overlap between the regulation of emotions and the regulation of perceived stress. This is because overwhelming emotions often both leads to, and is a result of, perceived stress (47). As such, it is timely to ask whether a self-regulatory function that CVA might load highly on is the internal regulation of perceived stress.

1.4 Cardiac vagal activity as a marker of perceived stress regulation

Perceived stress (i.e., psychological stress) can be defined as “any environmental or intrapsychic event or process that disturbs or threatens to undermine an individual’s goal trajectory, and that comes to operate as an error signal within a self-regulatory system” (20). This can be viewed as an internal aspect of self-regulation. Notably, it is not equivalent to the experience of stressful life events, which may or may not lead to perceived stress responses (20). Moreover, perceived stress is not directly equal to physiological stress responses, as reflected in for instance increased HR and cortisol secretion (see 48). Still, these constructs are closely related, in that an increase of one often leads to an increase in the other (see 49). This is an important foundation for the use of CVA — which directly reflects physiological stress responses — as an indirect marker of psychological stress regulation.

Individuals differ in their resources for regulation of perceived stress (see 47, 50), and factors underlying these differences appear to be reflected in CVA (12). In support of this notion, meta-analytic findings suggest that individuals with lower CVA are more vulnerable to perceive stress (12, 14), compared to individuals with higher CVA. Again, the Neurovisceral integration model might provide a neuroanatomical explanation for this: CVA indexes inhibition by the prefrontal cortex on the amygdala, which signals threat responses (7). As such, during efficient self-regulation, threat perception is inhibited by the prefrontal cortex (7). However, in situations that are perceived as uncertain or threatening — perhaps due to impaired self-regulation — the prefrontal cortex becomes less active (7). It as such “allows” stress responses signaled by the amygdala, which results in a vulnerability to perceive threats and consequently initiate stress responses (7). According to the Neurovisceral integration model, such prefrontal cortical hypoactivity should be reflected in a lower CVA (7), and this notion has been supported by meta-analytic findings from neuroimaging studies (14). Moreover, lower CVA has been associated with psychiatric disorders showing prefrontal hypoactivity and poorer recognition of safety signals, such as anxiety disorders, depression, and schizophrenia (7, 38, 39, 41). Therefore, lower CVA in these psychiatric disorders might be related to deficits in the regulation of perceived stress.

Deficits in the regulation of perceived stress could contribute to difficulties with self-regulation in general, due to the aforementioned notion that stress depletes an individual's self-regulatory resources (18). Consequently, although CVA might load most highly on the internal regulation of perceived stress, it might also indirectly index other aspects of self-regulation, including functions leading to external behavioral outcomes. This idea could correspond well with results from previous studies, where CVA not only has been related to the regulation of perceived stress, but also for instance executive functioning and behavioral regulation.

Although there is some evidence suggesting that CVA might serve as a marker of perceived stress regulation, the interpretation of such a marker is doubtlessly complicated. Notably, a systematic review investigating associations between self-reported perceived stress and CVA (indexed by HF-HRV) as assessed by ambulatory devices in daily life conditions, detected a significant association between the variables in almost 50% of the cases (51). This was superior to the associations between perceived stress and other cardiovascular measures, such as HR or blood pressure (51). Although these results are promising, CVA cannot currently be used unequivocally as a marker of perceived stress regulation. As the interactions between environmental, psychological and physiological factors are extremely complex (19), interpretation of IBI markers is likely complicated by associations with contextual factors. As such, to further investigate the use of CVA as a marker of perceived stress regulation, there is a need to clarify its relation to various contextual factors.

1.4.1 Relation to contextual factors: Perceived social support and sex

An individual's capacity for self-regulation is tied to the context in which they live, including social contexts (16, 52). Hence, the association between CVA and perceived stress regulation is likely related to social factors. This is supported theoretically by the framework of Porges' polyvagal theory, which suggests that CVA marks a tendency to engage socially (15, 53). Studies of these relations — CVA, perceived stress and perceived social support — might be particularly relevant for individuals with psychiatric disorders.

Individuals with psychiatric disorders rarely suffer purely from symptoms directly related to perceived stress (e.g. anxiety or depression, see 16), but often tend to experience social difficulties as well. For instance, they often report low perceived social support (54) — the perception of how one's social network provides stress coping resources (55). This has been associated with numerous negative health outcomes (56) and increased mortality (57), in fact perhaps to a larger degree than received social support (see 58). This could be because individuals often find their social networks aiding in self-regulation even when others are not present, for instance by imagining their potential reactions (16).

Lower perceived social support has been associated with a lower CVA (59-63). Thus, perceived social support might be important to take into consideration in the potential use of CVA as a marker of perceived stress regulation in psychiatric populations. Notably, in order to design effective studies of psychiatric samples, one needs to better understand these relations in the general population. Studies of non-psychiatric samples could therefore aid in developing hypotheses for future studies of psychiatric samples.

In non-psychiatric samples, experimental studies have reported positive associations between perceived social support and CVA during stress tasks (63, 64). Moreover, individuals with higher perceived social support have shown higher CVA reactivity in response to such laboratory stressors (60). There are, however, few studies of these associations as assessed by resting CVA and self-reports. This might provide complementing insights to studies of CVA reactivity, as resting IBI indices are considered to mark "trait" factors. As such, they might reflect how everyday situations — for instance related to perceived stress and perceived social support — play out in individuals' lives to a larger degree than reactive CVA.

It appears that only one self-report study has previously investigated the relations between CVA, perceived stress and social support (65). This study assessed a mixed measure of constructive coping that included social support seeking — which is somewhat related to perceived social support (see 66). The results showed that higher CVA associated with higher constructive coping (including social support seeking) in relation to intermediate and high, but not low, perceived stress levels (65). This suggests that social aspects should be considered in the interpretation of CVA as a marker of perceived stress regulation — as CVA also might associate with constructive coping, such as social support seeking, in individuals with perceived stress. This appeared to be particularly relevant for individuals with intermediate or high perceived stress, as often found in psychiatric disorders (17). Still, there is a need to confirm these findings, preferably using a pure measure of perceived social support.

In the study of CVA, stress and perceived social support, it has been noted that it is important to consider sex as a contextual factor (63). Still, it has rarely been investigated in such studies (see 60), although there are well-established sex-differences in the relations between the investigated factors. For instance, females use social support more consistently than males while stressed (67). They also generally display higher CVA (68), and higher CVA reactivity during stress, compared to males (31, 69). Sex differences in CVA have also been suggested in a neuroimaging study in which participants were subjected to cognitive stressors: The correlation between CVA and blood flow in brain regions central for autonomic regulation — the anterior insula, the anterior cingulate cortex, the orbitofrontal cortex, and the amygdala — was positive in females, but negative in males (70).

The mechanisms for the aforementioned sex differences might have originated during evolutionary processes, where an alternative to the traditional “fight-or-flight” response to stress (71) might have developed in females (72, 73). Considering that females traditionally have been responsible for children or been pregnant, fighting or fleeing might not have been the optimal response for increasing chances of survival during stress (73). Instead, a “tend-and-befriend” response might have developed. This involves the creation and maintenance of social networks, which aid in nurturant activities for the female and their offspring (73). Importantly, the tend-and-befriend response is considered to be mediated by oxytocin (72, 73) — a hormone which has been shown to increase CVA (74). This underlines the notion that the association between CVA and perceived social support in relation to stress might vary as a function of sex.

In sum, a study of the relations between CVA, perceived stress, perceived social support and sex could aid in the investigation of CVA as a marker of perceived stress regulation. This could contribute to elucidate whether and how contextual factors should be taken into consideration in the interpretation and use of this marker.

1.4.2 Applicability to psychiatric disorders: ADHD

The notion that CVA loads higher on the regulation of perceived stress compared to other aspects of self-regulation might be valid also in patient populations with psychiatric disorders, and thus have potential clinical applications. This notion might resolve previous inconsistencies in studies of CVA in ADHD — where a meta-analysis by Koenig et al. did not detect resting CVA alterations (46). Still, another meta-analysis by Robe et al. showed lower CVA immediately after experimental tasks in ADHD samples compared to controls (75). Although the Robe et al. study studied resting CVA, the fact that it was assessed in relation to an experimental task might complicate the interpretation of whether it in fact indexes "trait" responses. Still, there are indeed theoretical reasons to believe that CVA alterations occur in ADHD (see 76). This is because individuals with ADHD have been reported to shows deficits in self-regulation (21) and relative hypoactivity in prefrontal cortical areas (77), which according to the Neurovisceral integration model should be reflected in a lower CVA (7, 11, 37). In the meta-analysis reported by Koenig et. al., the authors proposed an explanation of their non-finding: That CVA alterations occur only in certain subgroups with ADHD. Thus, studies of ADHD samples as a whole — primarily characterized by externalizing symptoms — might not have detected CVA alterations compared to controls. The authors suggested one such subgroup which might display CVA alterations: Those with difficulties with emotion regulation — an internal process which is crucial for the regulation of perceived stress (47). Such emotion regulation difficulties are often found in children and adolescents with ADHD (78).

ADHD is a neurodevelopmental disorder occurring in approximately 5% of children and adolescents worldwide (79). The diagnosis is based on symptom levels of inattention and/or hyperactivity-impulsivity (35, 36) – often described as externalizing symptoms (77). Still, as many as 25-45% of children and adolescents with ADHD display symptoms of emotion dysregulation, which often are internalized (78).

Emotion dysregulation is defined as deficits in emotion regulation: The potential to influence which emotions one has, and how and when one experiences and expresses these emotions (80). Effective emotion regulation is a crucial aspect of self-regulation which depends on several processes, such as access to effective emotion regulation strategies (80). Such strategies are central for the regulation of perceived stress because overwhelming emotions often both leads to, and is a result of, perceived stress (47). As such, ineffective strategies for emotion regulation might lead to higher perceived stress (47).

The association between emotion regulation difficulties and lower CVA has frequently been reported in non-psychiatric samples (10, 25, 29). Furthermore, lower CVA has been found to associate with less use of coping strategies that are considered constructive, for instance social support seeking (65, 81) and cognitive reappraisal (82). As such, CVA might be related to an individual's access to effective emotion regulation strategies.

In children and adolescents with ADHD, experimental studies have suggested altered CVA reactivity during emotion regulation compared to controls (For a review, see 83, and see 84 for a study published after the review). Findings from studies of resting CVA, however, are inconsistent: One study reported no association between emotion regulation difficulties and CVA (85). Yet, another study showed that a dichotomous measure of emotion regulation difficulties associated with a lower CVA (86). Moreover, a last study concluded that CVA might reflect emotion regulation abilities and adaptive behavior in ADHD (87), based on correlations between CVA and anxiety symptoms, social difficulties and oppositional behavior. As such, a further elucidation of the relation between resting CVA and emotion regulation in children and adolescents with ADHD is required.

Inconsistent results in studies of resting CVA and emotion regulation in ADHD might have occurred because previous studies have assessed parent-reports (85-87).

Self-reports of emotion regulation might be more closely related to CVA, as they describe internal emotional processes. Such non-observable internal processes might be imprecisely captured by parent-reports. In support of this argument, a study found that self-reported information associated better with observed neurocognitive dysfunctions in ADHD compared to both parent- and teacher reports (88). However, studies of resting CVA and self-reported emotion regulation difficulties have not yet been performed in children and adolescents with ADHD. This could aid in clarifying how strongly CVA loads on perceived stress regulation — as reflected by a crucial process for such regulation; access to effective emotion regulation strategies — compared to other aspects of self-regulation, in a psychiatric disorder. As individuals with ADHD might show both internalizing and externalizing symptoms (89) related to deficits in self-regulation, it is a particularly relevant population in which to study which core aspects of self-regulation CVA marks.

1.5 Novel inter-beat interval indices as markers of self-regulation

Although one might hypothesize that CVA indices load more strongly on certain core self-regulatory functions — such as the regulation of perceived stress— than others, it is possible that IBI analyses could indeed provide information about self-regulation in general, and serve as a marker of both internalizing and externalizing symptoms, as proposed by the Neurovisceral integration model (7, 11, 37). However, the currently applied methods might not be sensitive enough to detect IBI alterations associated with all self-regulatory processes. This hypothesis was proposed by Koenig et al. in the meta-analysis of CVA in ADHD, as a possible explanation of their non-findings (46). Novel methods for IBI analysis might be more sensitive in detecting such alterations.

Deficits in self-regulation might occur due to spontaneous and transient changes in brain activity (see 90), for instance due to burst discharges of neurotransmitters (see 91), which might be reflected in IBI alterations occurring for short periods of time. IBI analyses based on linear models, such as the commonly applied time- and frequency domain methods, might not be sensitive enough to detect such alterations (5). This is because these methods compute IBI indices such as CVA from summary statistics of longer recordings: One minute is the minimum requirement, and the standard length is five minutes (92). Consequently, the computed IBI indices may be similar across two IBI series, despite originating from series with distinctly differently organized IBIs.

Another limitation with linear methods is based on the notion that IBI organization is considered to be nonlinear, due to the complexity of the mechanisms that regulate HR (93, 94). The Neurovisceral integration model underlines that activity of the CAN, which is reflected in IBIs, has features of a nonlinear system (11). As a result, relations between consequent IBIs cannot be plotted precisely along a straight line, as linear methods assume. Complex features of IBI organization might consequently go undetected by linear analyses. In contrast, nonlinear methods might increase the sensitivity, and possibly specificity, of IBI indices as markers of self-regulation.

Nonlinear methods based on concepts such as complexity, chaos and fractality have led to novel insights into IBI organization (94-96). These methods often provide the opportunity to investigate shorter segments of an IBI series at a time (see 96, 97). They might therefore be more sensitive to spontaneous or transient IBI alterations compared to linear methods. Moreover, one might hypothesize that the investigation of shorter IBI segments might reveal subtle disorder-specific IBI alterations. As such, while IBI indices from linear analyses are considered to mark general self-regulation across disorders (8, 9), nonlinear methods might detect specific IBI alterations in different psychiatric populations.

Although nonlinear methods provide important insights into IBI organization, the currently applied methods are not without limitations (see 96). Firstly, the analyses might be hard to compute or interpret (96). Some methods are further highly vulnerable to erroneousness if parameter choices are non-optimal, and others are sensitive to ECG lengths or artifacts (96). Other methods have been criticized for not utilizing all available data (96). In addition, some nonlinear approaches might reflect autonomic activity inaccurately (98). On account of such limitations, the development of nonlinear methods is still warranted to complement established analyses.

Graph theory is a promising mathematical field for application to nonlinear analyses of IBIs, which has led to important insights into various brain disorders in neuroimaging studies (99, 100). Such methods provide a novel mathematical perspective on IBI organization, as every IBI is represented in a graph: A set of nodes and edges. Each IBI is visualized as a point — a node — and similar IBIs are connected by a line — an edge (101; see figure 1.3). As such, a graph highlights similarities in IBIs. Different criteria of similarity — thresholds for defining IBIs as similar — provide different graphs that reflect various degrees of IBI similarity. In another nonlinear method, sample entropy, it is for instance customary to apply a threshold of the 20% of the standard deviation (SD) of the IBIs for defining IBIs as similar (102, 103).

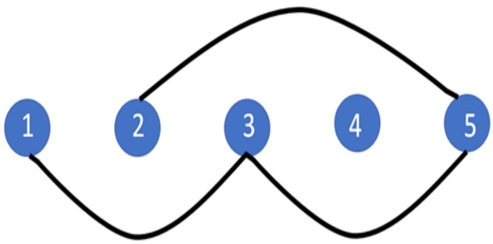

Figure 1.3. Example of a graph. An edge (black line) is introduced between two nodes (blue dots) if they are similar. Each node corresponds to an IBI. Node nr. 1, 3, and 5, and node nr. 2 and 5, are connected by edges. Figure retrieved from (3).

A graph theory-based, nonlinear method that might be applied to IBI series is the *similarity graph algorithm*. It has previously been used in studies of motor activity (104, 105). The algorithm investigates shorter segments of an IBI series — *time windows* — at a time. The values computed from each time window can then be averaged in order to compute an index representing the IBI series. By the time window approach, each IBI is compared to its nearest past and future. This beat-to-beat analysis might be more sensitive to spontaneous and transient changes than analyses of longer IBI series. Moreover, different time window sizes create different graphs. To describe the size of a time window, the term *neighbors* is used: The number (k) of preceding or subsequent nodes around the index node, i.e. the node considered by the algorithm at a given time (See figure 1.4). In

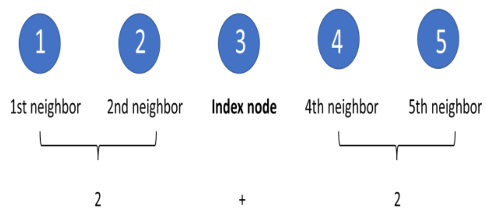

Figure 1.4 *A time window of 2+2 neighbors. The index node (3) has two rightmost neighbors (i.e. nodes) (1,2), and two leftmost neighbors (4,5).*

the similarity graph algorithm, practically every node in the IBI series serves as an index node, of which the time window "slides" and centers around. Thus, every (index) node has a total number of 2k neighbors, denoted as *k*+*k*. For instance, a time window of 2+2 neighbors includes two neighbors (i.e. nodes) on each side of the index node, and thus consists of five nodes (See figure 1.4). Consequently, such a time window considers a shorter IBI segment at a time than for instance a time window of 10+10 neighbors does.

There are numerous indices that can be deducted from graphs. Yet, previous graph theory-based investigations of IBI series attempting to differentiate two populations appear to have investigated only one index (106, 107). This might be insufficient in characterizing complex physiological systems such as IBI organization (see 108). The assessment of several IBI indices could provide a more complete insight into IBI alterations. The similarity graph algorithm assesses several indices familiar from graph theory. These reflect moment-to-moment IBI similarity: *Inter-relatedness*. As further detailed in the Methods section of the current dissertation, the indices of the similarity graph algorithm provide different perspectives on inter-relatedness or lack of inter-relatedness. In addition, two ratios of inter-relatedness across a longer compared to a shorter time window are calculated: Edges10+10/edges2+2 and edges10+10/edges5+5. These ratios might be viewed as the inverse of the LF/HF ratio from frequency analyses, although the use of time windows might provide more refined indices. The most sensitive of the graph theory-based ratios is likely edges10+10/edges2+2, which compares the inter-relatedness in two distinctly different time windows (i.e. 10+10 and 2+2 neighbors). The time windows investigated in edges10+10/edges5+5 differ less in terms of length (i.e. 10+10 and 5+5 neighbors), and the ratio might therefore be less sensitive.

Higher inter-relatedness might reflect altered ANS activity, similarly to a lower HRV or CVA. Supporting this notion, graph theory-based methods have found lower IBI complexity (i.e. higher similarity) in individuals with heart disease and the elderly (106, 107) — populations that often have a lower CVA (109, 110). Using the similarity graph algorithm, vagal alterations might be most accurately captured in time windows less than five seconds. This is because SNS effects are not rapid enough to influence IBIs substantially in this timeframe (see 24). As such, indices of inter-relatedness in time windows longer than five seconds might represent other aspects of ANS regulation, in addition to vagal modulation, on the heart. In sum, one could hypothesize that IBI indices from analyses of time windows less than five seconds are markers of self-regulation, similarly to CVA. However — possibly in contrast to CVA — these IBI indices might mark self-regulation in general, as they could be more sensitive in detecting IBI alterations compared to linear IBI analyses of CVA.

As linear time- and frequency domain analyses might not be sensitive enough to detect clinically relevant IBI alterations in the ADHD population (see 46), it is a highly relevant population for investigating IBIs with the similarity graph algorithm. Notably, self-regulatory deficits in ADHD are often considered to be caused by changes in noradrenaline and dopamine functioning (77, 91), which affect the ANS (111, 112). As dopamine often is discharged in bursts (91), these discharges might be reflected in spontaneous and transient IBI alterations. These might be detected by nonlinear methods such as the similarity graph algorithm. Consequently, studies of IBI series with the similarity graph algorithm might aid in the investigation of IBI indices as sensitive and specific markers of self-regulation.

2. Background and aims

As presented, IBI indices of CVA are often considered as markers of self-regulation (see 8, 9, 11). Still, there is a need to elucidate which core self-regulatory function(s) CVA marks. There are reasons to believe that CVA could load highly on the internal regulation of perceived stress. In this regard, there is a need to investigate how this marker is related to contextual factors, and whether it can be applied in patient populations with psychiatric disorders. Furthermore, improvement of the sensitivity and specificity of IBI indices as markers of self-regulation would support its clinical utility.

The objective of the current dissertation is to contribute to the investigation of CVA as a marker of self-regulation, and specifically of perceived stress regulation, compared to as a marker of behavioral regulation. As such, it investigates how this marker is related to contextual factors, and its applicability in samples with ADHD. In addition, it aims to investigate a novel method for IBI analysis, which derive indices that might reflect general aspects of self-regulation, related to both internalizing and externalizing symptoms. Specifically, we first performed a study of the associations between CVA and perceived social support in relation to perceived stress in males and females. Secondly, we studied the associations between CVA and emotion regulation — an important process for the regulation of perceived stress (47) — and CVA and behavioral regulation in adolescents with ADHD. Lastly, we investigated the use of a nonlinear, graph theory-based method for IBI analysis: the similarity graph algorithm. As the method had not been applied to IBIs previously, we investigated as a proof of concept whether it could differentiate a sample with psychiatric disorders, exemplified by ADHD, from controls. Future studies might further examine the sensitivity and specificity of the IBI indices derived from this method as markers of self-regulation.

In sum, the current dissertation might contribute towards the goal of improving the diagnostics, monitoring and treatment of the numerous psychiatric disorders that are associated with deficits in self-regulation.

2.1 Research questions and hypotheses for paper I

Overarching research question: How does CVA associate with contextual factors such as perceived social support and sex in relation to varying levels of perceived stress?

Hypothesis:

H1: Based on previous studies and the tend-and-befriend model, we expected that higher CVA would be associated with higher perceived social support specifically under conditions of higher, compared to lower, stress levels. We further expected that this association would be most prominent in females compared to males.

2.2 Research questions and hypotheses for paper II

Overarching research question: Is there an association between CVA and self-reported difficulties with emotion regulation— an important process for perceived stress regulation — in adolescents with ADHD and controls?

Sub-question:

1. If so, which facet(s) of emotion regulation difficulties are associated with CVA: Difficulties with acceptance of emotional responses, goal-directed behavior, impulse control, emotional awareness, access to effective emotion regulation strategies, or emotional clarity?
2. Is CVA associated with dimensional symptoms of inattention and hyperactivity-impulsivity and the diagnostic status of ADHD, as representations of behavioral regulation, in the same sample?

Hypotheses:

H1: Based on previous studies in normally developed samples, we expected that lower CVA would be associated with emotion regulation difficulties. Furthermore, we expected that this effect would be more prominent in the ADHD group compared to the control group.

H2: Based on previous studies in normally developed samples suggesting associations between CVA and emotion regulation strategies, we hypothesized that

among the different emotion regulation facets, lack of access to effective emotion regulation strategies would be most highly associated with CVA.
H3: Based on a meta-analysis reporting no CVA alterations in individuals with ADHD compared to controls, we did not expect CVA to associate significantly with dimensional symptoms of inattention and hyperactivity/impulsivity, nor with the ADHD diagnosis.

2.3 Research questions and hypotheses for paper III

Overarching research question: Can a novel method based on principles from graph theory, the similarity graph algorithm, differentiate IBI organization in adolescents with ADHD compared to controls?

Sub-questions:

1. Which criterion of similarity of the similarity graph algorithm best illustrates differences in IBI organization between adolescents with ADHD and controls?
2. Applying this criterion of similarity to the similarity graph algorithm in three different time windows, are there detected differences in IBI indices in adolescents with ADHD and controls? If so, which kinds of differences are detected, in which time window(s) and in which indices?
3. For comparison, which differences in IBI indices are detected by traditional time- and frequency domain analyses in the same sample?
4. In order to investigate the indices' specificity: How are the IBI indices from the time- and frequency domain analyses and similarity graph algorithm affected by comorbidities and symptoms of trait anxiety?

Hypotheses:
H1: We expected that IBI differences between the ADHD and control group would best be illustrated with a criterion of similarity of 20% of the SD of the IBIs. This hypothesis was based on previous work with another nonlinear method, sample entropy.
H2: We expected that the similarity graph algorithm would detect a higher inter-relatedness due to a higher number of similar IBIs in adolescents with ADHD compared to controls. This was based on the assumption that altered vagal

functioning in the ADHD group, which was expected based on previous studies and theoretical frameworks, could lead to less effective regulation of HR and thus more similar IBIs. We expected that analyses of time windows corresponding to less than five seconds would best illustrate these IBI differences. This was based on the notion that this time window would provide the most refined indices of vagal activity, because the vagus nerve exerts its actions more rapidly than the SNS. Furthermore, we expected these differences to be most prominent in IBI indices of higher inter-relatedness (i.e. edges, components maximum edges, cliques; Outlined in Methods section) compared to indices of lower inter-relatedness (i.e. missing edges, zero edges; Outlined in Methods section). This was based on the notion that it is generally easier to distinguish groups based on findings that are present rather than absent. Lastly, we expected to find a lower edges10+10/edges2+2 in the ADHD group — and that this ratio would be more sensitive than edges10+10/edges5+5 — as it was hypothesized to be inversely related to the LF/HF ratio, which is often higher in ADHD samples compared to controls (87, 113).

H3: Based on previous studies, we expected that IBI indices which do not primarily reflect vagal influence (i.e. SDNN, LF-HRV and LF/HF ratio; Outlined in the Methods section), would suggest altered ANS functioning in the ADHD group compared to controls. We did not expect indices reflecting vagal influence (i.e. RMSSD, HF-HRV; Outlined in the Methods section) to differ in the diagnostic groups, based on meta-analytic evidence (46).

H4: As traditional HRV indices are considered to index self-regulation across disorders, we expected results from time- and frequency domain analyses to be influenced by the number of comorbid disorders and symptoms of trait anxiety in the sample. We expected that the similarity graph algorithm might detect more disorder-specific IBI alterations, and hence that its indices would not be as affected by comorbidities and trait anxiety symptoms.

3. Methods

Followingly, the most relevant methodological aspects for the interpretation and discussion of the results will be described. For detailed methodological descriptions, tables and figures, the reader is referred to the attached printouts of the articles.

3.1 Methodological aspects overarching the dissertation

3.1.1 Common features of samples in paper II and III

The samples described in paper II and III are sub-samples from a common project, a follow-up study on emotion regulation in children and adolescents with ADHD (Stoppventgå; Department of Biological and Medical Psychology, University of Bergen). The project was conducted in two waves. As ECGs were collected only in the second wave, the sub-samples described in paper II and III are from this wave.

The sample of paper III comprised all participants in the second wave of the project, except from those who met exclusion criteria: A full-scale IQ of less than 75, loss of consciousness after a head trauma, suspected autism spectrum disorder, birth before gestation week 36, or ADHD symptoms in control participants. This applied to three participants (ADHD: *n*=1, Controls, *n*=2). The sample of paper II consisted of the same group as in paper II, except individuals who did not provide information on the Difficulties in Emotion Regulation Scale (DERS; outlined below) — who were excluded (See figure 3.1. for a flow chart of sample selections and retention rates)

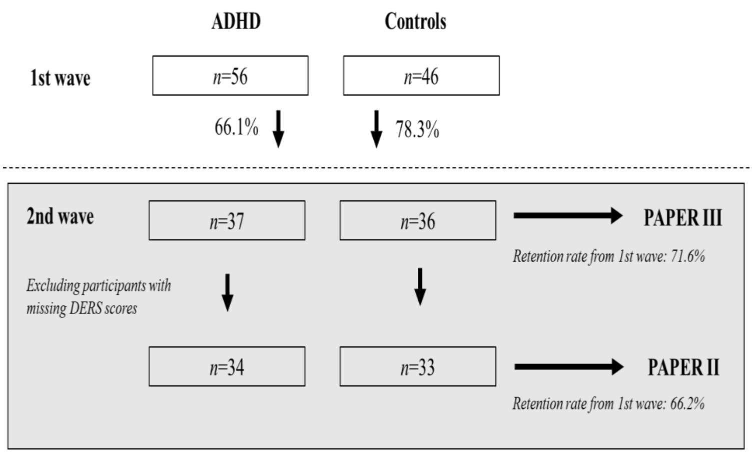

Figure 3.1 *Flow chart of selection of samples for paper II and III*

As participants in the studies of paper II and III were from a common sample, recruitment, diagnostic group assignment and comorbidities was similar for the two samples. These aspects will be described in the Methods section for paper II, before reference is made to this section in the description of methods for paper III.

3.1.2 Collection, preparation and analysis of IBI data

The following section will describe shared features of the collection, preparation and analysis of IBI data in the three papers of the current dissertation. Additional procedures will be described in the Methods sections of the respective papers.

Before initiation of the resting ECG recordings, participants were instructed to relax and not fall asleep. Three adhesive electrodes conducted the signal (simple lead II setup, sampling rate 1,000 Hz), while participants breathed spontaneously.

The Kubios HRV software was used in preparation and analysis of the ECG recordings (114). A frequency analysis with the Fast Fourier Transformation was performed, with activity in the HF band as the applied index of CVA (see 6). The frequency bands were transformed with their natural logarithm to approximate a normal distribution (6). Moreover, respiratory effects on IBIs (see 115) were indirectly derived (outlined below).

The Statistical Package for the Social Sciences (SPSS; IBM Corp., Armonk, NY, USA, 2016) was used to conduct statistical analyses.

3.1.3 Calculation of body mass index

In all studies of the current dissertation, the participants' body mass index (BMI) was calculated from measurements of height and weight, by the formula of weight/height^2 (kg/m^2). BMI has been shown to associate with IBIs (116).

3.1.4 Missing data

In all studies of the current dissertation, some data was missing due to missing responses from the participants or technical errors. Missing values were imputed with the sample mean. As missing items generally corresponded to less than 7% of the total number of observations for the variable in question, such imputation with the sample mean was considered adequate (see 117). The exception to the rule of imputation with the sample mean was for a missing ADHD-RS score for one participant with ADHD, which was imputed with the mean of the ADHD group.

3.1.5 Outliers and skewness

Outliers — i.e., values >±3SD than the sample mean — in IBI indices and total scores of the applied questionnaires were assessed in all studies of the current dissertation.

No outliers were detected in the questionnaire scores in any of the studies. The exception was the International Physical Activity Questionnaire (IPAQ; outlined below) applied in paper I, which displayed several outliers and a severely skewed distribution. It was therefore transformed into a categorical variable.

As for the IBI indices, an outlier in CVA was detected in the study of paper I. As we could not rule out that it had occurred due to underlying somatic issues, the participant was excluded. In addition, one outlier was detected in the graph-theory based indices of missing edges and cliques (outlined below) in the study of paper III. There were no previous studies of how these indices relate to heart function. We considered excluding the participants, but chose to include them to maintain sample size and thus statistical power, as we detected only one outlier in the graph theory-based indices that were examined in these participants. Moreover, as we considered the outliers to be realistic, we chose not to impute them with mean values. Moreover, the cliques displayed a severely skewed distribution in the sample. Therefore, we performed statistical analyses of the variable which did not assume a normal distribution.

We also detected one to three outliers in BMI values in paper II and III, which were imputed with the sample mean.

3.2 Paper I

3.2.1 Sample

The sample consisted of *n*=143 university students. The participants were 18-38 years old (mean age: 19.9), and 57% were male (*n*=90).

The study was based on cross-sectional data from the Ethnic Differences in the Experience of Noxious Stimuli (EDENS) study at the Ohio State University, USA, in which students were recruited by study advertisement. Inclusion criteria of the current sub-study were available data on CVA, the Medical Outcomes Study Social Support Survey (MOS; outlined below) and the Perceived Stress Scale (PSS; outlined below).

3.2.2 Procedure

Assessments were completed at one appointment or within one week. First, a baseline assessment and physical examination was completed, including measurement of weight and height, an ECG recording, and completion of a standard socio-demographic questionnaire on education and a set of other questionnaires (outlined below): The IPAQ, the Alcohol Use Disorders Identification Test (AUDIT), and the Drug Abuse Screening Test (DAST). Secondly, the participants underwent an extended psychological assessment, with questionnaires including the MOS and PSS.

3.2.3 Measures

Cardiac vagal activity

The general procedures for CVA assessment were described in “3.2: General Methodologic Considerations”. Specific for the current study, IBI indices were calculated from five-minute recordings. The root mean square of successive IBI differences (RMSSD), reflecting vagal modulation on the heart (5), was computed as an additional index of CVA. Moreover, mean HR, and EDR as a proxy of respiration was assessed, as these factors might influence CVA (5, 115).

Questionnaires

The short version of the MOS (118, 119) assessed perceived social support. Eight situations (“If you needed it, how often is someone available… , e.g. to turn to for suggestions about how to deal with a personal problem?”) were rated on a 5-point Likert-type scale from 1 (“none of the time”) to 5 (“all of the time”). The psychometric properties of the MOS have been characterized as excellent (120).

The short form of the Perceived Stress Scale (PSS; 121) assessed perceived stress. Participants were asked to rate 10 items about their thoughts and feelings the last month (e.g. “How often have you found that you could not cope with all the things that you had to do?”) on a Likert-type scale from 0 (“never”) to 4 (“very often”). The validity and reliability of the questionnaire has been found satisfactory (48).

Physical activity levels, which might influence IBIs (122), were assessed by the 4-item short form of the IPAQ (see https://sites.google.com/site/theipaq/, 123). Its validity and reliability is considered satisfactory (123, 124). In the IPAQ, participants reported how many sessions and the average time per session they had spent on physical activity the last week. This was reported for three intensity categories: sitting, moderate intensity, and vigorous intensity. The time spent on physical activity in these intensity categories was converted to metabolic equivalents (METs): A measure of energy expenditure during the activity (see 125). In the current study, the METs expended during vigorous intensity activities was chosen as the applied measure of physical activity.

Alcohol consumption was assessed by the AUDIT (126). Participants were asked to rate the 10 items (e.g. How often do you have a drink containing alcohol?) on a five-point Likert-type scale from “never” to “four times a week or more”. The AUDIT has shown good internal consistency (127) and sensitivity (128).

The short form of the DAST (129) was used to assess drug use. The participants answered “yes” or “no” to 10 items asking for drug-related behavior the last 12 months (e.g. “Have you used drugs other than those required for medical reasons?”). The DAST is considered to have adequate validity and internal consistency (130)

3.2.4 Statistical procedure

In preliminary analyses, independent samples t-tests investigated sex differences in CVA, age, BMI, mean HR, EDR, and PSS, MOS, AUDIT and DAST scores. Sex differences in IPAQ scores and educational levels were investigated by chi-square tests. Furthermore, bivariate correlations were assessed between CVA, MOS and PSS scores, age, educational levels, BMI, EDR, mean HR, and IPAQ, AUDIT and DAST scores. Moreover, to investigate the required sample size to detect expected statistical effects in the main analysis (outlined below), an a priori estimation of statistical power was performed. The estimated effect size was based on two comparable studies of the association between CVA and perceived social support (63, 65) which detected medium effect sizes (f^2 = 0.20 and 0.21, respectively). In the current study, we assumed a small to moderate effect size: $f^2 = 0.15$. We used the following predictors in g*power: CVA, PSS scores, sex, the interaction of these variables, and IPAQ scores (which covaried significantly with MOS scores, as reported in the Results section). The level of sufficient power was specified as $1-\beta = 0.80$, and the significance level as $\alpha = 0.05$.

As the main analysis, a moderation (i.e. interaction) analysis, with model 3 in the PROCESS v.3.5 macro (131) of SPSS was performed (see Figure 3.4). The moderation analysis examined the hypothesis that higher CVA would predict higher MOS scores only in relation to high and intermediate PSS scores, and that this would be detected specifically in females. As the independent variable, we used CVA, and MOS scores were used as the dependent variable. PSS scores and sex were included as moderators. Age, educational levels, BMI, EDR, mean HR, and IPAQ, AUDIT and DAST scores were added as covariates. In the final moderation analysis, these covariates would only be included if they displayed a significant covariation with the MOS scores. The moderation analyses consisted of a linear regression analysis, and an analysis of the effects of varying PSS scores on the relation between CVA and MOS. In the latter analysis, PSS scores were described for low (16th percentile), intermediate (50th percentile) and high (84th percentile) levels.

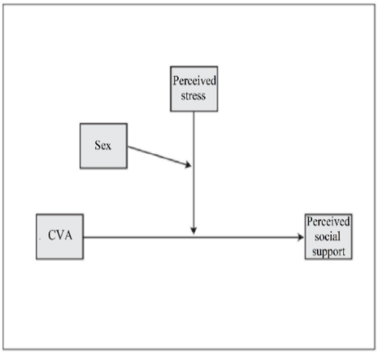

Figure 3.4. *The applied moderation model. Figure retrieved from (2).*

Lastly, we aimed to test a reversal of the model direction, to investigate if MOS scores predicted CVA. As such, the moderation analyses were repeated using MOS scores as the independent variable and CVA as the dependent variable.

3.3 Paper II

3.3.1 Sample

The sample consisted of adolescents with ADHD (*n*=34) and controls (*n*=33). The participants were between 11 and 17 years old, and 64% were male (*n*=43, of 67).

In the first wave of the project that the study was based on, child and adolescent psychiatry units in Bergen referred children with ADHD symptoms. Control participants were recruited from schools in the same geographical area. Participants were appointed to an ADHD or control group by a semi-structured diagnostic interview: the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (K-SADS; 132). The Norwegian translation, which has shown satisfactory divergent and convergent validity for ADHD diagnostics, was applied (133, 134). In the second wave of the study, the K-SADS was repeated to confirm the diagnostic group status that had been assigned in the first wave. Criteria from the Diagnostic and Statistical Manual of Mental Disorders – Fourth edition (DSM-IV) were used (135). The K-SADS interview also investigated comorbid diagnoses and physical activity levels. An experienced psychologist performed the interview, and another contributed to the final diagnostic assignment. All participants in the ADHD group still met the criteria for the diagnosis, except for one with symptoms in remission. The ADHD group had frequent comorbidities, and some psychiatric disorders (other than ADHD) were present in the control group.

3.3.2 Procedure

Participants using ADHD medication were asked to conduct a 48-hours washout period before participation, as such medication could influence IBIs (136) and emotion regulation (137). Assessments were performed over two days, in which participants and their parents took part. Test administrators were blinded to diagnostic group statuses.

ECGs were recorded on the first day of testing. To minimize circadian rhythm effects on IBIs (138), recordings were conducted between 9 a.m. and 1 p.m. Moreover, the participants' height and weight were measured. On the second day, the K-SADS interview was performed separately for the participants and their parents.

Parents completed several questionnaires, including the ADHD-RS, and adolescents a set of questionnaires including the STAI and the DERS (outlined below).

3.3.3 Measures

Cardiac vagal activity

The general procedures for CVA assessment were described in "3.2: General Methodologic Considerations". The following section describes details specific for the current study.

Nineteen participants used ADHD medication regularly. Most of them (*n*=17, 89.5%) completed a washout period of at least 24 hours. Two participants using methylphenidate had shorter washout periods: 18- and >12-hours, respectively. These time frames were acceptable as the half-life of methylphenidate is two to three hours, i.e., the active ingredient is eliminated after 10-15 hours (139, 140). Moreover, two participants with ADHD used lamotrigine, and one used sertraline. Both conducted a 48-hour washout period. One control participant used risperidone and melatonin, and did not conduct a washout period.

During the ECG recordings, participants were lying in a supine position. The ECG was recorded for six minutes, and the first five minutes were analyzed. Activity in the HF band was reported in normalized units (see 114). As a proxy for respiratory effects, the peak frequency of the HF band (HF peak; see 141) was computed.

Questionnaires

The DERS (142) is a self-report questionnaire assessing difficulties in emotion regulation. The Norwegian translation (143) was applied in the current study. Using a five-point Likert-type scale from 1 ("almost never") to 5 ("almost always"), 36 items (e.g. "I experience my emotions as overwhelming and out of control") were rated. The questionnaire yields a total score (DERS TOTAL), as well as scores on six subscales reflecting aspects of emotion regulation difficulties related to: a) Acceptance of emotional responses (NONACCEPTANCE); b) Goal-directed behavior (GOALS); c) Impulse control (IMPULSE); d) Emotional awareness (AWARENESS); e) Access to effective emotion regulation strategies (STRATEGIES); and f) Emotional clarity (CLARITY). The DERS has shown adequate to high internal consistency and satisfactory construct validity in adolescent samples (144, 145).

In the Norwegian translation (146) of the DSM-IV ADHD-RS (147), parents rated how well 18 statements of inattention (e.g. "Does not seem to listen when spoken to directly") and hyperactivity-impulsivity (e.g. "Has difficulty playing quietly") fitted their child. A four-point Likert-type scale from 0 ("Never") through 3 ("Very Often") was applied. Total scores indexed ADHD symptom severity. The ADHD-RS has shown high internal consistency and construct validity (148).

Physical activity levels

Adolescents reported their weekly engagement in sports and exercise in the K-SADS interview. Based on this information, physical activity levels were registered on a six-point Likert-type scale from 0 ("zero times a week") to 5 ("seven times or more a week"). The scoring norm was adapted from the Physical Activity Questionnaire for Adolescents (PAQ-A; 149), which has shown satisfactory convergent validity (149).

3.3.4 Statistical procedure

Preliminary analyses with the independent samples t-test investigated differences between the ADHD and control groups in age, sex, physical activity levels, BMI, ADHD-RS and DERS scores, HF peak and CVA. Moreover, bivariate correlation analyses between the DERS scores and CVA were performed. Similar analyses assessed whether the DERS scores and CVA, respectively, correlated with age, physical activity levels, BMI, ADHD-RS scores and HF peak.

Multiple linear regression analyses tested the hypothesis that lower CVA would predict higher DERS TOTAL and STRATEGIES scores. As dependent variables, the DERS TOTAL and the six subscale scores, respectively, were included. Diagnostic group status (ADHD/control) and CVA were used as predictors. Analyses controlled for effects of age, sex and HF peak by including these as predictors. The model was further adjusted for effects of physical activity levels and/or BMI if these variables correlated with CVA and/or DERS scores in the preliminary analyses. Based on Bonferroni correction for multiple testing, an alpha level of $p = .007$ (.05/7) was applied. As follow-up analyses, an interaction term of ADHD*CVA was added as a predictor of the DERS TOTAL and subscale scores in the regression model.

As supplemental analyses, the multiple linear regression analyses were first repeated in the ADHD and control groups separately. Secondly, controlling for effects of using a mixed participants control group, adolescents with psychiatric disorders in the control group were excluded, before the linear regression analyses for DERS scales that had been significantly predicted by CVA were repeated in the total sample.

3.4 Paper III

3.4.1 Sample

The sample consisted of *n*=37 adolescents with ADHD and *n*=36 controls between 10 and 18 years old. The majority of participants were male (*n*=48, 66% of the sample).

Information regarding recruitment, diagnostic group assignment and comorbidities is similar to the description in “3.3.1. Sample; Paper II”.

3.4.2 Procedure

The procedure of the current study is described in “3.3.2. Procedure; Paper II”.

3.4.3 Measures

Cardiac vagal activity

22 participants (60% of the ADHD group) used ADHD medication regularly. 20 of these (91%) performed a washout period of at least 24 hours. Two participants conducted shorter washout periods, and some participants used other medications as well (See 3.2.3. Cardiac vagal activity; Measures; Paper II).

The general procedures for CVA assessment were described in “3.2: General Methodologic Considerations”. Specific for the current study, participants were lying in a supine position as the ECG was recorded for six minutes. The number of IBIs in every ECG, based on the first five minutes of the recordings, was then counted. The lowest number of IBIs in any of the recordings corresponded to 237. As we aimed to assess a constant number of time windows in order to investigate the relative inter-relatedness of all the IBI series, the first 237 IBIs of every series was analyzed.

In addition to the similarity graph algorithm (outlined below), time- and frequency domain analyses were performed. Also in these analyses, we analyzed the first 237 IBIs of the recordings, although the standard is five minutes (5), to compare IBI indices from the different methods on a similar basis. The time domain analyses yielded indices of the SD of normal IBIs (SDNN) — reflecting total variability in HR — and RMSSD, reflecting vagal modulation on the heart (5). In the frequency domain analyses, activity in the HF and LF bands, and the LF/HF ratio, were calculated.

HF peak as a proxy of respiratory effects on IBIs was calculated (141). Moreover, as the use of specific frequency bands for children and adolescents has been suggested (150), the mean respiratory frequency in the sample was deducted by the ECG derived respiration (EDR), estimating respiration rate from R wave amplitudes (151). We would consider our use of adult frequency bands as supported if the respiration rate in the current sample was found to be within the normal range for adults: .20–.33 Hz (i.e. 12-20 breaths/min; 152).

Lastly, we performed IBI analyses with established nonlinear methods: Sample entropy (103) and recurrence quantification analyses (RQA; 153). These methods have often been applied to IBI series, of which they assess indices of complexity.

Questionnaires and physical activity levels

The STAI (154) is a self-report questionnaire of state and trait anxiety symptoms. The Norwegian translation (155) was applied in the current study. As anxiety disorders have been associated with alterations in IBI indices (38), the trait anxiety subscale (STAI-T) was applied. It consists of 20 items (e.g., "I worry too much over something that really doesn't matter") rated on a four-point Likert-type scale from 1 ("Almost never") to 4 ("Almost always"). The STAI has generally shown adequate internal consistency, test-retest reliability (154) and construct validity (156).

The questionnaires applied for ADHD symptoms is described in "3.3.3.: Measures; Paper II".

Physical activity levels

The method for assessment of physical activity levels is described in "3.3.3.: Measures; Paper II".

3.4.4 Statistical procedure

Graph theory principles and terminology

The following overview of graph theory principles and terminology and the similarity graph algorithm is based on the original publication of the method (104). Aspects described in the Introduction section of the current dissertation will be elaborated on.

A graph is a mathematical structure visualizing associations between objects. In relation to an IBI series, a graph is made up of a group of nodes and edges (if any): Every IBI corresponds to a *node*, and a two-element subset connecting two nodes is an *edge* (101). A connection by an edge imply that the nodes fulfill the *criterion of similarity,* a predefined threshold for defining two nodes (i.e. IBIs) as similar.

The similarity graph algorithm

The similarity graph algorithm is a nonlinear, graph-theory based method for analysis of time series. In the algorithm, every node function as an *index node* — the node considered by the algorithm at a given point in time — except for the first *k* and last *k* IBIs of the time series. The *k* denotion refers to the number of nodes on either side of the index node (i.e., neighbors) selected for analysis, making up a *time window*. The time window "slides" through the time series and centralizes on the index node, and the nodes and edges in this time window make up a *subgraph* (See figure 3.2). Nodes in the time window that fulfil the *criterion of similarity* will be connected by edges.

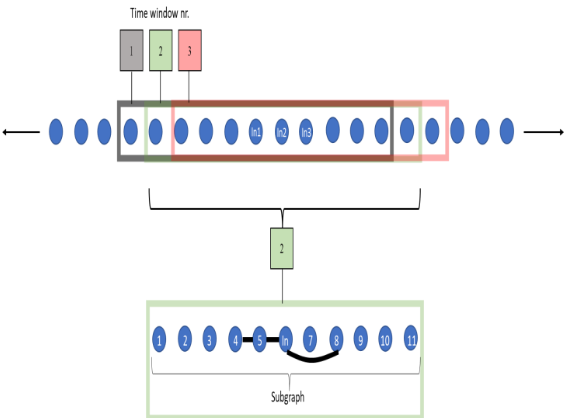

Figure 3.2. *Illustration of index nodes, time windows and subgraphs. Every node in the IBI series is applied as the index node (In1-3) considered by the similarity graph algorithm. The figure shows three time windows of 5+5 neighbors, with five nodes (i.e. neighbors) on each side of the index node. Nodes are connected by edges if they fulfil the criterion of similarity. This collection of nodes and edges is a subgraph. Figure retrieved from (4).*

In sum, an edge is created between two nodes if they a) meet the criterion of similarity, and b) are in the same time window. Based on its nodes and edges (if any), a graph exhibits topological properties that can be studied by well-known principles from graph theory. In the current study, the following indices were computed:

Edges: The mean number of edges in all time windows of the graph.

Components: A graph consists of separate components: A sequence of edges connecting a sequence of nodes. The different components are not interconnected by edges. A node with no edges is regarded as a component. The number of components in each time window of the graph, and their sum across the graph resulted in the index of *components* (See figure 3.3).

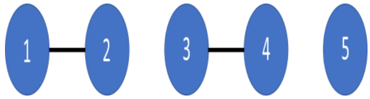

Figure 3.3 *Illustration of components. The subgraph consists of three components: Those including node nr. 1 and 2, nr. 3 and 4, and nr. 5, respectively.*

Missing edges: The number of subsequent nodes that were not connected by an edge in a given time window, summed up from all time windows in the graph.

Maximum edges: The highest number of edges in any time window of the graph.

Zero edges: The number of time windows of the graph in which none of its nodes were connected by edges.

C*liques*: A sequence of three nodes within the same time window that are connected by edges. The total number of cliques in the graph was counted.

Edges10+10/edges5+5 and edges10+10/edges2+2: Ratios comparing the number of edges detected in a graph using a) A relatively long time window and b) A relatively short time window. We suggest that these ratios are inversely related to the LF/HF ratio from frequency domain analyses. However, they are likely more refined in terms of reflecting ANS activity, as they are based on time window analyses.

The indices provide various perspectives of the *inter-relatedness* of an IBI series. A high inter-relatedness reflects a high number of similar IBIs, which might correspond to lower vagus modulation on the heart, leading to slower HR regulation. This could be reflected in several indices: A higher number of edges, maximum edges and cliques, and a lower number of components. Oppositely, a low inter-relatedness reflects a low number of similar IBIs, and thus suggestibly higher vagal modulation on the heart. This could manifest as a higher number of missing edges and zero edges. Importantly, time windows corrresponding to less than five seconds of the IBI series might provide the most refined indices of vagal modulation on heart.

Statistical analyses

In preliminary analyses, the independent samples t-test assessed differences between adolescents with ADHD and controls in age, BMI, HF peak, ADHD-RS and STAI-T scores. Chi-square tests investigated group differences in sex, physical activity levels and numbers of comorbidities. For the remainder of the statistical analyses outlined below, group differences were investigated with the independent samples t-tests — where the applied effect size measure was Cohen's d — unless otherwise stated.

First, we examined which criterion of similarity best illustrated IBI differences between the ADHD and control groups by investigating different approaches: A percentage-based (1-5%, i.e. 95-99% similarity) and a millisecond-based (msec; 5-50 msec). These analyses tested the hypothesis that group differences would be best illustrated with a criterion corresponding to 20% of the SD of the IBI series. A time window of 2+2 neighbors (corresponding to 2-5 seconds of the IBI series) was applied, based on the expectation that this would provide the most refined indices of vagal modulation on the heart. Analyses examined which criterion from the two approaches resulted in the largest effect size of a significant difference between the ADHD and control group. These two criteria were then compared by analyzing group differences in edges (with time windows of 2+2 neighbors and 10+10 neighbors) and edges10+10/edges2+2. The remaining similarity graph algorithm analyses applied the criterion that detected the largest effect size of a significant group difference.

Second, we aimed to investigate differences in inter-relatedness between the ADHD and control groups, and in which time windows such differences were most prominent. Hence, the similarity graph algorithm was applied to three different time windows: 2+2, 5+5 and 10+10 neighbors. The analyses tested the hypothesis that the ADHD group would show higher inter-relatedness compared to the control group, and that this finding would be prominent in a time window of less than five seconds (i.e. 2+2 neighbors). We compared this time window to two slightly longer time windows: 5+5 (6-13 seconds) and 10+10 neighbors (12-25 seconds). For all time windows, differences in edges, components, missing edges, maximum edges, zero edges and cliques between the ADHD and control group were investigated. The Mann-Whitney U test examined group differences in cliques. Group differences in edges10+10/edges*2*+2 and edges10+10/edges5+5 were also investigated. As post-hoc analyses, edges and edges10+10/edges*k*+*k* were calculated for time windows close to the window that had best illustrated group differences (*k*±1). The objective was to assess whether analyses of any of these time windows resulted in even larger effect sizes of group differences.

Third, time- and frequency domain analyses were performed, to investigate differences in HRV indices between the ADHD and control groups. These analyses tested the hypothesis that the ADHD group would display a lower SDNN and LF/HF ratio, a higher LF-HRV, and indifferent RMSSD and HF-HRV compared to controls.

Fourth, follow-up ANCOVAs were performed on the IBI indices that were significantly different in the ADHD and control groups, as computed by the similarity graph algorithm and time- and frequency domain analyses. The IBI indices, respectively, were used as the dependent variable, and diagnostic group status (ADHD/control) as the independent variable. The analyses included covariates, to assess effects of potential confounding factors on the IBI indices: A) Age, sex, BMI, physical activity levels and HF peak. Covariates that were found to significantly predict any of the indices were included in a final ANCOVA model, B) The number of comorbidities the participants had, and C) STAI-T scores.

Fifth, supplemental analyses were performed: A) To compare the similarity graph algorithm to established nonlinear methods, RQA and sample entropy analyses of differences between the ADHD and control groups were performed, B) Bivariate correlation analyses between edges10+10/edges2+2, edges10+10/edges5+5 and IBI indices from the time- and frequency domain analyses were conducted. The objective of the analysis was to aid in the physiological interpretation of the graph theory-based ratios, C) Bivariate correlation analyses with ADHD-RS scores and the significantly different IBI indices in the ADHD and control groups were performed, to investigate whether symptom levels of ADHD correlated with the indices. The reader is referred to the original publication of the paper for details about other supplemental analyses.

3.5 Ethics

All participants received detailed information about the aims of the projects and the planned procedures, and gave written informed consent in line with the Declaration of Helsinki. Parents of participants below 16 also signed written informed consent.

The study protocol for the study of paper I was approved by the Institutional Review Board Approval at the Ohio State University, Columbus, USA, study number 2017B0179. The data was legally cleared for analysis in Norway in line with the Personal Data Act ("Personopplysningsloven"), as confirmed by the Regional Committee for Medical Research Ethics of Western Norway. The study protocol for the study of paper II and III was approved by the Regional Committee for Medical Research Ethics of Western Norway, study number 2014/1304. This ensured that the studies were conducted in line with the Health Research Act ("Helseforskningsloven) and the Personal Data act.

4. Results

Please refer to the attached paper reprints for tables, figures and additional details.

4.1 Paper I

Preliminary analyses with the independent samples t-test detected no significant sex differences in age, educational levels, CVA, BMI, mean HR, EDR, PSS, IPAQ, AUDIT or DAST scores. However, females reported higher MOS scores than males. In the total sample, there were bivariate correlations between a higher CVA and lower PSS scores, higher IPAQ scores, and lower mean HR. CVA did not correlate with MOS scores. Higher MOS scores correlated with lower age and higher IPAQ scores, but not with PSS scores. The *a priori* power analysis suggested n = 109 as a minimum sample size to avoid type II errors (current sample size: n = 143).

In the main analyses, a moderation analysis investigated the hypothesis that higher CVA would predict higher MOS scores in relation to higher, compared to lower, PSS scores in females but not males. IPAQ scores covaried with MOS scores, and were included in the final model. Age, educational levels, EDR, mean HR, BMI, AUDIT and DAST scores were excluded from the model, as they did not covary with MOS scores. In the final model, PSS scores and sex moderated the association between MOS scores and CVA in relation to intermediate and high, but not low, PSS scores in females. In males, the relation was not moderated by any PSS levels. Similar results were found with the use of RMSSD as the applied index of CVA.

In the linear regression analysis of the final moderation model, there was a significant three-way-interaction of CVA, PSS and sex on MOS: Female sex, higher CVA and higher PSS scores associated with higher MOS scores. Moreover, an interaction effect between PSS scores and sex predicted MOS scores, by a more prominent association between higher perceived social support and lower perceived stress in females compared to males. In addition, higher IPAQ scores associated with higher MOS scores. However, there was no prediction of MOS scores by CVA, sex, PSS scores, or by the interaction effects of CVA and PSS scores, or CVA and sex.

Analyses of MOS scores as the independent variable and CVA as the dependent variable, with PSS scores and sex as moderators, did not yield significant results.

4.2 Paper II

Preliminary analyses with the independent samples t-test showed higher ADHD-RS scores in the ADHD group compared to controls. The ADHD group also reported higher DERS total and subscale scores compared to controls, except for on the AWARENESS subscale, in which there was no group difference. Moreover, controls reported higher physical activity levels than the adolescents with ADHD did. The groups did not differ in age, sex, BMI, HF peak or CVA. Bivariate correlations were found between lower CVA and higher DERS TOTAL, STRATEGIES, and CLARITY scores. CVA did not correlate with the other DERS subscales, ADHD-RS scores, age, BMI, or HF peak. CVA did, however, correlate positively with physical activity levels. DERS TOTAL correlated positively with ADHD-RS scores, and scores on all DERS scales correlated with all the other DERS scales. The exception was for AWARENESS, which correlated with CLARITY but no other DERS scales.

In the total sample, multiple linear regression analyses controlling for age, sex, HF peak and physical activity levels found a lower CVA to predict higher STRATEGIES scores. There was also a tendency of lower CVA predicting higher DERS TOTAL scores ($p = .019$). There was found no effect of CVA on NONACCEPTENCE, GOALS, IMPULSE, AWARENESS, or CLARITY scores. Diagnostic group status (ADHD/controls) predicted all DERS scales expect for AWARENESS, in that the ADHD group had higher scores. In addition, DERS TOTAL, STRATEGIES, and CLARITY— but not the other DERS scores — covaried with sex, in that girls reported higher scores. Furthermore, higher CLARITY and GOALS scores, but no other DERS scores, covaried with a higher HF peak. Physical activity levels or age did not correlate with any DERS scores. In follow-up regression analyses, there was no interaction effect of ADHD and CVA on any DERS subscales.

Supplemental multiple regression analyses perfomed in the ADHD and control groups separately found that CVA predicted higher STRATEGIES scores, but no other DERS scores, in the ADHD group. In the control group, no DERS scores associated with CVA. Lastly, after excluding controls with psychiatric disorders, CVA still predicted STRATEGIES scores in the total sample in multiple regression analyses.

4.3 Paper III

Preliminary analyses with the independent samples t-test found that the ADHD group had higher STAI-T and ADHD-RS scores, and number of comorbidities compared to the control group. Furthermore, controls reported higher physical activity levels than the adolescents with ADHD did. However, the ADHD and control groups did not differ significantly in age, sex, BMI, or HF peak. The mean EDR in the sample was .26 Hz, supporting the use of adult frequency bands in frequency domain analyses.

First, the similarity graph algorithm was applied with varying criteria of similarity to investigate which criterion best illustrated IBI differences between adolescents and controls. Significant differences in edges between the ADHD and control groups were detected only with the 1.5% criterion and the 10 msec criterion. For both criteria, there were significant group differences in edges in a time window of 2+2 neighbors, and in edges10+10/edges2+2. There were no significant differences in edges in a time window of 10+10 neighbors. The largest effect size was found for the 1.5% criterion, which was hence applied in the remaining analyses.

Second, differences between the ADHD and control groups in IBI indices from the similarity graph algorithm were systematically investigated in three different time windows. These analyses investigated group differences in inter-relatedness, and in which time window such differences were most prominent. In the time window of 2+2 neighbors, a higher number of edges, maximum edges and cliques, and a lower edges10+10/edges2+2 was found in the ADHD group compared to controls. There were no significant group differences in components, missing edges, or zero edges. When investigating time windows of 5+5 and 10+10 neighbors, respectively, none of the graph theory-based indices were significantly different in the ADHD and control groups. Post-hoc analyses of time windows of 1+1 and 3+3 neighbors, respectively, did not yield larger effect sizes of group differences than 2+2 neighbors did.

Third, time- and frequency domain analyses were performed, and differences between the adolescents with ADHD and controls investigated. There was detected a higher LF/HF ratio in the ADHD group compared to the control group. No significant group differences were detected in SDNN, RMSSD, LF-HRV or HF-HRV.

Fourth, follow-up ANCOVAs on significantly different IBI indices in the ADHD and control groups were performed in order to investigate whether the indices were affected by potential confounding factors. Results showed that A) With age, sex, physical activity levels, BMI and HF peak in the model, HF peak predicted edges10+10/edges2+2. The LF/HF ratio was predicted by physical activity levels and age. None of the IBI indices were predicted by sex or BMI. Consequently, HF peak, age and physical activity levels were included as covariates in the final ANCOVA model. Here, diagnostic group status still predicted all graph theory-based indices, i.e. edges, maximum edges, cliques and edges10+10/edges2+2, but not the LF/HF ratio, B) Controlling for the number of comorbidities the participants had, diagnostic group status still had a significant effect on maximum edges and edges10+10/edges2+2, whereas effects on edges, cliques and the LF/HF ratio were non-significant. The number of comorbidities covaried with the LF/HF ratio, but not with any of the graph theory-based indices, C) Controlling for STAI-T scores, diagnostic group status still predicted maximum edges, cliques and edges10+10/edges2+2. The effect on edges and the LF/HF ratio was nonsignificant. STAI-T scores and the LF/HF ratio covaried significantly, but the STAI-T scores did not covary with the indices from the similarity graph algorithm.

Fifth, results from supplemental analyses showed that: A) Comparing the similarity graph algorithm to other nonlinear methods, there were no significant differences between the ADHD and control groups in RQA or Sample entropy, B) Edges10+10/edges2+2 and edges10+10/edges5+5 correlated positively with each other, RMSSD and HF-HRV, and negatively with the LF/HF ratio, but not with SDNN or LF-HRV, and C) ADHD-RS scores, indexing ADHD symptom severity, correlated negatively with edges10+10/edges2+2 and positively with maximum edges. There were not detected any correlations between the ADHD-RS scores and the LF/HF ratio, number of edges or cliques.

5. Discussion

5.1 Main findings

In sum, the papers of the current dissertation support the notion that IBI indices of variability and similarity might be applied as markers of self-regulation in normally developed and psychiatric populations, and underline the importance of considering contextual factors when interpreting these markers. Results suggest that the index of CVA (as reflected in HF-HRV), might be more sensitive as a marker of perceived stress regulation, compared to as a marker of behavioral regulation. In contrast, IBI indices from the similarity graph algorithm might also reflect deficits in behavioral regulation, and as such complement insights from traditional IBI analyses.

In the next sections, a detailed discussion of the findings of each study will be given, before a further discussion of the results as a whole will follow.

5.1.1 Cardiac vagal activity and perceived stress in relation to contextual factors: Perceived social support and sex

Results from paper I suggest that perceived social support and sex are important contextual factors to consider in the interpretation of CVA as a marker of perceived stress regulation. The results showed that a higher CVA associated with higher perceived social support in females with higher, compared to lower, perceived stress levels. This association was non-significant in females with lower perceived stress levels and in males. As such, taking perceived social into account when interpreting CVA as a marker of perceived stress regulation seems to be highly relevant in females, particularly those with intermediate and high perceived stress levels.

The results showing that CVA, perceived social support, and perceived stress are inter-related were in line with expectations. Firstly, studies have shown a positive association between perceived social support and CVA (59, 61-63). Secondly, higher perceived social support is well-known to often alleviate perceived stress (67). Lastly, studies of all three variables — CVA, perceived social support and perceived stress — have suggested inter-relations: For instance, individuals with higher perceived social support have shown higher CVA and CVA reactivity during experimental stressors compared to individuals with lower perceived social support (60, 63, 64).

Our results are comparable to a previous study, in which higher CVA associated with higher levels of self-reported constructive coping (including social support seeking; 65). This association was significant only in individuals with higher, compared to lower, perceived stress levels. Although we applied a measure of perceived social support and considered sex differences, our study confirms these findings: Higher CVA associated with higher perceived social support, specifically in relation to higher, compared to lower perceived stress levels. Notably, both studies investigated CVA as a marker of a social contextual factor, and how stress levels influence this marker. Therefore, neither directly investigated CVA as a marker of perceived stress. Nevertheless, the results show that in relation to perceived stress, CVA might be associated with social contextual factors. As such, our study adds to the literature suggesting that when interpreting CVA as a marker of perceived stress regulation, one must consider that CVA also might reflect social contextual factors.

Our findings of a positive association between CVA and perceived social support specifically in females with intermediate and high perceived stress levels is in line with the "tend-and-befriend" theory (72, 73). Throughout evolution, an effective stress regulation strategy for females might have been to seek safety together with other individuals (72, 73). Importantly, this tend-and-befriend pattern is considered to be oxytocin-mediated (72, 73) — a hormone that has been shown to increase CVA (74). Consequently, sex-specific adaptations to stress might be reflected in CVA, also today. This notion was supported by a study of pregnant females: Those who reported frequent life stressors and higher levels of perceived emotional support (i.e., a subtype of perceived social support), showed higher CVA flexibility compared to females with lower levels of emotional support (157). In sum, results from the current and previous studies underline the importance of considering sex when interpretating CVA as a marker of perceived stress, particularly in relation to social contextual factors.

5.1.2 Cardiac vagal activity and emotion dysregulation in ADHD

The study of paper II showed that lower CVA associated with self-reported emotion regulation difficulties in a sample of adolescents with ADHD and controls.

Specifically, lower CVA associated with less access to effective emotion regulation strategies. Effective emotion regulation strategies are crucial for perceived stress regulation, in that overwhelming (i.e., under-regulated) emotions might increase perceived stress (47). This stress could deplete general self-regulatory resources (18) and further reduce emotion regulation. In contrast, effective strategies for emotion regulation reflect an ability to get “on top of” overwhelming emotional responses, and consequently down-regulate potentially stressful emotional reactions. Therefore, the findings of the current dissertation support the use of CVA as a marker of perceived stress regulation, as indirectly reflected in emotion regulation strategies, in an ADHD sample.

Previous studies of the associations between resting CVA and emotion regulation in children and adolescents with ADHD are few, and have relied on parent-reports (85-87). One of these studies showed an association between lower CVA and a dichotomous index of emotion regulation difficulties (86), in line with results from the current study. Still, there are inconsistent findings with regards to which domain of emotion regulation is associated with CVA: In non-ADHD samples, CVA has been associated most strongly with self-reported difficulties in emotional awareness (158), emotional impulse control and emotional clarity (159), as well as acceptance of negative emotions (160), respectively. However, these results cannot be directly compared to the findings in the ADHD sample of the current study. Thus, future studies are needed to further investigate which specific self-reported aspects of emotion regulation is associated with CVA in children and adolescents with ADHD.

No significant interaction effects between CVA and the ADHD diagnosis on emotion regulation difficulties were detected in the current study. However, analyses conducted separately in adolescents with ADHD and controls showed an association between CVA and emotion regulation strategies in the ADHD group but not in the control group. In other words, there was a tendency for CVA and emotion regulation difficulties to associate specifically in the ADHD group. As such, the non-significant interaction effect could have occurred based on a lack of statistical power due to a relatively small sample size. Alternatively, the tendency could have occurred because the ADHD group reported higher emotion regulation difficulties than the controls did.

Thus, the association might have been more easily detected in the ADHD group. The question of whether there is an interaction effect between CVA and the ADHD diagnosis on emotion regulation difficulties thus awaits clarification in future studies.

In the current study, CVA did not associate with the diagnostic status of ADHD or with parent-reported ADHD symptoms. This is at odds with the notion that CVA reflects general self-regulation (8-10), including behavioral regulation. On the other hand, the results support the hypothesis that CVA loads more specifically on the regulation of internal perceived stress, compared to external behavior. This could explain the previous inconsistencies in CVA studies (see 46) that have studied the ADHD population as a whole. As suggested in the current study, CVA alterations might occur in ADHD subgroups, such as those with difficulties in perceived stress and/or emotion regulation.

5.1.3 Novel inter-beat interval indices as markers of self-regulation

In the study of paper III, results suggested that the nonlinear, graph-theory based similarity graph algorithm could be a promising method for IBI investigations. The algorithm detected a higher inter-relatedness (i.e., more IBI similarity) in the ADHD group compared to the controls. This was found investigating time windows of 2-5 seconds, but not 6-13 or 12-25 seconds. This finding was in line with expectations, as time windows of less than five seconds might provide indices of “pure” vagal modulation on the heart, which is expected to be altered in ADHD (see 76).

The aforementioned results were detected with the use of a 1.5% criterion of similarity, which best illustrated differences between the ADHD and control groups. This finding is in line with previous studies of sample entropy (102, 103), where it is customary to apply a threshold of 20% of the SD of the IBIs for defining IBIs as similar. In the current IBI dataset, this formula results in the number of (70 * 0.20 =) 14. As the mean IBI length was approximately 900 msec, this threshold corresponds to approximately (14/900 =) 1.5% of the IBI lengths in the series — as demonstrated to be the most sensitive criterion of similarity.

There were several graph-theory based indices reflecting altered inter-relatedness in the ADHD group compared to controls: The ADHD group displayed a higher number of edges, maximum edges and cliques. However, the index of components — which similarly to the aforementioned indices reflects higher inter-relatedness — was unexpectedly non-significant. This could be due to a lack of statistical power. Moreover, the indices of missing edges and zero edges were non-significant — in line with our hypothesis that these indices, reflecting lower inter-relatedness, would be less sensitive to group differences in IBIs than the indices of higher inter-relatedness. Furthermore, there was a lower edges10+10/edges2+2 in the ADHD than the control group. As expected, this ratio correlated inversely with the LF/HF ratio. Although the interpretation of the LF/HF ratio has been debated (see 28), a higher LF/HF ratio is often considered to represent relatively low vagal activity of the ANS (5). Therefore, a lower edges10+10/edges2+2 — as detected in the ADHD group in the current study — might represent a similar construct. The findings might therefore be in line with previous studies reporting higher LF/HF ratios in ADHD samples (87, 113). Lastly, group differences in edges10+10/edges5+5 was non-significant, supporting the hypothesis that edges10+10/edges2+2 would be the most sensitive of the ratios.

Differences between the ADHD and control groups in HRV (including CVA) as deducted from linear analyses were non-significant. Moreover, no group differences were detected by the other nonlinear approaches, RQA and sample entropy. As such, the current study suggests that the similarity graph algorithm might provide complementing information to other IBI analyses.

5.2 Similarity and variability of IBIs as markers of self-regulation

Summarizing results from the papers of the current dissertation, they support indices of variability and similarity of IBIs as markers of self-regulation, and emphasize the importance of considering contextual factors in the interpretation of such markers. In order for such IBI indices to be effectively applied as clinical markers, they have to be modifiable in a dynamic and reliable manner in line with clinical progression (34). Results from the current dissertation support the notion that these indices indeed might vary as a function of clinical progression related to perceived stress regulation, although a causal direction cannot be established. Moreover, clinically applied biomarkers have to meet some additional criteria: Reproducibility, detectability and accessibility (34). The studies of the current dissertation cannot answer the question of whether these criteria are met. Still, previous studies suggest that CVA derived from resting ECGs are highly reproducible (30, 31). Future studies are required to investigate the reproducibility of the IBI indices from the similarity graph algorithm. However, the criteria of detectability and accessibility could be said to be generally met for IBI indices — as IBIs are easily detectable from ECG recordings, and such recordings are widely accessible.

Although IBI indices might have interesting clinical implications, one must bear in mind that biomarkers rarely (if ever) can reflect complex phenomena such as psychophysiology perfectly. In research, one tends to arrange aspects of human behavior into distinct components, in order to easier understand and study them (16). In reality, the boundaries between these constructs are not as strict, and constantly shifting (16). Moreover, the constructs interact with each other: Perceived social support might for instance be inter-related with emotion regulation, as adaptive emotion regulation is crucial for social functioning (see 161). In line with this, identification and acceptance of emotions in others is needed to correctly interpret facial expressions (see 162). Relatedly, CVA has been positively associated with accurate interpretation of emotional expressions (162). Consequently, deficits in emotion regulation could lead to lower perceived social support, further complicating the interpretation of CVA in relation to perceived stress and social contextual factors.

Moreover, it is crucial to bear in mind some methodological and ethical considerations in the interpretation of the results from the current dissertation.

5.3 Methodological considerations

5.3.1 CVA assessment

Applied index of CVA

An important question for the interpretation of the results in the current dissertation is whether the applied index of HF-HRV is a valid representations of CVA. We chose HF-HRV over another commonly applied index, RMSSD, because HF-HRV has been used as an index of CVA in studies that are central for the background of the current dissertation, for instance (46) and (9). HF-HRV has also been described as the most widely reported index of CVA (74). Although RMSSD is also commonly applied, the use of HF-HRV allowed for a more direct comparison to relevant studies. Moreover, guidelines for IBI assessment have stated that the use of HF-HRV is preferred compared to time domain analyses in short-term recordings (i.e. 5 minutes; 5) — as in studies of the current dissertation. Yet, a disadvantage is that HF-HRV might be more affected by respiratory effects compared to RMSSD (163, 164). Consequently, proxies of respiration were statistically controlled for in all studies. Yet, the most optimal study design would have been to assess respiration directly (e.g. with a respiration belt), or instruct participants in paced breathing.

Moreover, we performed measures to avoid disadvantages associated with analyzing just one index of CVA: In paper I, analyses were also performed with RMSSD as the applied index of CVA. Optimally, RMSSD should also have been examined in paper II. However, in paper III, both RMSSD and another time domain index (SDNN) were assessed, in addition to several indices from frequency domain analyses. This extensive examination was performed because the study was highly explorative. This likely ensured a more valid interpretation of the results than the analysis of only one such index would have provided.

Resting vs. reactive ECG recordings

In all studies, IBI indices were calculated from resting ECG recordings, which might complement insights from experimental studies. Resting CVA indices are considered as markers of a “trait” neurophysiological tendency of self-regulation (see 8, 9, 11, 15, 30). As such, these indices might represent how ANS activity plays out in participants’ everyday lives more accurately than CVA reactivity indices do (see 165). Thus, resting ECGs align well with the use of self-report questionnaires in the current studies. Particularly relevant for the study of paper I, the use of experimental studies to investigate social support and CVA has been criticized (see 58). This is because CVA reactivity depends on both task-related factors (e.g. the type of support provided), recipient-related factors (e.g. whether the participant has chosen to receive the support) and provider-related factors (e.g. the quality of the support provider-receiver relationship) (58). These factors represent possible sources of non-naturalistic settings and thus CVA responses. On the other hand, investigations of resting CVA in studies of social support, as in paper I, might inherently provide a more naturalistic insight as "trait" markers of responses in everyday life.

A further advantage of analyzing resting CVA is that it is quite well-established as a marker of "trait" self-regulation (see 8, 9, 11, 15, 30), while there is still some disagreement regarding what CVA reactivity represents (see 29). For instance in relation to emotion regulation, some consider CVA reactivity to represent the exertion of regulatory efforts, while others suggest that it reflects shifts in emotional experiences (29). Thus, a consensus on the interpretation of CVA reactivity awaits establishment.

Yet another advantage of assessing resting CVA is that the comparison of results across studies is less complex than for studies of CVA reactivity. For instance, a meta-analysis of CVA reactivity found that durations of the baseline-, preparation-, test-, and recovery phases for CVA assessment varied from as short as two minutes to as long as twenty minutes across studies, which could have caused variability in the results (165). Furthermore, CVA reactivity seems to vary in relation to the applied task, for instance whether the participants are subjected to cognitive or emotional stressors (166). Even within the same category, for instance psychosocial stressors, stress tasks that require verbal responses have been found to elicit different CVA responses compared to non-verbal tasks (167). Although there also is methodological variability across studies of resting CVA, there are fewer elements in the study design to vary without a task protocol, which allows for more accessible standardization of IBI collection in line with established guidelines (see 5, 23, 92).

Results of resting IBI indices from the current studies generally point in similar directions as previous studies of CVA reactivity. This supports the validity of the results. For instance, higher perceived social support has been associated with higher CVA and CVA reactivity in relation to stressful conditions (60), comparable to the results of higher resting CVA in paper I. Furthermore, emotion regulation has been associated with higher CVA reactivity (83), comparable to findings of resting CVA in paper II. As for paper III, results might suggest altered vagal modulation on the heart in adolescents with ADHD. These findings are in line with a meta-analysis showing lower CVA directly after an experimental task in children with ADHD compared to controls (75), and theoretical expectations (see 76).

5.3.2 Self-report questionnaires

Self-report questionnaires were applied to assess various constructs in the current studies: Emotion regulation difficulties, perceived stress, perceived social support, ADHD symptoms, physical activity levels, drug- and alcohol use. Self-reports provide an effective, inexpensive and widely available assessment of a construct, yet they have some important limitations (see 168). Firstly, self-reports depend on the honesty and introspective abilities of the participants (168). Furthermore, properties with the questionnaires — such as the phrasing of the questions and the design of the rating scales — could lead to biases (168). For instance, a rating scale from 1 to 10 could be subjected to an individuals' tendency to rate all items as extreme or mediocre (168).

Despite limitations, an argument in favor of self-reports is that some constructs are inherently subjective and cannot be as adequately described by alternative assessment methods. Such constructs include perceived social support and stress, which were studied in paper III. Firstly, as for perceived social support, it has been shown to be distinct from the more objective assessment of received support (see 58). This disparity has implications for experimental studies, in which the provision of support not necessarily corresponds to the support perceived by the participant. Moreover, perceived social support has consistently shown a beneficial influence on physiological outcomes such as cardiovascular disease, in contrast to received support (see 58). Thus, the assessment of perceived social support might contribute to more health-related insights than assessments of received support. Secondly, as for perceived stress, it is an inherently subjective construct which is known to vary as a function of sex (see 169). This is particularly relevant for the study of paper III. In this study, we assessed perceived stress by self-reports rather than subjecting all participants to the same experimental stressor. This approach is supported by the notion that females might perceive higher stress compared to males despite exposure to the same stressor (170), which further might influence the associations with CVA and perceived social support. Lastly, another argument for using self-reports in general is that it is important to explore how a construct is subjectively perceived by a participant, regardless of objective norms of what is considered a “difficulty” or “problem”. Such subjective experiences might be more easily detected by self-reports compared to other methods (e.g. neuropsychological testing), and could lead to the detection of aspects that are important to follow up for the individual in question.

Another strength with the use of self-reports is that they represent how the assessed constructs appear in participants’ daily lives to a higher degree than experimental studies — which are often non-naturalistic and situation-based (see 58). Another research design which meets the need for studying both naturalistic insights and subjective perceptions are ecological momentary assessments, in which participants provide several reports in real-time (171). This method is not subjected to the recall biases of self-report questionnaires (171). Although such assessments might be slightly more resource-demanding, it would have been an interesting alternative to our use of self-report questionnaires. More naturalistic insights might also have been provided by the application of more than one self-report questionnaire to characterize each assessed construct in the current studies. As we only used one self-report questionnaire to assess for instance emotion regulation, it may not have explicated the full complexity of how it presents in the participant’s daily lives.

Of importance to paper II and III in the current dissertation, it has been debated whether children, adolescents, and individuals with ADHD have sufficient introspective abilities to provide representative information in self-report questionnaires (see 172). This could serve as an argument for the use of parent reports as a complementing insight to self-reports. In the studies of the current dissertation, we applied parent-reports in the assessment of ADHD symptoms, as these often are externalized in children and adolescents. Hence, they are often easily observed by parents. Further supporting our use of parent reports of ADHD symptoms, diagnostic criteria applied in the studies were largely based on objective observations of behavior (135). In contrast, we applied self-reports in the assessment of emotion regulation difficulties in the same sample. This was based on the inherently subjective nature of several aspects related to emotion regulation difficulties. The notion that it is important to use self-reports in the study of such subjective constructs is supported by a study of children and adolescents with ADHD, in which self-reported anxiety symptoms — which are considered to be largely due to emotion regulation difficulties (173, 174) — associated more highly with observed neurocognitive dysfunctions compared to parent- and teacher reports (88).

Although self-reports are adequate for the study of inherently subjective psychological constructs, it might not have provided the most precise measures of physical activity levels, drug and alcohol use in the current studies. As these constructs are quite sensitive to social norms, the biases associated with self-reports might be higher than for other constructs. Thus, we should optimally have used more objective methods for assessing these variables (e.g. activity trackers, blood tests). However, this would have required substantially more resources and time on the study administrators' and participants' behalf, and would have been more ethically challenging. Furthermore, the scores computed from the aforementioned self-reports were applied statistically as covariates, and not main variables, supporting the use of a less refined assessment of these constructs. Moreover, none of these variables were included in any final statistical model, except physical activity levels.

In sum, we suggest that the use of self-reports in the studies of the current dissertation was adequate for the studies' research aims. Moreover, insights from these self-reports might complement findings from other kinds of assessments, including experimental studies, field assessments and parent- or teacher reports.

5.3.3 Potential proxy variables and confounding factors

A complicating factor in the interpretation of the current results, is the likely presence of proxy variables and confounding factors in the investigated associations. With regards to proxy variables, we have interpreted IBI indices as proxies of brain activity. Moreover, we have assumed that emotion regulation strategies at least partly might serve as a proxy for perceived stress regulation. There could also be other proxy variables involved in the current studies, which to a higher degree are undisclosed. Therefore, our assessment of one construct could in reality represent another construct closely related to it. For instance, perceived social support could as previously noted serve as a proxy for emotion regulation — which again could serve as a proxy for perceived stress regulation. On a similar note, all these constructs might be influenced by personality traits (175-177), another potential proxy variable which we did not control for in the current studies. There is also the possibility that perceived stress regulation — a main focus of the current dissertation — serves as a proxy for another psychological or physiological construct. For instance, one might ask whether individuals with higher CVA seem to adaptively regulate perceived stress because they a) in fact regulate perceived stress more adaptively, or b) have a lower biological stress response, which leads to lower perceived stress (37). In other words, the regulation of perceived stress could in fact serve as a proxy for a lower threshold to perceive stress.

Thus, the current studies cannot completely explicate the complexity of the investigated constructs. A full insight would require an assessment of multiple vagal and CNS responses, genetic factors, hormonal levels, extensive details related to the psychological constructs investigated, social contextual factors and numerous other variables. Acquiring such a complete insight must be said to not only be practically, but also theoretically, difficult because the variables interact with each other.

In an attempt to minimize the effect of confounding factors on the current results, several variables known to associate with IBI indices were statistically controlled for in the current studies: Age, sex, physical activity levels, BMI and respiration. A consistent finding was that physical activity levels associated with the key variables investigated, underlining the importance of controlling for this factor. Furthermore, psychiatric comorbidities were controlled for in the studies of paper I and II, in which we investigated adolescents with ADHD and frequent comorbidities, as well as some control participants with psychiatric disorders. As comorbidities is a particularly relevant factor to consider in the study of psychiatric disorders, the next section will discuss how this might have affected the current results.

Comorbidities

Individuals with ADHD are often diagnosed with a wide range of psychiatric disorders: Mood disorders, anxiety disorders, conduct disorder, oppositional defiant disorder, developmental disorders, tic disorders, substance use disorders, and learning disabilities (178, 179). In the study of ADHD samples, one option is to exclude participants with such comorbidities. This would decrease the risk for comorbid disorders serving as confounding variables of the investigated constructs. The question of how to address comorbidities in ADHD samples is particularly relevant with regards to CVA, which has been found to be altered in a wide range of psychiatric disorders (see 8, 9), including in comorbidities that are frequently found in individuals with ADHD. As such, one could argue that it is important to investigate associations between CVA and the ADHD diagnosis *per se*, without participants with comorbid disorders. Still, in the studies of paper I and II, the exclusion of participants with ADHD with comorbidities would to have led to a substantially smaller sample size with consequentially lower statistical power, and was considered as an inadequate option. The current studies therefore applied a naturalistic study design, comprising participants with ADHD and comorbidities. Further in line with this naturalistic design, control participants with psychiatric disorders that often are comorbid to ADHD (e.g. anxiety disorders, Tourette syndrome), were also included.

We aimed to control statistically for including participants with psychiatric disorders other than ADHD in the studies of paper II and III. In paper II, controls with psychiatric disorders were excluded in follow-up analyses. Importantly, main findings were still significant, suggesting that our use of a mixed-participants control group did not significantly alter the results. Still, as the ADHD group was characterized by frequent comorbidities which were not controlled for, the results are not necessarily generalizable to populations with "pure" ADHD or with other compositions of comorbid disorders. In paper III, follow-up analyses controlled for the number of comorbidities the participants had, and symptoms of trait anxiety — a trait often found in children and adolescents with ADHD (see 180). Interestingly, the IBI indices computed by the similarity graph algorithm were quite robust when controlling for the number of comorbidities and symptoms of trait anxiety in the sample. Still, there is a possibility that comorbid disorders might have influenced the results to a more complex degree than what was controlled for with this method. In particular, the number of comorbid disorders is a crude measure, which not necessarily reflects how complex psychological phenomena such as psychiatric disorders associate with IBIs. Therefore, comorbidities in the sample might still have influenced the results to some degree.

5.3.4 Statistical power

Paper I

In the study of paper I, the sample size of *n* = 143 can be considered moderate to large, compared to other studies that have investigated associations between CVA and aspects related to social support (e.g. 62, 65, in which n was ≈90). Although an *a priori* power analysis revealed that our sample size was sufficient, some non-significant associations in the study could possibly have gained significance in a larger sample.

Theorizing which non-findings that could have occurred due to a lack of statistical power, one might consider the non-significant interaction effects of the moderation analysis. For instance, a significant interaction effect of sex and CVA on perceived social support might have been expected based on other findings in the current study and the tend-and-befriend model (see 72, 73). In conclusion, future studies of larger samples are required to further elucidate the complex associations between CVA, perceived stress and perceived social support in males and females.

Paper II

The samples size of *n* = 67 in the study of paper II can be considered small to moderate. In comparison to the few previous studies of resting CVA and emotion regulation in children and adolescents with ADHD, it is quite small: These studies comprised samples of *n* = 99 (85) and *n* = 104 (86), respectively. Notably, the latter sample was described by the authors as “large” (86). However, in comparison to other studies of CVA in ADHD, the sample size could arguably be described as moderate (see meta-analysis by 46). Several of the samples in these studies comprised less than *n* = 50 participants. These relatively small sample sizes suggest that performing studies in which participants — and perhaps particularly adolescents with ADHD — are required to meet at a laboratory might be associated with recruitment challenges and resource demands. This could result in smaller sample sizes than what one would ideally prefer, as in the current study.

In CVA studies, it has been estimated that a sample size of 21, 61 and 233 participants per group is required to detect large, medium and small effect sizes of group differences, respectively (181). Thus, the respective ADHD and control groups of the current sample are above the recommended threshold for detecting large effect sizes, but below the threshold for detecting small and medium effect sizes. In retrospect, an a priori power analysis would have been informative for further elucidation of the statistical power of the study. Yet, as effect sizes in the relation between CVA and self-regulation, including emotion regulation, have been found to vary from small to large (10), we consider the possibility that not all statistical effects might have been detected in the current study, due to a lack of statistical power. For instance, this might apply to the non-significant interaction effect of ADHD and CVA on emotion regulation difficulties. The statistical power for detecting group differences was likely further reduced by the fact that the control group included adolescents with psychiatric diagnoses (other than ADHD) that have been associated with IBI alterations. In sum, future studies in larger samples of adolescents with ADHD and controls are needed to further investigate the associations between CVA and self-reported emotion regulation difficulties.

Paper III

As the sample of $n = 73$ in the study of paper III was largely based on the same sample as in paper II, the discussed considerations in the previous section also apply to the current sample: The sample can be considered as small to moderate, and could likely detect large (but not medium or small) effect sizes of group differences in traditional IBI indices. Possibly due to a lack of statistical power, we did not detect differences between the ADHD and control groups in traditional HRV indices of SDNN, LF-HRV, or the LF/HF ratio, as expected.

The investigation of the applicability of the similarity graph algorithm to IBI analyses in the current study was highly explorative. Therefore, the discussion of effect sizes and statistical power in relation to this algorithm is largely based on speculations. An *a priori* power analysis would generally have been informative in this regard but was challenging to perform in the current study — as no previous studies had applied a similar method for IBI analysis in an ADHD sample. An appropriate estimation of effect size was therefore considered to be unattainable. Still, based on theoretical reasoning, the similarity graph algorithm might provide larger statistical power compared to traditional IBI analyses. This is because the algorithm systematically analyzes every IBI in a time window in relation to every other IBI in the time window. Thus, a higher number of data points than what is provided by traditional analyses of IBI series is provided. In line with the notion that the algorithm provides relatively high statistical power, effect sizes for the detected group differences were moderate to large (Cohen's d*:* .50-1.00) in the current study. Furthermore, also supporting the statistical power of the similarity graph algorithm, significant group differences were detected despite a relatively short IBI recording length: 237 IBIs. The gold standard in validation of novel methods that assess complexity indices are 30 000 data points (see 182, 183)*,* which was not generated by our relatively short IBI series. Yet, the reason that longer data series are often required, is that the series need to gain enough complexity for the algorithm to identify group differences (183). The fact that such a data length was not required in the current study underlines the potential sensitivity of the similarity graph algorithm compared to traditional HRV analyses. Yet, there is a possibility that some of the significant results of the current study occurred due to sampling error or multiple testing. However, our findings were in line with *a priori* hypotheses, and appeared robust when controlling for potential confounding factors, reducing the risk for such type I errors.

5.3.5 Cross-sectional data and the question of causality

All studies of the current dissertation were cross-sectional in nature. Although such studies involve fewer challenges related to recruitment and follow-up, and are less expensive and resource-demanding compared to longitudinal studies, their inability to make causal inferences represents an important limitation.

Despite the fact that the current studies cannot prove any directions of causality, some speculations can be made in line with previous literature. Firstly, in paper I we hypothesized that higher CVA might facilitate an increase in perceived social support, as CVA might represent activation in brain networks that are important for social engagement (15) and "tend-and-befriend" behavior (73). This hypothesis was supported by a significant prediction of perceived social support by CVA. Notably, follow-up statistical analyses of perceived social support as a predictor of CVA did not yield significant results. Interestingly, in one study, CVA and social connectedness — a construct associated with perceived social support (see 184) — were found to prospectively enhance each other in a positive "upward spiral" (185). In addition to the relation between IBI indices and perceived social support, such complex reciprocal relations could possibly also occur in the associations between IBI indices and emotion regulation, ADHD symptoms, or perceived stress. Secondly, in paper II we hypothesized that CVA would predict emotion regulation as a proxy of perceived stress regulation, instead of the opposite relation. This expectation was based on prospective studies suggesting that lower CVA prospectively predicts emotion regulation difficulties, including depressive symptoms (186, 187). Furthermore, a study found that an HRV index similar to SNDD (i.e., SDRR) was altered before perceived stress was reported in ecological momentary assessments (188). These findings are theoretically sound, as CVA is considered to represent the activation of brain networks that lead to adaptive self-regulation (7, 11, 14). Thirdly, of relevance to paper III, one could for similar reasons expect that IBI indices would predict ADHD symptoms of behavioral dysregulation. However, the cross-sectional and highly explorative nature of this study entails that such a claim neither can be supported nor dismissed.

In conclusion, although speculations can be proposed, the question of causality in the studies of the current dissertation remains unanswered.

5.3.6 Validity

When interpreting the current results, it is essential to consider whether the findings are generalizable to other populations than those investigated.

A first important aspect with regards to the generalizability of the results is that in paper II and III, individuals with ADHD were recruited from psychiatric outpatient clinics. This resulted in a clinical sample (in contrast to a community sample). Thus, it increased the likelihood that individuals with more severe ADHD symptoms, which manifested at a relatively early age (potential participants were recruited when they were children), were included in the studies. Furthermore, the composition of ADHD diagnostic subtypes (predominantly inattentive, predominantly hyperactive-impulsive or combined subtypes; 35, 36) in the investigated samples might influence the generalizability of the results. In the recruited samples of paper II and III there were more participants diagnosed with the combined subtype of ADHD than the inattentive subtype, although in some studies the inattentive subtype has been reported to be more prevalent in the general ADHD population (see 189, 190). This is likely because hyperactivity-impulsivity symptoms are more apparent to other individuals than inattention symptoms are, and thus children showing such symptoms might be referred to psychiatric clinics at an earlier age. Consequently, the current findings cannot necessarily be generalized to other ADHD populations, such as those with "pure" ADHD, different subtype distributions, other kinds of comorbidities, or community samples. Furthermore, the different ADHD diagnostic subtypes could be related to different CVA alterations (see 46), although the sample sizes of the current studies did not allow for investigations of this notion.

A second element to consider with regards to the generalizability of the current results, is that a substantial number of the participants with ADHD in the studies of paper II and III used ADHD medication regularly. We aimed to rule out short-term effects of medication by asking participants to perform a wash-out period, which most of them conducted. Furthermore, stimulant medication for treatment of ADHD symptoms tend to normalize IBI indices towards values found in normally developed samples (see 136, 191). Therefore, the use of stimulant medication in the current studies have probably not resulted in any false group differences. Still, we cannot rule out any long-term effects of ADHD medication on IBI indices, emotion regulation difficulties or ADHD-symptoms, and consequently the findings are not necessarily generalizable to medication-naïve adolescents with ADHD.

A third important aspect to take into consideration regarding the validity of the current results is the ages of the participants: Young adults were investigated in the study of paper I, and adolescents in paper II and III. As CVA varies as a function of age (109, 192), the results cannot be generalized to other age groups than those investigated.

A fourth factor to take into consideration when interpreting the validity of the current results, is the sex of the participants. This is clearly illustrated in the study of paper I, in which different associations between CVA and perceived social support in relation to stress were found for males and females. Of relevance to the current studies, there are also well-established sex differences in CVA (68, 193), ADHD symptoms (194, 195) and emotion regulation (196, 197). In the studies of paper II and III the majority of participants were male (64-66% of the total sample), and although analyses were controlled statistically for effects of sex, the results have to be replicated in sex-specific subgroups before the findings can be generalized to both males and females.

Finally, one last aspect which might alter the generalizability of the results is the ethnicity of the participants. As ethnicity is typically unaccounted for in Norwegian research studies for political-ethical reasons, we did not take this factor into consideration in the current studies. Still, controlling for ethnicity is more common in some international research environments. For instance, meta-analyses have detected ethnic differences in CVA (198). In all studies of the current dissertation, the majority of participants were of Caucasian ethnicity, and thus generalizations to other ethnic populations have to be performed with caution.

As a whole, a careful interpretation of the results of the current dissertation is recommended, as they do not necessarily generalize across populations, ages, sexes or ethnicities.

5.4 Ethical considerations

An important ethical consideration when performing a research study is the load related to assessment for the participants involved. In the current studies, assessments were non-invasive and have likely not led to any substantial pain or discomfort. Still, the completion of self-report questionnaires, diagnostic interviews and ECG recordings required substantial time and mental capacity. This is perhaps particularly relevant for the adolescents with ADHD, who typically have difficulties focusing on monotonous tasks. To account for this mental strain, regular breaks were organized during the procedures of the studies, and participants were encouraged to ask for more breaks if needed.

Additional ethical considerations are required in research studies of children and adolescents. This is of relevance to the studies of paper II and III. In these studies, it was crucial to ensure that the adolescents understood what they consented to by participating in the study. Therefore, they and their parents were thoroughly informed about the project and the assessments they would undergo, including any burden or pain associated with the procedures. This information was provided both orally and in writing, in an accessible language. Participants were further asked if they had any questions or concerns. Another aspect of the procedure in these studies which also is of ethical importance, is that the participants were followed by the same test administrator throughout the day of assessments. This procedure was implemented to ensure a feeling of connectedness and safety for the participants.

The potential tolls associated with participation in a research study must always be weighed against the scientific value of the research. Despite some potential strain associated with participation in the current studies, the results increase the knowledge of IBI indices in relation to self-regulation. This is hopefully considered to have a scientific value, which can benefit the participants of the studies in the long term.

5.5 Potential implications for the psychiatric field

While bearing in mind the discussed methodological aspects, one could speculate in potential clinical implications of the current results — although importantly, none of the current studies are clinical, and the implications therefore are largely hypothetical. Nevertheless, as alterations in brain activity are found in numerous psychiatric disorders (20), IBI indices might be applied to the field of psychiatry. The following section will mainly focus on the IBI indices' potential applicability as diagnostic, response and monitoring markers of self-regulation, before a brief discussion of their use as prediction, prognosis or susceptibility/risk markers will be given.

Firstly, IBI indices might potentially be applied as diagnostic markers — markers used to detect or confirm a disorder (34) — and might as such accommodate some shortcomings of the current categorical, symptom-based psychiatric diagnostic system (see 199): As a given number of symptoms result in a given diagnosis, there are large variations in symptomatology even within the same diagnosis (34). These variations are further amplified by the presence of comorbidities that interact with each other (34). This complexity could be more precisely described in the diagnostic system by integrating several levels of analysis. For instance, the Research Domain Criteria (RDoC) prompts to take into consideration biology, behavior and social interaction in the diagnostics of psychiatric disorders (200). With regards to the biological aspect of such multi-level diagnostics, there seems be a biological factor which could mark a predisposition to developing self-regulatory deficits and as such psychiatric disorders: A *p* (psychopathology)-factor (201). This *p-factor* has been suggested to have neurological roots (201), such as changes in brain function. As IBI indices are considered to reflect brain activity, they might potentially represent such a p-factor.

If one were to use IBI indices as diagnostic markers of psychiatric disorders, the applied methods' sensitivity in detecting clinically relevant IBI alterations is essential. Notably, previous studies have often detected associations between CVA and psychological constructs such as self-regulation of small to moderate effects (see 10). These relatively low effect sizes could have occurred because the associations between CVA and such constructs are extremely complex, and influenced by contextual factors. Importantly, this applies to the use of markers in the field of psychiatry in general, and is not specific for CVA. Still, based on results of the current dissertation, the similarity graph algorithm might provide IBI indices that are more sensitive as markers of self-regulation. Furthermore, one might hypothesize that the similarity graph algorithm could detect disorder-specific patterns of vagal alterations — for instance in anxiety disorders compared to ADHD. This would be in contrast to traditional IBI indices, which are considered as transdiagnostic markers of self-regulation and psychopathology (8, 9). Yet, IBI indices from the similarity graph algorithm have not been investigated in relation to ANS functioning, and their interpretation is therefore highly explorative. Moreover, the indices' reproducibility awaits further investigation before they can be reliably applied as "trait" markers.

Secondly, IBI indices could serve as response markers: Markers that are modified in response to a clinical treatment (34). This could be particularly relevant for treatments that increase vagal activity, and as such indirectly influence the brain (see 37) — for instance biofeedback treatment and vagal stimulation. The use of these methods has been supported for various somatic and psychiatric disorders (202-204). Biofeedback treatment was first studied in the 1990s (see 205). It consists of live ECG monitoring, while the individual in treatment attempts to maximize their CVA, typically by slow breathing maneuvers (see 205). The individual could for instance be provided feedback on their CVA levels by visualizing matching to patterns of peaks and valleys (205). Of relevance to the current dissertation, biofeedback treatment has been shown to improve emotion regulation (206), the regulation of perceived stress (207), as well as general mental health in children and adolescents (202). Furthermore, such changes seem to be reflected in increased functional connectivity of the prefrontal cortex and the amygdala (208). As for vagal stimulation, it has been investigated since the late 19th century (203), and can for instance be executed by devices positioned at the auricle of the ear (see 209). This has been shown to have positive effects on emotion regulation (209). Consequently, biofeedback and vagal stimulation might be cost-effective treatments of psychiatric disorders. Of relevance to the current dissertation, it might for instance be applied in children and adolescents with ADHD and emotion dysregulation, or individuals (perhaps particularly females) with psychiatric illnesses, high perceived stress and low perceived social support.

Thirdly, IBI indices could be applied as monitoring markers: Markers analyzed at different points in time in order to monitor the status of a disorder (34). For instance, one could compute IBI indices at several time points during a treatment program. An increase in IBI indices of vagal activity might then generally be interpreted as improved self-regulation. Importantly, these IBI indices would have to be interpreted together with the individual's symptom levels. In line with the focus of the current dissertation, adolescents with ADHD and emotion dysregulation might be a relevant population for such monitoring. As results from the current dissertation suggest that these adolescents tend to have insight into their own emotion regulation difficulties, they could potentially benefit from an integrated assessment of self-reports, observational parent- and teacher reports, and IBI indices.

Lastly, one might speculate in whether IBI indices could serve as predictive, prognostic or susceptibility/risk markers. Predictive markers are markers which predict which individuals are more likely to experience an effect after being exposed to a medicine or environmental agent (34). The use of IBI indices as such a marker is supported by a study in which individuals with anxious depression and higher CVA showed better outcomes from medical treatment compared to others with the same diagnosis and lower CVA (210). Next, prognostic markers identify the likelihood for individuals diagnosed with a disorder to develop a clinical event, such as death, disorder progression or recurrence, or another medical condition (34). It is well-established that lower CVA is a risk factor for somatic disorders, such as those related to immune dysfunction and cardiovascular disease, and mortality (110). As such, IBI indices might serve as a prognostic marker of somatic disease and mortality in individuals with psychiatric disorders. With regards to susceptibility/risk markers, they characterize the risk of developing a disorder, often before the onset of clinical symptoms or signs (34). The use of IBI indices as such a marker is for instance supported by a study showing that lower CVA prospectively predicted depressive symptoms (211) (which could be due to deficits in general self-regulation and/or the regulation of perceived stress, see 16). Interestingly, the risk for developing depression for individuals with lower CVA only occurred under conditions without social support (211) — again underlining the importance of considering contextual factors in the use of IBI indices.

In sum, IBI indices have the potential for wide application in the psychiatric field. It is still likely that they could be more applicable as one kind of marker (i.e. for instance diagnostic or response marker) compared to others. However, before these markers can be applied clinically, extensive further research is required.

5.6 Conclusions and recommendations

The studies of the current dissertation support the notion that IBI indices of variability and similarity might be applied as markers of self-regulation — also in psychiatric disorders such as ADHD — and underline the importance of considering contextual factors in the interpretation of these markers. Such contextual factors include, but are not in any way restricted to, sex and perceived social support. With regards to which core aspects of self-regulation the IBI indices might mark, CVA (as reflected in HF-HRV) seemed to load higher on internal perceived stress regulation, compared to the regulation of external behavior as exemplified by ADHD symptoms. On the other hand, indices derived from the nonlinear similarity graph algorithm might be more sensitive to other aspects of self-regulation, including behavioral regulation.

These findings align well with the purpose of the current dissertation: To clarify which self-regulatory functions IBI indices of vagal activity mark — both in a sample from a non-psychiatric population and in ADHD samples — and how they relate to contextual factors, as well as investigating novel methods which might increase the sensitivity and specificity of such markers. The dissertation as such confirms and extends previous findings from IBI studies, and provides further insight into how physiological, psychological and social functions are interconnected.

Extensive research is required to further investigate the potential use of IBI indices as markers of self-regulation. Future studies would benefit from longitudinal designs, as well as manipulations of the investigated factors (i.e. vagal activity, emotion regulation, perceived social support, ADHD symptoms, perceived stress), to establish causal directions. Moreover, results from the current studies — suggesting that CVA reflects perceived stress regulation to a higher degree than behavioral regulation — need replication in other and larger samples (i.e. other ages, sexes, ethnicities, psychiatric disorders), in order to establish their validity. Also the results from the explorative study of the similarity graph algorithm require extensive further validation. Future studies investigating the similarity graph algorithm's sensitivity and specificity in distinguishing different psychiatric diagnoses are encouraged. Furthermore, studies should investigate the relations between these graph theory-based IBI indices and autonomic activity. In addition, further studies of how contextual factors at biological, cognitive, behavioral and social levels influence IBI indices as markers of self-regulation are required. Lastly, investigations of the clinical application of IBI indices in various areas of the psychiatric field are needed. These joint efforts could hopefully contribute to increased life quality and improved function levels for the many individuals diagnosed with psychiatric disorders throughout the world.

Source of data

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Appendices

Appendix I

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ORIGINAL RESEARCH REPORT

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Vagally mediated heart rate variability, stress, and perceived social support: a focus on sex differences

Elisabet Kvadsheim[a], Lin Sørensen[b], Ole B. Fasmer[a,c], Berge Osnes[b,c], Jan Haavik[c,d], DeWayne P. Williams[e], Julian F. Thayer[e,f] and Julian Koenig[g]

[a]Department of Clinical Medicine, University of Bergen, Bergen, Norway; [b]Department of Biological and Medical Psychology, University of Bergen, Bergen, Norway; [c]Division of Psychiatry, Haukeland University Hospital, Bergen, Norway; [d]Department of Biomedicine, University of Bergen, Bergen, Norway; [e]Department of Psychological Science, University of California, Irvine, CA, USA; [f]Department of Psychology, Ohio State University, Columbus, OH, USA; [g]Faculty of Medicine, University of Cologne, and Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Cologne, Cologne, Germany

ABSTRACT

Higher vagally mediated heart rate variability (vmHRV), reflecting vagal activity as indexed by heart function and lower stress vulnerability, is associated with higher perceived social support. Seeking social support is an adaptive stress response, and evolutionary theories suggest that females use this strategy more than males. The current study investigated the hypothesis that higher vmHRV is related to higher perceived social support under conditions of higher, relative to lower, stress, and that this association is most prominent in females. A healthy student sample ($n = 143$; 82 males, 61 females; mean age 19.9) completed the short version of the Medical outcomes study social support survey (MOS) and the Perceived stress scale (PSS). Activity in the high frequency band of heart rate variability (HF-HRV), deducted from five-minute resting electrocardiogram (ECG) recordings, indexed vmHRV. A moderation analysis was conducted, with PSS and sex as moderators of the association between vmHRV and MOS. Statistical effects were adjusted for age, education, physical activity, body mass index (BMI), alcohol and drug use, ECG-derived respiration (EDR), and mean heart rate. Higher PSS scores moderated the association between vmHRV and MOS in females but not males. Lower PSS scores did not moderate the relation between vmHRV and MOS. This suggests that higher vmHRV is associated with higher perceived social support under conditions of higher stress in females but not males, consistent with evolution of different stress management strategies in the sexes. The results may have implications for individualized intervention strategies for increasing vmHRV and perceived social support.

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Introduction

Activity of the vagus nerve of the autonomic nervous system (ANS) as indexed by heart function is reflected in vagally mediated heart rate variability (vmHRV), deducted from resting electrocardiogram (ECG) recordings. Owing to its test–retest reliability (Bertsch et al., 2012; Tarkiainen et al., 2005), resting vmHRV is often used as a "trait" marker of a neurophysiological tendency to self-regulate and engage socially (Porges, 2007; Thayer & Lane, 2000). Supporting this, higher vmHRV has been associated with adaptive stress coping abilities (Kim et al., 2018), such as the increase of perceived social support (Fabes & Eisenberg, 1997; Gerteis & Schwerdtfeger, 2016; Holt-Lunstad et al., 2007; Kao et al., 2014; Schwerdtfeger & Schlagert, 2011) – defined as the perception of how one's social network provides stress coping resources (Cohen, 2004). Furthermore, studies have found positive associations between perceived social support and flexible vmHRV changes during experimental stressors (Goodyke et al., 2021; Maunder et al., 2012; Schwerdtfeger & Schlagert, 2011).

Notably, there are sex differences in social support behavior during stress (Taylor et al., 2000), which might be reflected in vmHRV. The association between vmHRV and perceived social support might therefore relate differently to stress in males and females. For instance, social support has been found to be more consistently used during stress in females compared to males (Taylor, 2007). Moreover, females display higher vmHRV (Koenig & Thayer, 2016), and higher vmHRV flexibility during experimental stressors (Li et al., 2009; Sato & Miyake, 2004). Similar sex differences in vmHRV during stress have also been suggested in a neuroimaging study. In this study, there was found a positive correlation between blood flow in brain regions important for

CONTACT Elisabet Kvadsheim elisabetkvadsheim@hotmail.com Department of Clinical Medicine, University of Bergen, Jonas Lies vei 87, 5021 Bergen, Norway

Supplemental data for this article can be accessed here.

autonomic regulation and vmHRV during stress in females, whereas the association was negative in males (Nugent et al., 2011).

The aforementioned sex differences might originate from evolutionary processes. Compared to the "fight-or-flight" stress response, the use of social networks aiding in nurturant activities for oneself and the offspring – i.e. "tend-and-befriend" behavior (Taylor et al., 2000) – might have been more favorable for females. This was supported in a self-report study of stress and social support (Kneavel, 2021). The tend-and-befriend pattern might be mediated by oxytocin (Taylor et al., 2002), which increases vmHRV (Kemp et al., 2012). In line with this behavioral pattern, a study found that vmHRV increased in females and decreased in males during a stress task (Adjei et al., 2018). However, sex differences in the association between vmHRV and perceived social support in relation to self-reported stress have not been investigated previously.

In the current study, we investigated how self-reported stress and sex moderated the association between vmHRV and perceived social support – assessing whether vmHRV could reflect sex differences in tend-and-befriend behavior. As such, we hypothesized that higher vmHRV would associate with higher perceived social support specifically under conditions of higher, relative to lower, stress levels, and that this association would be most prominent in females.

Materials and methods

Design

Data for the present analyses were derived from the Ethnic Differences in the Experience of Noxious Stimuli (EDENS) study. The EDENS study is a cross-sectional study including behavioral pain testing while various psychophysiological measures were simultaneously recorded. In addition, participants completed several self-report questionnaires. Here, we only report on resting state ECG recordings in association with selected self-report measures, addressing the main hypotheses of the manuscript. Data on the Perceived Stress Scale (PSS) and the Medical outcomes study social support survey (MOS), as well as relevant HRV variables (i.e. HF-HRV and RMSSD; *outlined below*), were included. The authors state that they have reported all measures, conditions, data exclusions and how they determined their samples sizes in the current sub-study. In the EDENS study, several measures were assessed that are not reported in the current manuscript, as these were not included in the data analysis of the current study. The study protocol was approved by Institutional review board approval at the Ohio State University, Columbus, USA (Study number 2017B0179).

Study site and population

The sample comprised 143 individuals between 18 and 38 years of age (mean age 19.94; SD 2.72*;* Table 1). The majority of participants were male ($n = 82$, 57.8%). University students at the Ohio State University, USA, were recruited by study advertisement.

Procedure

Minimum requirements for participation in the study involved two assessments (baseline and physiological assessment, and extended psychological assessment), which was completed at one appointment or within one week. Participants were given a detailed explanation of the procedure and the aim of the study, and then gave written consent to participation in accordance with the Declaration of Helsinki. The students received partial course credits for participating in the study, or if not applicable, 15$(13€). Upon completion of the consent form, the baseline assessment and initial physical examination were completed, or a future appointment was scheduled. The baseline assessment included the completion of several questionnaires, including a standard socio-demographic questionnaire on education (*What is the highest level of education you have completed?).* The item was scored on a scale from 1 (12th grade or less) through 5 (Masters or Doctorate degree). Participants also completed the International physical activity questionnaire (IPAQ), the Alcohol use disorders identification test (AUDIT), and the Drug abuse screening test (DAST; *outlined below*). The initial physical examination included assessment of height and weight, and a 5-minute ECG recording (*outlined below*). Subsequently, all participants underwent an extended psychological assessment where a set of questionnaires was administered, including the MOS and the PSS.

Measures

Physiological measures

A 5-minute ECG recording was obtained from each participant in a seated position. Participants were instructed to relax and try not to fall asleep while breathing spontaneously. The ECG signal (sampled at 1000 Hz) was conducted through three adhesive electrodes and assessed using a 16-channel bioamplifier (*Nexus-16; Mind media B.V.; Roermond-Herten, The Netherlands*), and the recording software Biotrace+ (*Mind media B.V., Roermond-Herten: Mind Media BV, 2004*). The inter-beat-interval time series was written and exported from BioTrace + in a single text file for each participant and further analyzed using the Kubios HRV analysis software, version 3.0.0 (Tarvainen et al., 2014). Automatic artifact corrections – which are commonly used, and supported under adequate recording conditions such as those in the current study (Laborde et al., 2017) – were performed on 32 of the recordings (22 males; 10 females; mean corrections: 4.04% of the recordings). An autoregressive model with smoothness priors detrending in Kubios HRV was used for spectral analysis, yielding a power spectrum displaying activity in the low frequency (LF; .04–.15 Hz) and high frequency (HF; .15–.4 Hz) domain. Although also influenced by other physiological systems, HF power (i.e. HF-HRV) mainly reflects vagal activity as indexed by heart function (Thayer et al., 2010). The square root of the mean squared differences of successive R-R intervals (RMSSD), also considered to mark vagal activity (Thayer et al., 2010) as indexed by heart function, was also calculated. HF-HRV was chosen as the primary measure of vmHRV (the term vmHRV will refer to HF-HRV in

Table 1. Descriptive statistics for males and females.

Variable	Males ($n = 82$)	Females ($n = 61$)	t	$\chi2$	p	φ	d	CI
Background information								
Age (years)	20.17 (3.25)	19.62 (1.72)	1.20		0.23		0.20	[−0.13, 0.54]
Educational level	2.59 (0.74)	2.43 (0.72)		4.57	0.33	0.18		[−0.016, 0.33]
IPAQ	2.09 (0.65)	1.90 (0.54)		5.70	0.058	0.20		[−0.037, 0.35]
BMI (kg/m^2)	25.41 (4.75)	24.33 (5.08)	1.31		0.19		0.22	[−0.11, 0.56]
AUDIT	5.55 (3.85)	4.31 (4.17)	1.84		0.070		0.31	[−0.02, 0.65]
DAST	1.61 (1.02)	1.44 (1.07)	0.95		0.34		0.16	[−0.17, 0.50]
Questionnaire scores								
MOS	28.90 (7.01)	31.77 (6.52)	−2.49		0.014*		−0.42	[−0.76, −0.09]
PSS	22.01 (5.41)	22.87 (5.93)	−0.90		0.37		−0.15	[−0.49, 0.18]
vmHRV	6.89 (1.23)	6.93 (1.30)	−0.21		0.84		−0.032	[−0.37, 0.30]
EDR (Hz)	0.23 (0.06)	0.23 (0.06)	−0.84		0.40		0.00	[−0.33, 0.33]
Mean HR	74.66 (10.17)	76.81 (12.51)	−1.13		0.40		−0.19	[−0.53, 0.14]

Note. Data are given as mean (SD). Degrees of freedom (df) were 145 for all variables, expect for IPAQ, where df was 2. IPAQ scores were re-categorized as low (1; ≤16th percentile), intermediate (2; 16th–84th percentile), and high (3; ≥84th percentile). CI: 95% confidence interval of *d or* φ, respectively. AUDIT: Alcohol use disorders identification test; BMI: Body mass index; DAST: Drug abuse screening test; IPAQ: International physical activity questionnaire; MOS: Medical outcomes study social support survey; PSS: Perceived stress scale; vmHRV: Vagally mediated heart rate variability, indexed by high frequency power (HF-HRV). HF-HRV values were log-transformed; EDR: Electrocardiogram (ECG) derived respiration; Mean HR: Mean heart rate (beats/min).
*= $p < 0.05$ **= $p < 0.01$.

the following Methods and Results sections, unless otherwise specified), as it has been acknowledged as the most commonly reported index of vmHRV (Kemp et al., 2012). We used ECG-derived respiration (EDR) to control for respiratory frequency (Tarvainen et al., 2014). In addition, we assessed mean HR. The height (cm) and weight (kg) of each participant was recorded. These measures were used to derive their body mass index (BMI, by *kg/m²*), which has been related to vmHRV (Koenig et al., 2014). The BMI value was missing for one participant (male).

Questionnaires

The short version of the *MOS* (Hays et al., 1995) measured perceived social support. The short form has shown excellent psychometric properties similar to those of the original 19-item instrument (Hays et al., 1995). The questionnaire comprises 8 situations (*If you needed it, how often is someone available…*, e.g. *who understands your problems?*). Thus, it aims to measure the perceived availability, if needed, of social support. The situations are rated on a 5-point Likert scale from 1 (*none of the time*) to 5 (*all of the time*). A higher total score indicates higher perceived social support.

The *PSS* (Cohen et al., 1983) is a widely used self-report instrument to evaluate an individual's level of perceived stress. In the current study, the 10-item short form was used. In general, the psychometric properties of the 10-item PSS have been found to be superior to those of the 14-item PSS (Lee, 2012). The PSS has shown adequate reliability and validity, and has often been assessed in students (Lee, 2012), as in the current sample. Asking for feelings and thoughts regarding the last month, it assesses the extent to which a person perceives life as stressful (e.g. *How often have you found that you could not cope with all the things that you had to do?).* The questionnaire uses a Likert-type response scale from 0 (never) to 4 (very often). Higher PSS scores indicate a higher level of perceived stress.

The short form of the *IPAQ* (see https://sites.google.com/site/theipaq/, Craig et al., 2003) was used to assess the participants' physical activity levels. The IPAQ's measurement properties have been evaluated in 12 countries, where it has shown good reliability and acceptable validity (Craig et al., 2003). The 4-item short-form of the IPAQ collects information on the time (i.e. number of sessions and average time per session) spent on physical activities with moderate intensity, vigorous intensity, and sitting, reported from the last seven days. This is converted to metabolic equivalents (METs) expended at the various intensities per week. In the current study, the number of METs/week for vigorous intensity was chosen as the applied measure of physical activity levels. The reason for this was that vmHRV is related to maximum oxygen intake (VO_{2max}; Goldsmith et al., 1997), and to elicit significant changes in VO_2max it is necessary to perform physical activity at 75 percent or more of heart rate maximum (i.e. vigorous intensity; Burke & Franks, 1975).

The participants' alcohol consumption, which has been associated with vmHRV (Cheng et al., 2019), was assessed by the *AUDIT* (Babor et al., 2001). The AUDIT is used to identify persons with hazardous and harmful patterns of alcohol consumption, and has been found to provide an accurate measure of risk across sex, age, and cultures (Babor et al., 2001). Items are rated on a five-point Likert-type scale ranging from "never" to "four times a week or more". A higher total score corresponds to a higher alcohol consumption. The 10-item short form of the *DAST* (Skinner, 1982) was used to assess drug use – which also has been related to vmHRV (D'Souza et al., 2019) – within the sample. This is a well-validated instrument, which has been shown to have acceptable internal consistency (Skinner, 1982). Referring to the last 12 months, participants were asked to answer "yes" or "no" to a set of questions, which were then scored according to the scoring norm. A higher total score corresponds to a higher degree of problems related to drug use. We also assessed participants' smoking habits in the current study, however few participants ($n = 3$, 2.0%) reported that they currently smoked daily, and smoking was consequently not included as a covariate in the current analyses.

Statistical analysis

Statistical analyses were performed in the Statistical package for the social sciences version 24.0 (*SPSS; IBM Corp., Armonk,* *NY, 2016*). Missing values were imputed with the sample mean. Due to missing data linking vmHRV to self-report scores and demographic information, four participants were excluded from the study. Outliers, defined as values $> \pm 3$ standard deviations (SD) from the mean of the total sample, would as a general rule be corrected with the value of ±3SD of the sample mean (for handling of missing data and outliers, see Supplemental Figure 1). To approximate a normal distribution, vmHRV data were log-transformed (Thayer et al., 2010). If other variables were found to be severely skewed, they would be converted to percentile-based categorical variables: low (1; $\leq$16th percentile), intermediate (2; 16th–84th percentile) and high (3; $\geq$84th percentile). If the variable had been exactly normally distributed, the applied percentiles would translate to the mean –1SD, the mean, and the mean +1SD. However, the percentile-based approach avoids risking that one of the measures could be outside of the range of measurement, as with a SD-based approach (Hayes, 2017). The same rationale was applied for reporting PSS scores at these specific percentiles in the moderation analyses (*outlined below*).

We performed preliminary analyses of sex differences in age, vmHRV, MOS, PSS, BMI, AUDIT, DAST, EDR, and mean HR with the independent samples *t*-tests. Chi-square tests were used for analyses of sex differences in educational levels and IPAQ scores. Effect sizes were estimated with Cohen's *d* in the *t*-tests, and with phi (φ) in the chi-square tests. Bivariate correlation analyses were conducted in the total sample, and for males and females, respectively, for correlations between vmHRV, MOS and PSS, age, educational levels, IPAQ, BMI, AUDIT, DAST, EDR and mean HR.

As our main analysis, we conducted a moderation (i.e. interaction) analysis in the PROCESS v.3.5 macro (Hayes, 2017) in SPSS. An a priori estimation of statistical power was performed in order to determine the sample size needed to estimate statistical effects in the moderation model. To our knowledge, no comparable prior studies had included two moderators in their model and the estimation was therefore based on two similar, prior studies using different statistical models (Fabes & Eisenberg, 1997; Schwerdtfeger & Schlagert, 2011). These studies revealed medium effect sizes ($f^2 = 0.20$ and 0.21, respectively). Considering that the current study applied two moderator variables, which tend to decrease effect sizes, and that there were few studies to provide a robust estimate of the expected effect size, we assumed a small to moderate effect size concerning the association between vmHRV and perceived social support: $f^2 = 0.15$. Using g*power, we specified all eight predictors in the model: vmHRV, PSS scores, sex, the interaction of the aforementioned variables, and IPAQ scores (which were shown to covary significantly with the MOS scores, as described in the Results section). We specified the conventional level of sufficient power to be $1-\beta = 0.80$ and a significance level of $\alpha = 0.05$. The analysis revealed that a total number of $n = 109$ participants would be a required minimum to avoid a type II error in our study, which is below our sample size of $n = 143$.

In the moderation analysis, model 3 was chosen as our applied model (Figure 1). This investigated the hypothesis that higher vmHRV would be associated with higher MOS scores only under conditions of higher PSS scores, and that this moderation would be found specifically in females. In the moderation model, vmHRV was included as the antecedent (i.e. independent, X) variable, MOS scores as the consequent (i.e. outcome, Y) variable, and PSS scores (W) and sex (Z) as moderators. A preliminary moderation analysis – performed to investigate which potential confounding factors would be included in the final model – included age, educational levels, IPAQ, BMI, AUDIT, DAST, EDR, and mean HR as covariates. Importantly, the effect of these covariates would only be controlled for in the final statistical model if they were shown to covary significantly with MOS scores in this preliminary model. In a conditional effects part of the final moderation analysis, effects of varying PSS scores on the relation between vmHRV and MOS were investigated. Here, PSS scores were reported at low (16th percentile), intermediate (50th percentile), and high (84th percentile) levels (while still being included as a continuous variable in the statistical model). To investigate whether these results were comparable when using another vmHRV index, we repeated the analyses with RMSSD as the antecedent variable. Furthermore, a linear regression analysis was included in the moderation analyses. Lastly, to test an alternative direction of our model, to account for the fact that perceived social support could predict vmHRV, we repeated the moderation analyses with MOS scores as the antecedent variable and vmHRV as the consequent variable.

Results

Preliminary analyses

There were detected three outliers for BMI values (one male, two females), and one for vmHRV (male). The BMI values were corrected with the value of ±3SD of the sample mean. The vmHRV outlier was a very low value, and we could not rule out that this had occurred due to underlying somatic issues. Therefore, we decided to deviate from our original rule of correcting outliers, and instead excluded this participant. No outliers were detected for educational levels, MOS, PSS, AUDIT or DAST scores, EDR or mean HR. The distribution of the IPAQ scores was severely skewed, and the scores were therefore re-categorized as low, intermediate, and high.

The preliminary analyses showed that there were no significant sex differences in age, educational levels, vmHRV, PSS, IPAQ, BMI, AUDIT, DAST, EDR, or mean HR (Table 1). Females reported significantly higher MOS scores than males. In the total sample, bivariate correlations analyses showed that higher vmHRV correlated with lower PSS scores, higher IPAQ scores and lower mean HR (Table 2). However, vmHRV was not correlated with MOS scores in the total sample. Higher MOS scores correlated with lower age and higher IPAQ scores. In males, higher vmHRV correlated with lower PSS scores and lower mean HR, but vmHRV was not correlated with MOS scores. Higher MOS scores correlated with higher IPAQ scores and lower educational levels. In females, higher vmHRV correlated with higher MOS scores, lower EDR and lower mean HR, but not with PSS scores. MOS and PSS scores were not correlated in any of the samples.

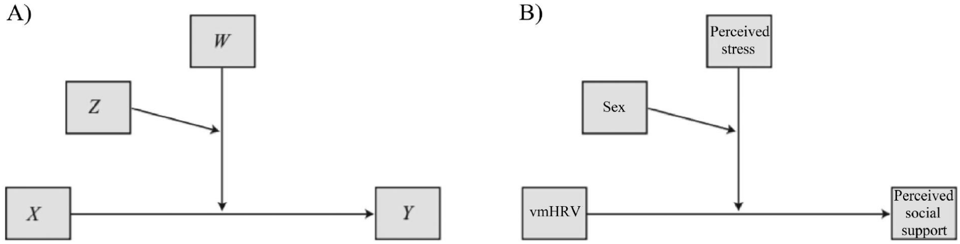

Figure 1. Model for multiple moderation analysis, as A) designed by Hayes, and B) applied in the current study. Note. vmHRV: Vagally mediated heart rate variability, indexed by high frequency power (HF-HRV).

Table 2. Bivariate correlations among main study variables in the total sample and the two sexes, respectively.

	MOS	PSS	Age	Educational level	BMI	IPAQ	AUDIT	DAST	EDR	Mean HR
Total sample										
vmHRV	0.076	−0.21*	−0.082	0.067	−0.074	0.17*	0.098	0.071	−0.16	−0.49**
MOS		0.0080	−0.17*	−0.15	−0.11	0.21*	−0.022	−0.015	0.079	−0.12
PSS			−0.060	0.000	0 .14	−0.16	0.060	0.038	0.098	0.12
Males										
vmHRV	−0.12	−0.34**	−0.12	0.026	−0.0060	0.15	0.12	0.15	−0.032	−0.35**
MOS		0.067	−0.20	−0.22*	−0.025	0.30**	−0.014	0.014	0.029	−0.15
PSS			−0.059	−0.017	0.098	−0.20	−0.0040	0.021	0.18	0.12
Females										
vmHRV	0.33**	−0.054	0.010	0.13	−0.15	0.22	0.083	−0.021	−0.31*	−0.65**
MOS		−0.11	−0.035	0.0030	−0.18	0.16	0.042	−0.016	0.11	−0.15
PSS			−0.047	0.041	0.21	−0.082	0.16	0.072	−0.0050	0.11

Note. AUDIT: Alcohol Use Disorders Identification Test; BMI: Body Mass Index (kg/m^2); DAST: Druge Abuse Screening Test; EDR; Electrocardiogram (ECG) derived respiration; IPAQ: International Physical Activity Questionnaire; MOS: Medical Outcomes Study Social Support Survey; Mean HR: Mean heart rate; PSS: Perceived Stress Scale; vmHRV: Vagally mediated heart rate variability, indexed by high frequency power (HF-HRV). *= $p < 0.05$ **=$p < 0.01$.

Moderating effects

To test the hypothesis that higher vmHRV would be associated with higher MOS scores only under conditions of higher PSS scores specifically in females and not males, we performed a moderation analysis (Table 3). In the preliminary moderation model, IPAQ scores covaried significantly with MOS scores. Therefore, IPAQ scores were included in the final model. Age, educational level, BMI, AUDIT, DAST, EDR, and mean HR did not covary significantly with MOS scores and were not included in the final model.

The conditional effects results of the final model showed that inclusion of both PSS scores and sex moderated the association between MOS scores and vmHRV, specifically for higher (i.e. intermediate and high) PSS scores in females and not males (Figure 2). Low PSS scores alone did not moderate the relation in either females or males. The same results were found when using RMSSD as an index of vmHRV.

The linear regression results from the moderation analysis, including all key variables, found that the three-way-interaction of vmHRV, PSS scores, and sex significantly predicted MOS scores, where female sex, higher vmHRV, and higher PSS scores were associated with higher MOS scores. Furthermore, the interaction term of PSS scores and sex significantly predicted MOS scores. Specifically, there was found a more prominent relationship between lower stress levels and higher perceived social support in females compared to males. Neither vmHRV, PSS scores, nor the interaction of vmHRV and PSS scores associated with MOS scores. Furthermore, there were no significant main effects of sex, or interaction effect of sex and vmHRV on MOS scores. Lastly, IPAQ scores significantly predicted MOS scores, in that higher IPAQ scores were associated with higher MOS scores.

Using MOS scores as an antecedent variable and vmHRV as a consequent variable with PSS scores and sex as moderators did not yield significant results.

Discussion

The aim of the current study was to investigate if a positive relation between vmHRV and perceived social support was dependent on (i.e. moderated by) an interaction between stress levels and sex. The expectation was confirmed in that we found a significant three-way interaction of vmHRV, perceived stress and sex on perceived social support. Specifically, investigating how this association was related to various stress levels, we found that higher vmHRV was associated with higher perceived social support only in females and not males under conditions of higher stress levels. For lower stress levels, vmHRV was not associated with perceived social support in any of the sexes.

The results, indicating sex differences in the association between perceived social support and vagus nerve activity as reflected in vmHRV in relation to stress, are in line with evolutionary theories. As females have often been responsible for offspring, a tend-and-befriend response to stress – involving the creation and maintenance of social networks aiding in nurturant activities (Taylor et al., 2000) – might generally

Table 3. Moderation analyses of prediction of MOS.

	Model summary							
	R^2	F	df	Coeff.	SE	t	p	CI
vmHRV	0.44**	3.98	134	5.08	5.91	0.86	0.39	[−6.61, 16.77]
PSS				2.78	1.85	1.51	0.13	[−0.87, 6.43]
vmHRV*PSS				−0.36	0.27	−1.37	0.17	[−0.89, 0.16]
Sex				40.57	25.41	1.60	0.11	[−9.70, 90.83]
vmHRV*Sex				−4.77	3.57	−1.34	0.18	[−11.83, 2.29]
PSS*Sex				−2.35	1.11	−2.12	0.0357*	[−4.55, −0.16]
vmHRV*PSS*Sex				0.31	0.16	1.98	0.0496*	[0.0006, 0.63]
IPAQ				2.65	0.92	2.89	0.0045**	[0.84, 4.46]
	Conditional effects of the focal predictor at the values of the moderators							
	PSS	**Percentile**		**Effect**	**SE**	***t***	***p***	**CI**
Males ($n = 82$)	17	16th		−0.51	0.83	−0.61	0.54	[−2.16, 1.14]
	22	50th		−0.75	0.62	−1.21	0.23	[−1.98, 0.48]
	28	84th		−1.04	1.01	−1.03	0.30	[−3.03, 0.95]
Females ($n = 61$)	17	16th		0.066	0.82	0.081	0.94	[−1.55, 1.69]
	22	50th		1.40	0.64	2.17	0.031*	[0.13, 2.67]
	28	84th		3.00	0.88	3.41	<0.001**	[1.26, 4.73]

Note. Coeff.: Regression coefficient. CI: 95% confidence interval for the regression coefficient and effect, respectively. MOS: Medical outcomes study social support survey; vmHRV: Vagally mediated heart rate variability, indexed by high frequency power (HF-HRV); PSS: Perceived stress scale; vmHRV*Sex: Interaction term of vmHRV and sex; PSS*Sex: Interaction term of PSS and sex; vmHRV*PSS*Sex: Three-way interacion of vmHRV, PSS and sex; IPAQ: International physical activity questionnaire; Focal predictor: vmHRV. *= $p < 0.05$; **= $p < 0.01$.

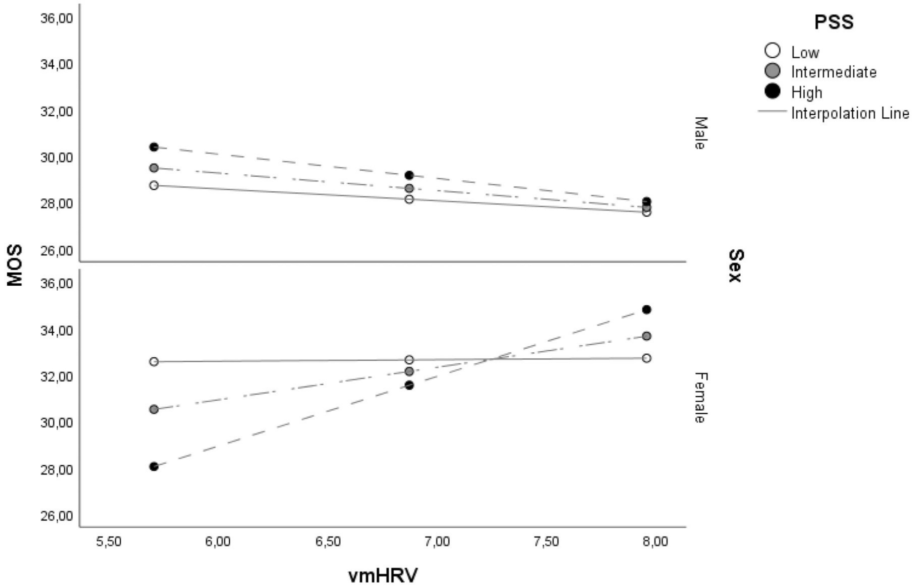

Figure 2. Regression lines for males and females on the association between vmHRV and MOS scores in relation to varying PSS scores. Note. vmHRV: Vagally mediated heart rate variability, indexed by high frequency power (HF-HRV). MOS: Medical Outcomes Study Social Support Survey; PSS: Perceived Stress Scale. Low, intermediate and high PSS scores correspond to the 16th, 50th and 84th percentiles, respectively. *$p \leq 0.05$ **$p \leq 0.01$.

have been favored over the classic "fight-or-flight" response. Whereas the fight-or-flight response typically involves adrenaline and noradrenaline signaling, the tend-and-befriend pattern is partially mediated by oxytocin (Taylor, 2007; Taylor et al., 2000). Importantly, the female sex hormone estrogen potentiates the effects of oxytocin (Taylor et al., 2000). Furthermore, higher oxytocin levels are associated with increased vagal activity (Kemp et al., 2012), likely through oxytocin-type neurons from the paraventricular nucleus communicating with cardiovagal neurons in the nucleus tractus solitarius, the dorsal motor nucleus of the vagus, and the nucleus ambiguous (see Kanthak et al., 2016). This leads to an increase of vagal outflow especially during stress, as supported in a study where social support associated with higher vmHRV in the preparation for and during a stress task only in individuals with a gene variant increasing the effect of oxytocin (see Kanthak et al., 2016), Such an increase in vagus activity as indexed by heart function, reflected by higher vmHRV, is considered to represent activity of a neural network including the prefrontal cortex and amygdala (Thayer & Lane, 2000). This network is important for adaptability and self-regulatory functions (Thayer & Lane, 2000), which might include social support seeking as a stress regulation strategy.

Associations between key variables in our study were largely in agreement with previous literature. Firstly, females reported higher perceived social support than males, as expected. Secondly, also as expected, vmHRV was more strongly associated with perceived social support under conditions of higher, relative to lower, stress levels. This is in line with a self-report study investigating the relation between vmHRV and a mixed measure of constructive coping (including social support seeking) in both sexes (Fabes & Eisenberg, 1997). Lastly, the relationship between higher perceived social support and lower stress levels was more prominent in females compared to males, which could be due to perceived social support reducing stress more effectively in females.

Contrary to expectations, when including all key variables in the same moderation model, some interaction effects (e.g. sex and vmHRV, and vmHRV and stress) were non-significant predictors of perceived social support. This underlines the importance of investigating vmHRV, stress and sex in a total model in relation to perceived social support, as the variables moderate each other. Therefore, although we found non-significant associations between some of the key variables considered separately, this does not question the validity of the total model (Hayes, 2017). Further contrary to our expectations, we did not find higher vmHRV in females compared to males. As vmHRV shows age-dependent sex differences – with lower vmHRV in girls compared to boys, and higher vmHRV in females compared to males (Koenig et al., 2017; Koenig & Thayer, 2016) – this developmental shift in vmHRV could cause less apparent sex differences in a young adult sample such as ours, explaining the respective non-finding.

In contrast to the majority of previous studies, which have relied on experimental data, our results are derived from what is often considered "trait" assessments of vagal function and self-reports. Self-reports might more accurately represent how perceived social support and stress affect participants in their daily lives, compared to experimental studies that often are non-naturalistic and situation-based (see Uchino et al., 2011). Self-reported perceived stress further accounts for that females might experience higher stress and thus greater ANS activation compared to males despite exposure to the same stressor (Tamres et al., 2002) – possibly due to effects of menstrual cycles (Simon et al., 2021). In addition, self-reports might contribute to health-related insights, as perceived support has consistently shown a beneficial influence on psychological and physiological outcomes such as cardiovascular disease, in contrast to received support (Uchino et al., 2011). Furthermore, we assessed vmHRV derived from resting ECGs, which is considered to reflect "trait" vagal activity as indexed by heart function to a large degree. This notion is supported by studies showing that resting vmHRV has a high test–retest reliability (Bertsch et al., 2012; Tarkiainen et al., 2005), and by widely supported theoretical frameworks that consider resting vmHRV as a neurophysiological marker of a tendency to self-regulate and engage socially (Porges, 2007; Thayer & Lane, 2000). Moreover, our results point in similar directions as experimental data assessing "state" vagal responses to stress protocols, in that higher vmHRV and flexible vagal reactivity under stressful conditions are associated with higher perceived social support (Goodyke et al., 2021). As such, our results are likely valid. Taken together, our study might provide important complementing insights to experimental studies.

Regarding limitations of the study, we did not control for effects of varying estrogen levels in relation to menstrual cycles, which has been shown to influence the relation between vmHRV and stress (Simon et al., 2021). However, estrogen levels are generally higher in females than males. Thus, if all females in the current study had been in the same menstrual (i.e. follicular/luteal) phase, we expect that we still would have found a more prominent association between higher vmHRV and higher perceived social support in females compared to males. Furthermore, our cross-sectional design leaves us unable to draw on causal inferences. However, our findings are supported by theoretical frameworks such as Porges' influential polyvagal theory, which suggest that higher vmHRV allows for adaptive social engagement (Porges, 2007). In addition, there is evidence that vmHRV prospectively predicts feelings of social connectedness (Kok & Fredrickson, 2010). While an opposite relation is possible, where perceived social support influences vmHRV, using perceived social support as a predictor of vmHRV in our moderation analyses did not yield significant results. Thus, the question of causality is not yet clearly elucidated. In addition, our study cannot fully explicate the complexity of the investigated constructs. Further insight might be provided by assessing multiple markers of ANS responses, possible genetic contributing factors such as oxytocin gene receptor polymorphism (see Kanthak et al., 2016), sub-categories of perceived social support (see Taylor, 2007), which sex social support is perceived to be provided from (see Taylor, 2007), and accounting for the fact that social support might not universally have a positive effect (see Kneavel, 2021; Taylor, 2007).

Our results might have important theoretical and clinical implications. Firstly, they add to the literature underlining the importance of assessing sex differences in the relations between vmHRV and psychological functioning (see e.g. Simon et al., 2021). This could also apply to studies of psychiatric illnesses, where differences in ANS regulation in relation to stress could explain sex-based variations in symptomatology (see Klein & Corwin, 2002). Secondly, we found that physical activity levels predicted perceived social support, in line with previous literature (e.g. Mendonça et al., 2014). This highlights the importance of controlling for the possible confounding effects of physical activity in the current research field. Thirdly, our results give reason to suspect that increasing vmHRV, for example by vagus nerve stimulation or biofeedback, could be an intervention strategy for individuals with high perceived stress and low perceived social support (see Porges, 2007). Interventions for increasing vmHRV could be a cost-effective prevention for the wide range of morbidities and increased mortality associated with lower vmHRV (Thayer & Lane, 2007), higher stress (Cohen et al., 2007) and lack of social support (Holt-Lunstad et al., 2010). Our findings suggest that such interventions may be especially relevant in females, which could contribute to targeted therapy.

Possibly, such interventions could also be relevant for psychiatric illnesses, which often represent the activation of evolutionary defense strategies, especially in contexts with few indications of social support (see Petrocchi & Cheli, 2019). Yet, the generalizability of the findings in the current study awaits future validation in other and larger populations.

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Data availability statement

The data that support the findings of this study are available on request from JK.

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Appendix II

frontiers in Psychiatry

ORIGINAL RESEARCH
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Lower Cardiac Vagal Activity Predicts Self-Reported Difficulties With Emotion Regulation in Adolescents With ADHD

Elisabet Kvadsheim[1], Ole Bernt Fasmer[2], Berge Osnes[1], Julian Koenig[4,5], Steinunn Adolfsdottir[1,6], Heike Eichele[1], Kerstin Jessica Plessen[7,8] and Lin Sørensen[1]*

[1] Department of Biological and Medical Psychology, University of Bergen, Bergen, Norway, [2] Department of Clinical Medicine, University of Bergen, Bergen, Norway, [3] Haukeland University Hospital, Bjørgvin District Psychiatric Centre, Bergen, Norway, [4] Section for Experimental Child and Adolescent Psychiatry, Department of Child and Adolescent Psychiatry, Centre for Psychosocial Medicine, University of Heidelberg, Heidelberg, Germany, [5] University Hospital of Child and Adolescent Psychiatry and Psychotherapy, University of Bern, Bern, Switzerland, [6] Department of Visual Impairments, Statped West - National Service for Special Needs Education, Bergen, Norway, [7] Child and Adolescent Mental Health Center, Capital Region Psychiatry, Copenhagen, Denmark, [8] Department of Clinical Medicine, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

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***Correspondence:**
Elisabet Kvadsheim
elisabet.kvadsheim@uib.no

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Objective: To investigate the relation between cardiac vagal activity (CVA), a measure of autonomic nervous system (ANS) flexibility, and self-reported emotion regulation (ER) difficulties in adolescents with attention-deficit/hyperactivity disorder (ADHD) and controls.

Methods: The sample comprised 11–17-year-old adolescents with ADHD (n=34) and controls (n = 33). Multiple linear regression analyses investigated the relation between CVA, as indexed by high frequency heart rate variability (HF-HRV), and ER difficulties as assessed by the Difficulties in Emotion Regulation Scale (DERS). Supplemental analyses were performed in ADHD and control groups separately. Analyses assessed effects of body mass index (BMI), physical activity levels, and HF peak as a surrogate of respiration on CVA.

Results: Lower CVA was associated with ER difficulties, and specifically with limited access to effective ER strategies. When investigating the relation between CVA and ER in the ADHD and control groups separately, there was a tendency of lower CVA predicting limited access to effective ER strategies in the ADHD group, and not in the control group.

Conclusion: The results suggest that lower CVA, i.e., reduced ANS flexibility, in adolescents with ADHD and controls is associated with self-reported ER difficulties, and specifically with limited access to effective ER strategies. There was a tendency for lower CVA to predict limited ER strategies only in the adolescents with ADHD and not controls.

Keywords: attention-deficit/hyperactivity disorder, heart rate variability, cardiac vagal activity, emotion regulation, autonomic nervous system, difficulties in emotion regulation scale

INTRODUCTION

Individuals with attention-deficit/hyperactivity disorder (ADHD) are characterized by difficulties with self-regulation (1), including emotion regulation [ER; (2)]. ER is the ability to influence which emotions one displays, at what time, and how they are experienced and expressed (3). In children and adolescents with ADHD, it is estimated that 25–45% have difficulties with ER (4). In healthy individuals, such ER difficulties are linked to inflexibility of the autonomic nervous system (ANS), often indexed by lower resting state cardiac vagal activity [CVA; (5–7)]. In ADHD, the link between ER difficulties and lower CVA is not as clear. This might be due to the reliance on parent-reports of ER difficulties in previous studies (8, 9). In the current study, we aimed to investigate the relation between self-reported ER difficulties and level of CVA in adolescents with ADHD.

Emotions—multifaceted processes involving changes in experiential, behavioral, and physiological responses (10)—are not always appropriate and in accordance with environmental demands, emphasizing the importance of ER for adaptive behavior and achievement of long-term goals (3). ER difficulties might be due to "bottom-up" psychological mechanisms such as orienting to emotional, salient stimuli and reward processing, or due to poorer "top-down" regulation (2). This might manifest as atypical allocation of attention to emotionally valenced stimuli, emotional lability (11, 12), and socially unsuitable behavior (2). The experience of emotions are associated with varying degrees of physiological arousal generated by the ANS (13). Flexibility of the ANS increases the capacity to rapidly modulate physiological and emotional states in accordance with situational demands. This flexibility is thought to depend on vagal modulation and to be reflected by CVA, derived from the variance in consecutive heart beat intervals (heart rate variability). CVA has been suggested as a transdiagnostic marker of ER difficulties (14). In healthy adults, self-reported ER difficulties have been associated with lower CVA, and specifically with difficulties with emotional impulse control, emotional clarity (15), and acceptance of negative emotions (16). Further, in a group of adolescents with and without psychiatric disorders (not ADHD), self-reports of difficulties with emotional awareness associated with lower CVA (17). Also of relevance, tendencies of worrying (18), anxiety disorders (19), and clinical depression in children and adolescents (20) are associated with lower CVA.

In ADHD, ER difficulties are shown to have a negative influence on well-being and self-esteem, peer relationships, school performance (21), and social skills (22). Further, such difficulties are linked to general anxiety disorder (23) and major depressive disorder [MDD; (24)], which are comorbid in about 20–30% of children and adolescents with ADHD [(22, 25)]. Only few studies have investigated the association between ER difficulties and CVA in children and adolescents with ADHD, and these have used parent-reports of ER difficulties. One such study found no association between CVA and ER difficulties (8), however another study found a dichotomous index of ER difficulties to be associated with lower CVA (9). Further, Griffiths et al. (26) found correlations of CVA with parent-reported symptoms of anxiety, social difficulties, and oppositional behavior in children and adolescents with ADHD. They concluded that CVA seem to reflect level of ER and adaptive behavior. However, no study has investigated whether CVA is associated with self-reported ER difficulties in children or adolescents with ADHD. As self-reports might capture internal, non-observable aspects of ER, they could provide information that parent reports do not detect. This is supported by a study in children and adolescents with ADHD, where self-reported symptoms of anxiety associated better with observed neurocognitive dysfunctions than parent- and teacher reports (27). Further, children with ADHD and controls have been found to report higher health-related quality of life (HRQoL) than HRQoL assessed by parent reports. This highlights the importance of also investigating self-reports in the study of psychophysiological aspects in children and adolescents with ADHD.

In the current study, we investigated the association between CVA and self-reported ER difficulties in adolescents with ADHD, and without ADHD (i.e. control group). We applied a naturalistic study design by including control participants with mental health disorders that often are comorbid to ADHD, such as anxiety disorders and Tourette Syndrome (TS). We hypothesized that lower CVA would be associated with ER difficulties, and expected this effect to be more prominent in adolescents with ADHD. We further explored the association between CVA and specific aspects of ER: acceptance of emotional responses, goal-directed behavior, and impulse control in relation to emotions, emotional awareness, access to effective ER strategies, and emotional clarity. We hypothesized that ER strategies would be the domain most closely associated with CVA, as CVA has been found to associate with neural activity during reappraisal and response modulation (28). This suggests that individuals displaying low CVA have difficulties with recruiting prefrontal brain areas necessary for modulation of amygdala activity during explicit ER. Further supporting our hypothesis, lower CVA has been associated with less use of constructive coping strategies (29) and interpersonal ER strategies such as support seeking (30).

METHODS

Design

The current study is a cross-section of a follow-up project on ER in children with ADHD [studies from first wave: (31–33). The retention rate from the first assessment was 66.2% (ADHD= 60.7%, controls=71.7%); **Supplemental Figure 1**). The study protocol was approved by the Regional Committee for Medical Research Ethics of Western Norway (Study number: 2014/1304).

Participants

The sample comprised 34 adolescents with ADHD and 33 controls between 11 and 17 years of age (**Table 1**). The majority of participants were male (*n*= 43; 64.2%). Children with suspected ADHD were originally referred from child and

TABLE 1 | Descriptive statistics for ADHD and control groups.

Variable	ADHD (*n* = 34)	Controls (*n* = 33)	t	χ2	*df*
Background information					
Gender (n, %)				.0080	1
Male	22 (64.7)	21 (63.6)			
Female	12 (35.3)	12 (36.4)			
Age (years)	14.34 (1.52)	14.62 (1.15)	.86		65
IQ	93.65 (10.27)	106.94 (10.63)	5.21**		65
BMI (kg/m^2)	22.62 (5.73)	21.11 (2.91)	-1.37		49
Physical activity (times/week)	1.68 (.81)	2.70 (1.18)		16.68**	5
HF peak (Hz)	-1.41 (.25)	-1.36 (.21)	.85		65
Questionnaire scores					
DERS TOTAL	84.56 (23.83)	67.60 (15.36)	-3.47**		57
NONACCEPTANCE	11.05 (5.70)	8.19 (2.92)	-2.59*		50
GOALS	14.41 (5.57)	11.21 (4.42)	-2.60*		65
IMPULSE	13.82 (6.48)	9.33 (4.29)	-3.35**		57
AWARENESS	19.02 (4.83)	18.25 (5.10)	-.63		65
STRATEGIES	15.50 (6.75)	12.24 (4.09)	-2.40*		55
CLARITY	10.79 (3.71)	8.33 (2.79)	-3.06**		65
ADHD-RS	25.81 (10.86)	5.21 (6.67)	-9.38**		55
CVA (HF-HRV (n.u.))	1.78 (.12)	1.82 (.11)	1.55		65

*ADHD, Attention Deficit Hyperactivity Disorder; BMI, Body Mass Index; HF peak, Peak of high frequency heart rate variability; DERS TOTAL, Difficulties in Emotion Regulation Scale total score; NONACCEPTANCE, Non-acceptance subscale; GOALS, Goals subscale; IMPULSE, Impulse control subscale; AWARENESS, Emotional awareness subscale; STRATEGIES, Emotion regulation strategies subscale; CLARITY, Emotional clarity subscale; ADHD-RS, ADHD Rating Scale; CVA, Cardiac Vagal Activity; IQ scores were collected in the first assessment of the study, when participants were 8-12 years old. *= p.<.05 **= p<.01.*

TABLE 2 | ADHD subtypes and comorbid diagnoses.

	ADHD (n, %)	Controls (n, %)
ADHD subtype		
IA	13 (38.2)	-
HI	1 (2.9)	-
C	20 (58.8)	-
TS	12 (35.3)	5 (15.2)
Anxiety disorders	10 (29.4)	2 (6.1)
ODD	7 (20.6)	–
OCD	2 (5.9)	1 (3.0)
MDD	1 (2.9)	–
Eneuresis	1 (2.9)	–
Epilepsy	1 (2.9)	–
Chronic motor tics	1 (2.,9)	–
Transient motor tics	1 (2.9)	–
Anorexia	–	1 (3.0)

IA, Inattentive subtype; HI, Hyperactive-impulsive subtype; C, Combined subtype; TS, Tourette Syndrome; ODD, Oppositional Defiant Disorder; OCD, Obsessive-Compulsive Disorder; MDD, Major Depressive Disorder.

adolescent psychiatry units, and were assigned to either an ADHD or control group after identification with a semi-structured diagnostic interview: "the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version" [K-SADS; (34)]. Full-Scale IQ was measured with the Wechsler Intelligence Scale for Children —fourth version [WISC-IV; (35)]. In the follow-up assessment, the K-SADS was re-administered. All adolescents in the ADHD group still met the criteria for an ADHD diagnosis, except one participant having ADHD symptoms in remission (See **Table 2** for ADHD subtypes and comorbid disorders). Among participants in the control group, some comorbid conditions other than ADHD were present. Nineteen adolescents with ADHD were using stimulant medication. Parent reports on the DSM-IV ADHD rating scale [ADHD-RS; (36)] assessed the symptom distribution in the ADHD and control groups in both study assessments (37).

Measures

Cardiac Vagal Activity

CVA was derived from electrocardiogram (ECG) recordings. Participants were lying in a supine position and were instructed to relax while trying not to move or fall asleep. Participants were breathing spontaneously while the ECG was recorded for 6 minutes with a simple lead II setup at a sampling rate of 1,000 Hz. An A/D converter (Biopac, MP36, Biopac System Inc., Santa Barbara, CA) was used to obtain the signal, which was recorded with Biopac 4.0 BSL (Biopac Systems Inc. Santa Barbara, CA). The signal was conducted through three adhesive Ag/AgCl electrodes (T815 Dia. 55).

The first 5 minutes of the ECG recordings were subject to preparation and analysis in Kubios HRV version 2.2 (38). Inter-beat intervals (IBI) were calculated, and, if necessary, corrected. A total of 11 IBI corrections were made (.4–1.5% of IBIs in the corrected recordings), in six recordings (ADHD, *n* = 3; controls, *n* = 3). Additionally, one R-R interval containing an extra systole was removed from a recording.

Recordings were subject to a frequency analysis with the Fast Fourier Transformation, yielding a power spectrum displaying activity in the very low frequency (VLF, < .04 Hz), low frequency (LF,.04–.15 Hz) and high frequency (HF,.15–.4 Hz) domain (39). HF power was chosen as the applied measure of CVA (40). We assessed HF power in normalized units (n.u.), which corrects for the influence of VLF-HRV, and thus can be regarded as a measure of pure vagal influence (41). We used HF peak to indirectly control for respiratory frequency (42).

Emotion Dysregulation

The Difficulties in Emotion Regulation Scale [DERS; (43)] is a 36-item self-report questionnaire assessing ER difficulties. Items are rated on a five-point Likert-type scale from 1 ("almost never") to 5 ("almost always"). In addition to a total score (DERS TOTAL), the questionnaire yields information on ER difficulties relating to different facets: (i) Non-acceptance of emotional responses (NONACCEPTANCE; e.g. "When I'm upset, I become embarrassed for feeling that way"; Cronbach's α in the current sample =.88); (ii) Difficulties engaging in goal-directed behavior (GOALS; e.g. "When I'm upset, I have difficulty focusing on other things"; α=.89); (iii) Impulse control difficulties (IMPULSE; e.g. "When I'm upset, I feel out of control"; α=.92); (iv) Lack of emotional awareness (AWARENESS; e.g. "I am attentive to how I'm feeling"; α=.75); (v) Limited access to ER strategies perceived as effective (STRATEGIES; e.g. "When I'm upset, it takes me a long time to feel better"; α=.85); and (vi) Lack of emotional clarity

(CLARITY; e.g. “I am confused about how I feel”; α=.73). High scores reflect a high level of emotional dysregulation.

A Norwegian translation of the DERS was used (44) Although originally developed for adults, the DERS has been validated in adolescent samples, where it has shown satisfactory to excellent internal consistency (Cronbach's α=.72-.89) and adequate construct validity (45, 46). Further, it has been applied in a study of an adolescent ADHD sample (47).

In the current sample, six participants (ADHD: *n*=3, Controls: *n*=3) had one through five missing item scores, in total 14 missing scores.

Physical Activity and Body Mass Index (BMI)

Engagement in physical activity was assessed during the K-SADS, as the adolescents reported in “Other activities; Sport and Exercise”. Physical activity levels were scored on a six-point Likert-type scale from 0 (“zero times a week”) to 5 (“seven times or more a week”), as for Question 1 of the Physical Activity Questionnaire for Adolescents [PAQ-A; (48)]. The PAQ-A scoring norm was applied due to its convergent validity (49). Physical activity levels in between two categories were lowered to the nearest category. Further, BMI was calculated from measured height and weight as weight/height^2 (kg/m^2). BMI could not be calculated for four participants (ADHD, *n*=2; Controls, *n*=2), as they declined to get weighed or due to technical errors.

Procedure

Adolescents and their parents participated in assessments conducted over two days. Test administrators were blinded to group status. On the first day, participants received information about the project and the assessments they would undergo. Then, ECG recordings were conducted between 9 a.m. and 1 p.m., to control for the effect of circadian variation on CVA (50). To rule out short-term effects of centrally stimulating medication for ADHD on heart rate (51) and ER, 89.5% of the adolescents with ADHD on stimulants (*n*=17, of 19) conducted a washout period of at least 24 h. This equals minimum five half-lives of the stimulant medication (52). On the second day, adolescents and their parents were separately interviewed with the K-SADS and filled out questionnaires. Participants received a reimbursement of £80 ($105).

Statistical Analysis

Statistical analyses were performed in the Statistical Package for the Social Sciences version 24.0 (SPSS; IBM Corp., Armonk, NY, 2016). CVA data were log-transformed to approximate a normal distribution (53). We performed preliminary analyses with independent samples t-tests of group differences in distribution of age, gender, ADHD-RS scores, DERS scores, CVA, HF peak, Full-Scale IQ, and on HRV power spectrum values. Bivariate correlations were conducted between age, BMI, physical activity, HF peak, CVA, and DERS scores. No outliers, defined as values ± 3*SD* from the mean, were detected for DERS TOTAL, ADHD-RS, or CVA. Missing ADHD-RS data from one participant with ADHD was imputed with the sample mean. Missing BMI values were replaced with the sample mean and missing DERS values by the sample means of the respective items.

To test the hypothesis that lower CVA would be related to higher self-reported ER difficulties, we conducted multiple linear regression analyses. The DERS TOTAL score and the DERS subscale scores were used as dependent variables, respectively. CVA and diagnostic status were included as predictors. Analyses were adjusted for effects of age, gender, and HF peak. The regression model were adjusted for BMI [see (54)] and physical activity [see (55)] by including these as predictors, when a significant correlation between these variables and CVA and/or DERS subscale scores were found in the preliminary analyses. To adjust for multiple testing, we performed Bonferroni correction (56), yielding a corrected alpha level of p =.007 (.05/7). In follow-up regression analyses, an interaction term of ADHD*CVA (CVA scores were centralized with z scores for the interaction term) was added as a predictor to test for interaction effects between ADHD and CVA on ER difficulties (57, 58). As supplementary analyses, we conducted the same multiple linear regression analyses separately for the ADHD and control groups. We also performed follow-up analyses excluding participants in the control group with psychiatric disorders, to control for effects of a mixed participants control group.

RESULTS

Preliminary Analyses

There were no differences in age nor gender between the ADHD and control group (**Table 1**). Parents reported higher ADHD-RS scores in the ADHD group compared to the control group. There were no significant differences in ADHD-RS scores between samples from the first and second assessment of the study. Further, control participants reported significantly higher physical activity levels than adolescents with ADHD did. There were no significant differences in BMI or HF peak between the groups. Bivariate correlation analyses showed that lower CVA was associated with higher DERS TOTAL, STRATEGIES, and CLARITY scores (**Supplemental Table 1**). Higher CVA also correlated with higher physical activity. CVA did not correlate with age nor BMI. Further, independent samples t-test showed no group difference in CVA. The ADHD group reported higher total scores and subscale scores on the DERS, except for on AWARENESS. No gender effects were found on CVA (**Supplemental Table 2**). The only gender effect that appeared in relation to DERS scores was that girls reported higher CLARITY scores than boys (Mean values: girls = 11.04; boys = 8.77; t = 2.67, p=.009). There were no group differences in CVA power spectrum values (**Supplemental Table 3**).

Predicting Emotion Dysregulation by CVA

To test the effect of CVA on ER difficulties, we conducted multiple linear regression analyses (**Table 3**), which showed that lower CVA predicted higher STRATEGIES scores (**Figure 1**). There was a tendency for lower CVA to also predict higher DERS TOTAL scores. No effects of CVA were detected on NONACCEPTENCE, GOALS, IMPULSE, AWARENESS, or

TABLE 3 | Prediction of CVA on DERS scores.

Predictor	R^2	df	F	p	β
DERS TOTAL					
ADHD	.311	(6, 66)	4.52*	.0020**	.39
Age				.89	.015
Gender				.021*	-.25
HF peak				.063	.21
Physical activity level				.69	.053
CVA				.019*	-.28
NONACCEPTANCE					
ADHD	.19	(6, 66)	2.33*	.017*	.32
Age				.67	.052
Gender				.057	-.23
HF peak				.17	.17
Physical activity level				.70	.055
CVA				.20	-.17
GOALS					
ADHD	.20	(6, 66)	2.41*	.030*	.29
Age				.92	.013
Gender				.12	-.18
HF peak				.043*	.25
Physical activity level				.81	-.033
CVA				.25	-.15
IMPULSE					
ADHD	.20	(6, 60)	2.43*	.0060**	.37
Age				.83	-.025
Gender				.19	-.15
HF peak				.50	.082
Physical activity level				.82	.032
CVA				.19	-.17
AWARENESS					
ADHD	.036	(6, 60)	.37	.39	.13
Age				.67	-.057
Gender				.78	.036
HF peak				.99	-.0010
Physical activity level				.27	.17
CVA				.34	-.12
STRATEGIES					
ADHD	.28	(6, 60)	3.82**	.064	.24
Age				.92	.011
Gender				.025*	-.25
HF peak				.13	.18
Physical activity level				.99	.000
CVA				.0030**	-.37
CLARITY					
ADHD	.35	(6, 60)	5.29**	.0030**	.37
Age				.30	.11
Gender				.0020**	-.33
HF peak				.015*	.27
Physical activity level				.772	.037
CVA				.050	-.23

*ADHD, ADHD group diagnostic status; HF peak, Peak of high frequency heart rate variability; CVA, Cardiac Vagal Tone. *= p< .05 **= p< .01.*

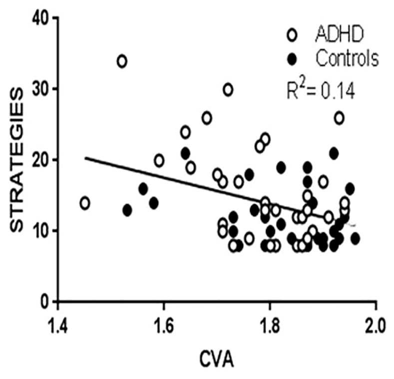

FIGURE 1 | Scatterplot with regression line of STRATEGIES and CVA.

CLARITY. The diagnostic status of ADHD predicted higher scores on DERS TOTAL and all DERS subscales except for AWARENESS. Gender covaried with DERS TOTAL, STRATEGIES, and CLARITY, in that girls had higher scores. Higher HF peak covaried with higher CLARITY and GOALS. Neither age nor physical activity covaried with any of the DERS scores. Follow-up regression analyses adding the interaction term ADHD*CVA did not detect an interaction effect between ADHD and CVA on any of the DERS scores. Conducting the same multiple regression analyses separately in the diagnostic groups showed that lower CVA predicted higher STRATEGIES in the ADHD group (**Supplemental Table 4**; **Supplemental Figure 2**). CVA did not predict any other DERS scores in the ADHD group, and did not predict any DERS scores in the control group. Lastly, excluding controls with comorbid disorders, we found that CVA still predicted STRATEGIES (p=.004) in multiple linear regression analyses.

DISCUSSION

The aim of the present study was to investigate whether CVA, an index of autonomic flexibility, was associated with self-reported ER difficulties in adolescents with ADHD and controls. Results showed that, as expected, lower CVA significantly predicted higher STRATEGIES scores. There was also a tendency for lower CVA to predict higher DERS TOTAL scores. This indicates that level of CVA reflects the ability to use effective strategies for ER. Results also showed that the ADHD diagnosis associated with higher DERS scores, and thus higher ER difficulties, except for in relation to lack of emotional awareness. However, no interaction effects between level of CVA and ADHD on the DERS scores were found. When analyzing the association between CVA and DERS separately in the diagnostic groups, we found that lower CVA was associated with higher STRATEGIES scores in the ADHD group and not in the controls. Thus, despite the non-significant interaction effect between CVA and ADHD, there was a tendency for CVA to be associated with less access to effective ER strategies specifically in the ADHD group.

Our results suggest that level of CVA is important for the use of ER strategies perceived as effective by the individual experiencing and expressing the emotions. The STRATEGIES subscale measures the flexible use of situationally appropriate strategies (43). This finding is in line with a study of young adults, where higher CVA was associated with the use of constructive coping strategies during stressful events (29). Further, a study of adolescents found higher CVA to be associated with a tendency of seeking support from others in times of suffering, an important way to modulate and alleviate emotions (30). Lower CVA has been suggested to be associated with difficulties in recruiting prefrontal brain areas necessary for modulation of activity in amygdala during explicit ER, such as reappraisal and response modulation (28). Such prefrontal cortical regions have been found to display altered structural and functional maturation in ADHD (2, 4), and activity in these areas are hypothesized to be reflected in CVA (7). Through this network, inappropriate timing and magnitude of emotional experiences and behavioral reactions might be associated with reduced vagal modulation (5).

In our study, the small sample size, and as such low statistical power, may restrict the detection of interaction effects between ADHD and level of CVA on DERS scores. Despite this restriction, when looking into the relation between CVA and ER for the ADHD and control groups separately, we found a significant effect of CVA on ER (i.e., STRATEGIES) only in the adolescents with ADHD. This indicates that despite a lack of a significant interaction effect, there is a tendency of lower CVA to associate with higher ER difficulties specifically in the ADHD group. Since there are few studies of CVA and ER in ADHD, it is still not clear whether lower CVA predicts ER difficulties specifically in ADHD. As far as we know, only one previous study has found parent-reported emotion dysregulation to be associated with lower CVA in children and adolescents with ADHD (9). Further, another study found lower CVA in children and adolescents with ADHD to be associated with higher levels of anxiety, social difficulties, and oppositional behavior (26). It is possible that lower CVA could be related to ER difficulties in general, and not specifically for individuals with ADHD. In the current study, adolescents with ADHD reported higher levels of ER difficulties than controls, as also reported by parents in previous studies (2). It is therefore important to be aware of the association between ER difficulties and inflexible autonomic functioning among adolescents with ADHD, whether this association is specific for ADHD or not. This is because lower CVA might have implications for not only psychological, but also somatic health, morbidity, and longevity (59).

Previous studies of CVA and DERS in young, healthy adults have found CVA to be associated with less emotional impulse control and emotional clarity (15), and less acceptance of negative emotions (16). A longitudinal study over three years of children with and without psychiatric conditions found increasing CVA to be associated with fewer ER difficulties as measured by the DERS, and specifically with higher emotional awareness (17). The inconsistency in results between studies of CVA and DERS shows that it is not clear on a subscale level which specific ER domain are associated with CVA. In our study, we investigated a different age group than two of the previous studies. Our results are therefore not directly comparable to the previous findings.

Our results, indicating that lower CVA and thus inflexibility of the ANS is associated with limited access to ER strategies among adolescents with ADHD and controls, might have important theoretical and clinical implications. Our study contributes to the understanding of which specific domains of ER are related to CVA, which could support theoretical models of the relation between neural and psychological processes. Further, this might lead to increased focus on biofeedback treatment, which has been suggested to increase ER abilities [e.g. (60)]. Also, our finding of a significant association between level of CVA and self-reported ER difficulties shows that adolescents with ADHD seem to have insight into their own problems, which could be a good starting point for psychotherapeutic interventions. Further, future studies of adolescent ADHD samples might benefit from using self-reports instead of only observational reports from parents or teachers when investigating relations between ER difficulties and neurobiological variables such as CVA.

The current study has several strengths and limitations. One strength is that we have included possible confounders on CVA such as physical activity levels (55), BMI (54), and HF peak (42). Further, we controlled for circadian influences on CVA. Regarding limitations, we only used one questionnaire to assess ER difficulties, which may not capture the entire complexity of the concept. In addition, although we asked participants to conduct a washout period of ADHD medications to rule out short-term effects, we cannot rule out possible longer-term effects of such medication on CVA (51, 61, 62) and/or ER (63). Further, the mixed control group of adolescents with and without psychiatric diagnoses other than ADHD may have contributed to lower statistical power in detecting group differences. Finally, the study is cross-sectional in nature. Although there is evidence suggesting that lower CVA prospectively predicts emotion dysregulation (64), there is a possibility that difficulties in ER could influence CVA. Future research on the field would benefit from longitudinal designs, as well as studies designed to manipulate ER, to examine this notion.

DATA AVAILABILITY STATEMENT

Datasets are available on request. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Regional Committee for Medical Research Ethics of Western Norway, study number 2014/1304. All subjects

gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Regional Committee for Medical Research Ethics of Western Norway.

AUTHOR CONTRIBUTIONS

LS and KP designed the study. LS, SA, EK, and HE were involved in data collection. EK and LS, and BO performed data acquisition and analyses. EK and LS wrote the first draft, and JK, OF, BO, HE, and KP contributed to manuscript editing. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00244/full#supplementary-material

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Appendix III

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ORIGINAL ARTICLE

PSYCHOPHYSIOLOGY SPR WILEY

Innovative approaches in investigating inter-beat intervals: Graph theoretical method suggests altered autonomic functioning in adolescents with ADHD

Elisabet Kvadsheim[1] | **Ole Bernt Fasmer**[1,2] | **Erlend Eindride Fasmer**[3] | **Erik R. Hauge**[2] | **Julian F. Thayer**[4] | **Berge Osnes**[5,6] | **Jan Haavik**[2,7] | **Julian Koenig**[8] | **Steinunn Adolfsdottir**[5,9] | **Kerstin Jessica Plessen**[10] | **Lin Sørensen**[5]

[1]Department of Clinical Medicine, University of Bergen, Bergen, Norway

[2]Division of Psychiatry, Haukeland University Hospital, Bergen, Norway

[3]Independent Researcher, Harstad, Norway

[4]Department of Psychological Science, University of California, Irvine, Irvine, California, United States

[5]Department of Biological and Medical Psychology, University of Bergen, Bergen, Norway

[6]Bjørgvin District Psychiatric Centre, Haukeland University Hospital, Bergen, Norway

[7]Department of Biomedicine, University of Bergen, Bergen, Norway

[8]Faculty of Medicine, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Cologne, University of Cologne, Cologne, Germany

[9]Department of Visual Impairments, Statped - National Service for Special Needs Education, Bergen, Norway

[10]Division of Child and Adolescent Psychiatry, Department of Psychiatry, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland

Correspondence
Elisabet Kvadsheim, Department of Clinical Medicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway.
Email: elisabetkvadsheim@hotmail.com

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Abstract

Cardiac inter-beat intervals (IBIs) are considered to reflect autonomic functioning and self-regulatory abilities and are often investigated by traditional time- and frequency domain analyses. These analyses investigate IBI fluctuations across relatively long time series. The similarity graph algorithm is a nonlinear method that analyzes segments of IBI time series (i.e., time windows)—possibly being more sensitive to transient and spontaneous IBI fluctuations. We hypothesized that the similarity graph algorithm would detect differences between Attention-Deficit/Hyperactivity Disorder (ADHD) and control groups. Resting electrocardiogram (ECG) recordings were collected in 10–18-year-olds with ADHD (n = 37) and controls (n = 36). IBIs were converted to graphs that were subsequently investigated for similarity. We varied the criterion for defining IBIs as similar, assessing which setting best distinguished ADHD and control groups. Using this setting, we applied the similarity graph algorithm to time windows of 2–5, 6–13 and 12–25 s, respectively. We also performed traditional IBI analyses. Independent samples t tests assessed group differences. Results showed that a 1.5% criterion of similarity and a time window of 2–5 s best distinguished adolescents with ADHD and controls. The similarity graph algorithm showed a higher number of edges, maximum edges and cliques, and lower edges10+10/edges2+2 in the ADHD group compared to controls. The results suggested more similar IBIs in the ADHD group compared to the controls, possibly due to altered vagal activity and less effective regulation of heart rate. Traditional analyses did not detect any group differences. Consequently, the similarity graph algorithm might complement traditional IBI analyses as a marker of psychopathology.

KEYWORDS

ADHD, autonomic nervous system, graph theory, heart rate variability, interbeat interval, nonlinear

1 | INTRODUCTION

Heart rate variability (HRV) indexes autonomic activity by assessing differences in consecutive inter-beat intervals (IBIs; Camm et al., 1996) of an electrocardiogram (ECG) recording. HRV is often analyzed by time- and frequency domain methods (Thayer et al., 2010) based on linear models, although the adaptive mechanisms that regulate heart rate (HR) are considered to be nonlinear (see Huikuri et al., 2003; Rajendra Acharya et al., 2006). Further, HRV indices typically depend on calculations from one to five minutes of an IBI series (Camm et al., 1996). As the indices are expressed as mean values or summary statistics from relatively long recordings (Camm et al., 1996; Shaffer & Ginsberg, 2017), they can be similar despite being from recordings with distinctly differently organized IBIs. Hence, valuable information about IBI organization might go undetected by use of linear methods.

IBIs are influenced by complex regulatory systems causing frequent spontaneous fluctuations in HR, leading to nonlinearity of IBI organization (see Huikuri et al., 2003; Rajendra Acharya et al., 2006). Nonlinear methods based on concepts such as chaos, fractality, and complexity have as such led to important insights into IBIs dynamics (de Godoy, 2016; Henriques et al., 2020; Voss et al., 2009), although not without limitations (see Henriques et al., 2020). These methods might be hard to compute or interpret, or vulnerable to erroneousness if parameter choices are non-optimal (Henriques et al., 2020). Others are highly sensitive to ECG length or artifacts, or do not utilize all data (Henriques et al., 2020). Some methods might also reflect autonomic activity inaccurately (Cepeda et al., 2018). On account of such limitations, the development of nonlinear methods is still warranted.

Graph theory is a promising mathematical field for application to nonlinear methods, which has provided new insights into various brain disorders in neuroimaging studies (e.g., Ahmadlou et al., 2012; Bullmore & Sporns, 2009; Stam & Reijneveld, 2007). As opposed to assessing values across an entire IBI series, graph theory allows for the investigation of smaller segments of the time series. These *time windows* provide information about moment-to-moment IBI fluctuations, as represented in a *graph*. The term "*graph*" refers to the visualization of a set of *nodes* and *edges* (Kleinberg & Tardos, 2006). Each IBI is visualized as a point—a node. Similar IBIs are connected by a line—an edge. Thus, a graph highlights similarities in IBIs (Figure 1). Different *criteria of similarity*—thresholds for defining IBIs as similar—provide different graphs. There are numerous indices that can be deducted from such graphs. Yet, previous graph theory-based studies of IBIs appear to have performed analyses of only one specific variable (Choudhary et al., 2019, 2020). This might be insufficient for characterizing complex physiological systems such as IBI organization (see Voss et al., 2009).

In the current study we applied a graph theory-based, nonlinear method that might complement other IBI analyses*:* the *similarity graph algorithm*. It has previously been applied in studies of motor activity (Fasmer et al., 2018, 2020). The algorithm assesses several indices familiar from graph theory in relatively short time windows, providing information about moment-to-moment IBI similarity - termed *inter-relatedness*. As further detailed in the method section, the indices represent different perspectives on inter-relatedness and lack of inter-relatedness. A ratio of inter-relatedness across a longer compared to a shorter time window is also calculated: edges10+10/edges2+2. This might be compared to the inverse of the previously criticized (Billman, 2013) low frequency/high frequency (LF/HF) ratio from HRV analysis (Camm et al., 1996), although our use of time windows might provide a more refined index.

We suggest that a higher inter-relatedness reflects altered ANS activity, similar to a lower HRV. Supporting this, graph theory-based methods have found lower IBI complexity (i.e., more similarity) in individuals with heart disease and the elderly (Choudhary et al., 2020, 2019), which are populations that often have a lower

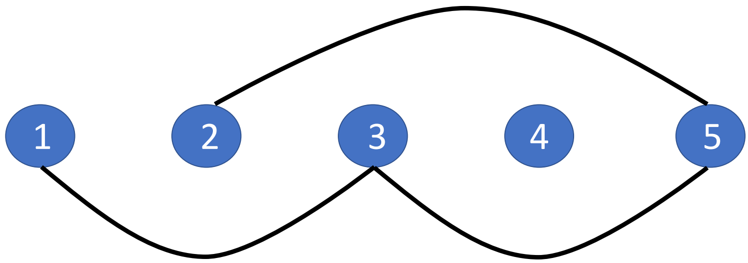


FIGURE 1 Illustration of nodes and edges in a graph. We introduce an *edge* (black line in the figure) between two *nodes* (blue dots 1–5 in the figure) if they are similar to each other and within the same time window. Each node corresponds to an IBI in the time series. The time window in the figure consists of five nodes. Node nr. 1, 3, and 5, as well as node 2 and 5, are connected by edges

HRV (Camm et al., 1996; Thayer & Lane, 2007; Voss et al., 2012). Of the ANS components, the vagus nerve is crucial for adaptive changes in HR and variability in IBIs (Berntson et al., 1997). The vagus nerve has a rapid course of action, compared to sympathetic activity that has a peak effect after about five seconds (Nunan et al., 2010). As such, higher inter-relatedness in shorter time windows (i.e., less than five seconds) might reflect vagal alterations most accurately, whereas longer time windows might also represent other ANS functions. Vagal alterations indexed by lower vagally mediated HRV have been associated with less effective self-regulatory abilities and general psychopathology (Beauchaine, 2015; Beauchaine & Thayer, 2015; Holzman & Bridgett, 2017). This is likely because activity in brain areas important for adaptability, such as the prefrontal cortical areas and amygdala, is connected to ANS centers in the brainstem and reflected in vagal modulation on the heart (Thayer & Lane, 2000). Possibly, such self-regulatory abilities and vulnerability to psychopathology might also be reflected in inter-relatedness indexes of vagal modulation.

As traditional IBI indices have been associated with self-regulatory abilities and general psychopathology (Beauchaine, 2015; Beauchaine & Thayer, 2015; Holzman & Bridgett, 2017), we investigated as a proof of concept if the similarity graph algorithm could detect IBIs differences between adolescents with Attention-Deficit/Hyperactivity Disorder (ADHD) and controls. A meta-analysis concluded with no differences in vagally mediated HRV in individuals with ADHD compared to controls (Koenig et al., 2016). Still, lower vagal activity has been associated with ADHD symptoms such as inattention, impulsivity, behavioral disinhibition, and difficulties with goal-directed behavior (see Rash & Aguirre-Camacho, 2012), giving reason to suspect vagal alterations in this group. Further, ADHD is likely characterized by altered noradrenaline and dopamine functioning (Sharma & Couture, 2014; Tripp & Wickens, 2009), which affects the ANS (LeBouef et al., 2020; Thorner, 1975). Importantly, dopamine is discharged in bursts (Tripp & Wickens, 2009), which might manifest as transient and spontaneous IBI alterations that could be detected in analyses of shorter time windows.

In the current study, we first investigated which criterion of similarity best illustrated differences between the ADHD and control group with the similarity graph algorithm. In line with previous work with the sample entropy (SampEn) method (Hauge et al., 2011; Richman & Moorman, 2000), we expected group differences to be most prominent for a criterion of similarity corresponding to 20% of the standard deviation (*SD*) of the IBIs. Second, we ran the algorithm for three different time windows in the ADHD and control group, investigating inter-relatedness. Our hypothesis was that the ADHD group would display higher inter-relatedness, and that this would be most prominent in shorter time windows (i.e., less than five seconds), which might provide the most refined indices of vagal alterations. We expected indices of higher inter-relatedness to be more sensitive to such vagal alterations than indices of lacking inter-relatedness, as it is generally easier to distinguish groups on a present rather than absent occurrence. Further, we expected to find lower edges10+10/edges2+2 in the ADHD group compared to controls, as LF/HF ratios tend to be higher in ADHD samples (Griffiths et al., 2017; Tonhajzerova et al., 2009). Third, HRV differences between the ADHD and control groups were investigated. Our expectation was that indices of vagal activity (i.e., RMSSD, HF-HRV) would not show group differences, in line with meta-analytical evidence (see Koenig et al., 2016). Still, we expected other indices to reflect ANS alterations in ADHD (i.e., lower SDNN, higher LF-HRV and LF/HF ratio). Fourth, we investigated if significantly different IBI indices were confounded by comorbidities or anxiety symptoms. We hypothesized that these variables would affect the HRV indices, which are associated with emotion dysregulation and general psychopathology (Beauchaine, 2015; Beauchaine & Thayer, 2015; Holzman & Bridgett, 2017), but not the graph theory-based indices. The latter indices might be more sensitive to subtle alterations and capture the complexity of IBI organization more accurately, and thus the detected alterations might be more ADHD-specific.

2 | METHOD

2.1 | Design and procedure

The current study is cross-sectional in design, investigating data from the second wave of a follow-up project on ADHD (See Supporting Information for details). The study protocol was approved by the Regional committee for medical research ethics of western Norway (Study number: 2014/1304). Participants received a reimbursement of £80 ($115).

Test administrators were blinded to group status throughout the two-day assessments. On the first day, adolescents and their parents received extensive information about the project and procedures. ECG recordings were conducted between 9 a.m. and 1 p.m. to control for effects of circadian variation on IBIs (Malpas & Purdie, 1990). On the second day, adolescents and their parents were separately interviewed with a semi-structured diagnostic interview (K-SADS; *outlined below*). This interview reviewed the diagnostic group statuses (ADHD/control) that had been assigned during similar interviews in the first wave of the project and assessed current comorbid diagnoses. Parents also completed the DSM-IV ADHD-rating scale (ADHD-RS; *outlined below*) for assessment of dimensional ADHD symptoms. Further, factors that have been associated with HRV (Gutin et al., 2005; Koenig et al., 2014; Tsang et al., 2015) and often differ in ADHD and control groups (Cook et al., 2015; Cortese et al., 2008; Fliers et al., 2013; Sharma et al., 2011) were examined: Physical activity levels as assessed during the diagnostic interview; body mass index (BMI) computed from height and weight measurements; and anxiety symptoms assessed by adolescent reports on the State-Trait Anxiety Inventory (STAI; *outlined below*).

2.2 | Participants

2.2.1 | Recruitment

In the first wave of the project, children with suspected ADHD were referred from child and adolescent psychiatry units in the Bergen municipality, Norway. Controls were recruited from schools in the same geographical area. An equal age and sex distribution in the ADHD and control groups was strived for. Participants in the first wave were asked by mail to take part in the second wave, and signed written informed consent in accordance with the Helsinki declaration. Exclusion criteria were a full-scale IQ score of <75, suspected autism spectrum disorder, former head trauma with loss of consciousness, or birth before gestation week 36.

2.2.2 | Diagnostic assessments

Subjects were assigned to an ADHD or control group by use of the Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version (K-SADS; Kaufman et al., 1997). The Norwegian translation was used (Sund & Aalberg, 2009), which has shown adequate convergent and divergent validity for ADHD diagnostics (Villabø et al., 2016). Diagnostic and Statistical Manual of Mental Disorders – Fourth edition (DSM-IV) criteria were applied (American Psychiatric Association, 2000). Interviews were performed by one of two experienced psychologists. The non-interviewing psychologist provided a second opinion on the participants' diagnoses.

2.3 | Sample properties

The sample comprised $n = 37$ adolescents with ADHD and $n = 36$ controls between 10 and 18 years of age (mean age 14.38; *SD* 1.51; Table 1). The majority of the participants were male ($n = 48$, 65.75%). All adolescents in the ADHD group met the criteria for the diagnosis, except for one who had ADHD in remission. Participants with ADHD were diagnosed as primarily inattentive, hyperactive-impulsive or combined subtypes, and were frequently diagnosed with comorbid disorders (See Table 2 for comorbidities and ADHD subtypes in the sample). Twenty two (59.46%) of the adolescents with ADHD used ADHD medication. Except for one who used atomoxetine hydrochloride, all were on central stimulants (nine used fast-acting formulas, nine used extended-release formulas and four a combination or alternation between the two). Further, two participants with ADHD used lamotrigine, and one used sertraline. One control participant used risperidone and melatonin.

2.4 | Measures

2.4.1 | Inter-beat intervals

To rule out short-term effects on IBIs (Buchhorn et al., 2012), participants were asked to conduct a washout period of medication 48 h prior to participation. 90.91% of the adolescents on ADHD medication ($n = 20$, of 22)

TABLE 1 Descriptive statistics for ADHD and control groups

Variable	ADHD (n = 37)	Controls (n = 36)	t	χ^2	df
Sex (n, %)				.68	1
Male	26 (70.27)	22 (61.11)			
Female	11 (29.73)	14 (38.88)			
Age	14.19 (1.74)	14.58 (1.21)	−1.11		71
BMI	22.37 (5.59)	21.01 (2.91)	1.30		54.52
Physical activity levels	1.73 (.77)	2.62 (1.20)		16.56**	5
ADHD-RS	26.33 (10.83)	5.91 (7.32)	8.96**		53.76
STAI-T	31.11 (7.08)	27.77 (4.79)	2.36*		71
Comorbidities	1.16 (1.12)	.33 (.63)		13.63**	4
HF peak	.25 (.063)	.26 (.051)	-1.05		69.00

Note: Comorbidities: The number of comorbidities each participant had. HF peak: Peak frequency of the high frequency band, as a proxy for respiration. Physical activity levels were scored on a Likert-type scale from 1 (lowest) to 5 (highest). Data are given as (mean, *SD*).

Abbreviations: BMI, Body Mass Index; ADHD-RS, ADHD Rating Scale; STAI-T, State-Trait Anxiety Inventory, trait anxiety subscale.

*$p \le .050$;; **$p \le .010$.

TABLE 2 Comorbid diagnoses and ADHD subtypes

	ADHD (n = 37)	Controls (n = 36)
ADHD subtype		
IA	11 (29.73)	–
HI	1 (2.70)	–
C	25 (67.57)	–
TS	13 (35.14)	7 (19.44)
Anxiety disorders	10 (27.03)	3 (8.33)
ODD	10 (27.03)	–
CD	1 (2.70)	–
OCD	2 (5.41)	2 (5.56)
MDD	2 (5.41)	–
Eneuresis	2 (5.41)	–
Epilepsy	1 (2.70)	–
Chronic motor tics	1 (2.70)	–
Transient motor tics	1 (2.70)	–
Anorexia nervosa	–	1 (2.78)

Note. Table displays number of participants (% of diagnostic group).

Abbreviations: C, combined subtype; CD, conduct disorder; HI, hyperactive-impulsive subtype; IA, inattentive subtype; MDD, major depressive disorder; OCD, obsessive-compulsive disorder; ODD, oppositional defiant disorder; TS, tourette syndrome.

conducted a washout period of minimum 24 h. Two participants on methylphenidate had washout periods of 18 and >12 h, respectively. Although shorter than requested, these timeframes were acceptable as methylphenidate has a half-life of two to three hours (i.e., the active ingredient is eliminated after 10–15 h; Ito, 2011; Kimko et al., 1999). Both participants on lamotrigine conducted a (48-h) washout period, while the participant on risperidone and melatonin did not.

Resting ECG recordings were assessed. As resting IBI indices have shown excellent test-retest reliability (Bertsch et al., 2012; Li et al., 2009), they might reflect "trait" neurophysiological tendencies of self-regulation (Beauchaine & Thayer, 2015; Porges, 2007; Thayer & Lane, 2000). Therefore, they could generalize to situations in everyday life to a larger degree than "state" IBI responses during experimental protocols (i.e., vagal reactivity; Balzarotti et al., 2017). Further, comparing our results to previous studies was more convenient with the use of resting ECGs, as they are often based on standardized protocols (Berntson et al., 1997; Camm et al., 1996).

Before initiation of the ECG recording, adolescents were instructed to lie down and relax while trying not to move or fall asleep. The ECG was recorded for six minutes as participants were breathing spontaneously. A simple lead II setup at a sampling rate of 1000 Hz was used. An A/D converter (Biopac, MP36, Biopac System Inc., Santa Barbara, CA) obtained the signal, which was conducted through three adhesive Ag/AgCl electrodes (T815 Dia. 55) and recorded with Biopac 4.0 BSL (Biopac Systems Inc. Santa Barbara, CA). The IBIs were manually inspected in Kubios HRV version 2.2 (Tarvainen et al., 2014), where 11 IBI corrections were made (.4%–1.5% of IBIs in the corrected recordings) in six recordings (ADHD, n = 3; controls, n = 3). One extra systole was removed. Then, the number of IBIs in the first five minutes of every series was calculated (See Supporting Information Figure S1 for flow chart of IBI analysis). The lowest IBI number in any of the series—237—was analyzed. This was due to the need to investigate the same number of time windows with the similarity graph algorithm in every IBI series, to compare their relative inter-relatedness. Although five-minute ECG recordings is the standard in HRV analyses (Camm et al., 1996), we used the first 237 IBIs of the recordings also in these analyses, in order to compare all IBI indices on equal premises.

The similarity graph algorithm (*outlined below*) was applied to the IBI series. We also performed time domain analyses of the *SD* of normal IBIs (SDNN) and the root mean square of successive RR interval differences (RMSSD). SDNN indexes total variability in HR, and RMSSD reflects vagal modulation (Camm et al., 1996). Further, a frequency analysis with the Fast Fourier Transformation yielded a power spectrum of activity in the LF (0.04–0.15 Hz) and HF (0.15–0.4 Hz) bands (Camm et al., 1996), expressed in non-normalized units. Activity in the LF band is frequently interpreted as sympatho/vagal activity, and the HF band indexes vagal activity (Camm et al., 1996). We also calculated the LF/HF ratio, which is—although debated (Billman, 2013)—often considered to index sympatho/vagal balance, in that a low LF/HF ratio reflects vagal dominance of the ANS (Camm et al., 1996). Further, respiration rates were estimated by the ECG-derived respiration, based on changes in R-wave amplitude (Moody et al., 1985). We assessed whether respiration rates were within the normal range for adults (.20–.33 Hz; McCance & Huether, 2018), supporting our use of adult frequency bands (see Shader et al., 2018). Lastly, we assessed peak frequencies of the HF band, HF peak, reflecting respiratory effects on IBIs (Grossman et al., 1991; Thayer et al., 2002).

2.4.2 | Physical activity levels

Engagement in sports and exercise was reported by the adolescents in the K-SADS interview, and physical activity levels were thereafter scored on a five-point scale from 1 ("zero times a week") to 5 ("seven times or more a week"). The scoring norm was adapted from the Physical Activity Questionnaire for Adolescents (PAQ-A; Kowalski et al., 2004) due to its convergent validity (Kowalski et al., 1997). Data reported between two categories were lowered to the nearest category (see Sallis et al., 1996). One participant in the current study did not provide information on physical activity.

2.4.3 | Body mass index

The participants' BMI was calculated by dividing weight in kg by height in meters squared. In the current study, it was impossible to calculate BMI for four participants (ADHD, $n = 2$; Controls, $n = 2$) as they declined to be weighed or due to a technical error with the scale.

2.4.4 | ADHD symptoms

ADHD symptoms were indexed by total scores on the Norwegian translation (Kvilhaug et al., 1998) of the parent-reported ADHD-RS (DuPaul et al., 1998). Eighteen items about symptom levels of inattention (e.g., "Is forgetful in daily activities") and hyperactivity-impulsivity (e.g., "Talks excessively") were rated on a four-point Likert-type scale from 0 ("Never") through 3 ("Very Often"). The ADHD-RS has shown high internal consistency (Cronbach's α in the current sample: .96) and adequate validity (DuPaul et al., 1998). ADHD-RS data were missing for one participant (with ADHD) in the current study.

2.4.5 | Anxiety symptoms

The Norwegian translation (Haseth et al., 1990) of the STAI (Spielberger et al., 1983) assessed self-reported symptoms of trait anxiety (STAI-T). The inventory has shown adequate internal consistency (Cronbach's α in the current sample: .87), test-retest reliability (Spielberger et al., 1983), and construct- and concurrent validity (Spielberger, 1989). The STAI-T score is based on 20 items (e.g., "I feel nervous and restless") rated on a four-point Likert-type scale from 1 ("Almost never") to 4 ("Almost always"). In the current study, STAI-T scores were missing for three participants with ADHD and two controls.

2.5 | Measures

2.5.1 | Graph theory

The overview of graph theory principles and description of the similarity graph algorithm are based on the original publication of this method (Fasmer et al., 2018). There are some additional features and adaptations to the analysis of IBI data, as outlined below.

Graphs are mathematical structures that model relations between objects. *A* graph $G = (V, E)$ consists of a collection V of nodes (vertices) and a collection E of edges (if any) (Bondy & Murty, 2008). An edge $e \in E$ is a two-element subset of V that associates two nodes: $e = \{u, v\}$, for some u, $v \in V$ (Lian, 2000). A *subgraph* is a graph formed from a subset of the nodes and edges (if any) of G.

2.5.2 | The similarity graph algorithm

We apply a heuristic algorithm that is nonlinear and not chaos-based that transforms a time series into a *similarity graph* $G = (V, E)$ (see Fasmer et al., 2018. Program code can be accessed at https://github.com/erlfas/SimilarityGraph). In the current study every IBI in a time series is represented by a node in the graph. Thus, in a time series S each node u_i in V, $1 \leq I \leq n$, corresponds to the element $x_i \in S$, and the node u_i is assigned a weight equal to x_i. An edge between two nodes signifies that the nodes fulfill the *criterion of similarity*: that the difference in IBIs is below a predefined threshold. This similarity is calculated as $\max(x_i, x_j)/\min(x_i, x_j)$ or $\max(x_i, x_j)-\min(x_i, x_j)$.

In the current study, every node in the graph will be the *index node* considered by the algorithm (Figure 2). Thus, every IBI will be analyzed in relation to a given section of other IBIs in the time series: a *time window* (Figure 2). To describe the size of a time window, we use the term *neighbors*: the number (k) of preceding or subsequent nodes around the index node. Thus, every node has a total number of 2k neighbors, denoted as k+k. When analyzing the IBI series, the applied time window "slides" and centers around every index node, except for the first k and last k IBIs of the time series. Different time windows create different *subgraphs* (Figure 2), which may reveal different properties of the underlying time series.

In sum, we introduce an edge between two nodes if and only if they fulfill the criterion of similarity and are within the same time window. The number of edges in a given time window reveal how similar the index nodes are to the other nodes. Minimum similarity is revealed when the index node has no edges. Conversely, maximum similarity is revealed when the index node has an edge to every other node in the time window. In the current study, graphs with a higher number of similar IBIs are described as having higher *inter-relatedness*.

The described methods create a graph that can be studied by well-known algorithms from graph theory (See Figure 3 for the transformation of an IBI series to graphs and subsequent indices). The indices investigated in the current study and their possible interpretation in terms of inter-relatedness and vagal activity are described in Table 3. The index of bridges is more complex to interpret (e, an edge of G, is a bridge if G–e has more components than G; see Fasmer et al., 2020), and was thus included in supplemental analyses.

2.5.3 | Additional nonlinear analyses

To compare the similarity graph algorithm to other nonlinear methods, we performed recurrence quantification analyses (RQA) and analyses of SampEn. Both methods assess complexity and have frequently been applied to IBI data (Henriques et al., 2020).

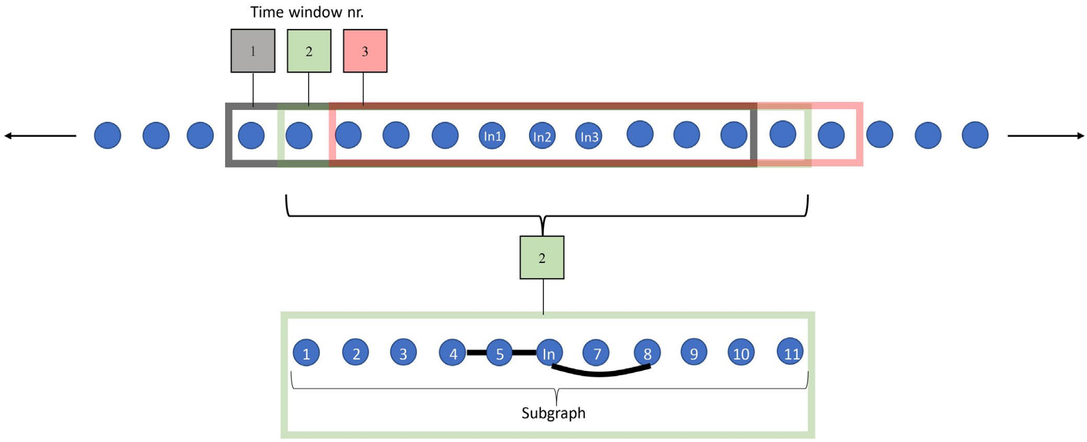
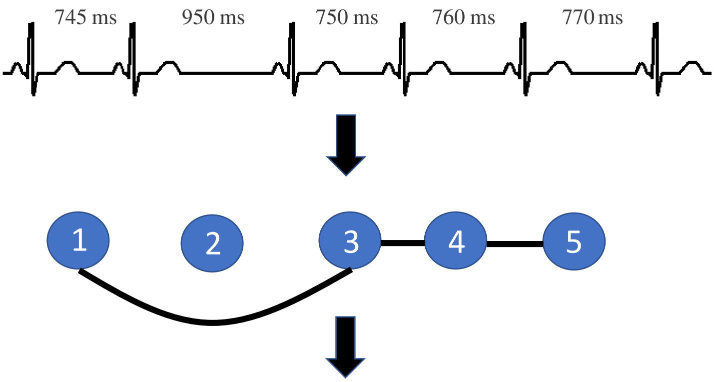


FIGURE 2 Illustration of the concept of index nodes, time windows and subgraphs. The figure illustrates three time windows of 5+5 neighbors. Each node in the time series is used as an index node (In1–3) considered by the similarity graph algorithm. Five nodes (i.e., neighbors) on the left and right side of the index node, respectively, make up a time window of 5+5 neighbors. Within the time window, nodes are connected by edges based on the similarity of the nodes. This collection of nodes and edges within a time window makes up a subgraph



Index	Value
Edges	3
Components	2
Missing edges	2
Max edges	3
Zero edges	0
Cliques	1

FIGURE 3 Illustration of the procedure for deriving graph theoretical indices from inter-beat intervals (IBIs). First, IBIs are collected. Then, the IBIs are converted to a graph, where nodes that fulfill the criterion of similarity (in the current figure: 1.5%) and are within the same time window are connected by edges. Lastly, the values of the graph theory-based indices are derived from the properties of the graph

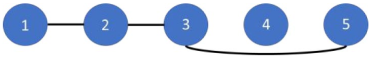
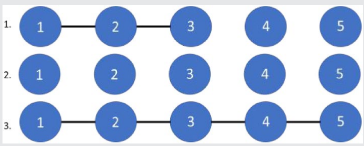
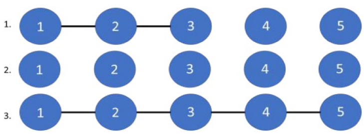
2.6 | Statistical analyses

Statistical analyses were performed in the Statistical Package for the Social Sciences version 24.0 (SPSS; IBM Corp., Armonk, NY, 2016). HRV frequency bands were transformed with their natural logarithm to approximate a normal distribution (Ellis et al., 2008). We examined potential outliers, i.e., values $> \pm 3$ *SD* from the mean, in variables used as covariates (i.e., age, sex, BMI, physical activity levels, HF peak, number of comorbidities and STAI-T scores). Outliers were imputed with the value of the sample mean ± 3 *SD*. Missing data were imputed with the sample mean, or the mean of the ADHD group (for the missing ADHD-RS score). Differences between the ADHD and control groups in age, sex, BMI, physical activity levels, ADHD-RS and STAI-T scores, number of comorbidities, and HF peak were investigated with the independent samples *t* test or chi-square tests. Unless otherwise noted, differences between the ADHD and control group in the remaining analyses were investigated with independent samples *t* tests, with Cohen's *d* as an effect size measure.

First, to investigate which setting of the similarity graph algorithm best accentuated IBI differences between the ADHD and control groups, we ranged criteria of similarity from 1% to 5% (i.e., 95%–99% similarity) and 5 to 50 ms. Differences in edges between the ADHD and control groups were assessed. We used a time window of 2+2 neighbors, hypothesized to provide the most refined index of vagal activity. The settings that yielded the largest effect size of a significant difference from the percentage-based and msec-based approaches were then compared. Here, we investigated differences in edges between the ADHD and control groups for time windows of 2+2 and 10+10 neighbors, and edges10+10/edges2+2. The criterion yielding the largest significant effect size in any index was applied in the remaining analyses.

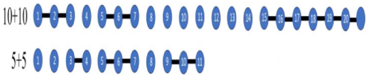
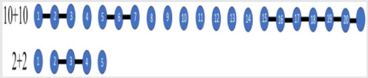
Second, we ran the similarity graph algorithm with three different time windows to test the hypothesis that we would find higher inter-relatedness in the ADHD group compared to the control group. Based on our hypothesis that vagal activity might be captured best in a time window of 2–5 s, which we aimed to compare to two longer time windows, we applied values of $k = 2$, $k = 5$ and $k = 10$. This corresponded to approximately 2–5, 6–13, and 12–25 s, respectively (mean IBI: 900.61 ms; range: 606–1261 ms). In these time windows, we investigated differences between the ADHD and control groups in the average number of edges, components, missing edges, maximum edges, zero edges, and cliques. We also calculated edges10+10/edges5+5 and edges10+10/2+2. Group differences in cliques were investigated with the Mann-Whitney *U* test. As post-hoc analyses, we investigated differences in edges and ratios derived from the number of edges in two different time

TABLE 3 Overview of graph theory-based indices calculated in the current study, and their suggested physiological interpretation in terms of inter-relatedness

Index	Definition	Illustration	Interpretation of high value
Edges	A two-element subset that associates two nodes that a) fulfill the criterion of similarity, and b) are located within the same subgraph (i.e., time window). The index refers to the mean of the number of edges in all subgraphs	 *The subgraph has three edges: Those connecting nodes nr. 1, 2 and 3, and nr. 3 and 5*	↑ Inter-relatedness ↓ Vagal activity
Components	A graph can be divided into separate *components*. The nodes in each component are connected by edges and the different components are not interconnected by edges. A node with no edges is itself a component. We are for each subgraph interested in the total count of these components	 *The subgraph consists of three components: Those including node nr. 1 and 2, nr. 3 and 4, and nr. 5, respectively*	↓ Inter-relatedness ↑ Vagal activity
Missing edges	The total count of the number of nodes that are nearest neighbors in a subgraph and do not have an edge connecting them	 *The subgraph has two missing edges: There are no edges between node nr. 2 and 3, or nr. 3 and 4*	↓ Inter-relatedness ↑ Vagal activity Comparable to RMSSD (see Fasmer et al., 2020)
Maximum edges	The highest number of edges found in any subgraph of the time series	 *The number of maximum edges is four. Subgraph 1 has two edges, subgraph 2 has zero edges, and subgraph 3 has four edges*	↑ Inter-relatedness ↓ Vagal activity
Zero edges	The number of subgraphs with zero edges	 *The number of subgraphs with zero edges is one: Subgraph 1 has two edges, subgraph 2 has zero edges, and subgraph 3 has four edges*	↓ Inter-relatedness ↑ Vagal activity
(3-) Cliques	The total count of subsets of three nodes in which every pair of distinct nodes are connected by an edge (i.e., a sequence of three nodes with similar values)	 *The subgraph has one clique: The one consisting of nodes nr. 2, 3, and 4*	↑ Inter-relatedness ↓ Vagal activity

(Continues)

TABLE 3 (Continued)

Index	Definition	Illustration	Interpretation of high value
Edges10+10/ edges 5+5	The ratio between the number of edges detected when analyzing 10+10 neighbors (i.e., the longest time window applied in the current study) and 5+5 neighbors (i.e., the intermediate-length time window applied in the current study)	 *Edges10+10/edges5+5 is 2. The time window of 10+10 neighbors has ten edges, and the time window with 5+5 neighbors has five edges (i.e., 10/5)*	Relatively ↑ vagal activity of total ANS activity Inverse of LF/HF ratio Less refined than edges10+10/ edges2+2
Edges10+10/ edges2+2	The ratio between the number of edges detected when analyzing 10+10 neighbors (i.e., the longest time window applied in the current study) and 2+2 neighbors (i.e., the shortest time window applied in the current study)	 *Edges10+10/edges2+2 is 5. The time window of 10+10 neighbors has ten edges, and the time window with 2+2 neighbors has two edges (i.e., 10/2)*	Relatively ↑ vagal activity of total ANS activity Inverse of LF/HF ratio More refined than edges10+10/ edges5+5

windows, between the ADHD and control group in time windows close to the k value ($k \pm 1$) that had best distinguished the groups, to investigate if this yielded even larger effect sizes.

Third, we performed time- and frequency domain analyses to investigate HRV differences between the ADHD and control groups. This tested the hypothesis that we would find a lower SDNN, and a higher LF-HRV and LF/HF ratio in the ADHD group compared to controls, but no significant differences in RMSSD or HF-HRV.

Fourth, we performed follow-up ANCOVAs on significantly different IBI indices in the ADHD and control groups from the graph theory-based and HRV analyses, investigating potential confounding effects. The IBI indices, respectively, were used as the dependent variable, and diagnostic group status (ADHD/control) as the independent variable. As covariates, we used (A) Age, sex, BMI, physical activity levels, and HF peak. Covariates that significantly predicted any of the indices were included in a final ANCOVA model, (B) The number of comorbid disorders each participant had, and (C) STAI-T scores.

Fifth, supplemental analyses assessed (A) Differences between the ADHD and control groups in RQA and SampEn, to compare results from the similarity graph algorithm to other nonlinear approaches, (B) Bivariate correlations between HRV indices, edges10+10/edges2+2 and edges10+10/edges5+5, aiding in the interpretation of the ratios, (C) The probability of an index node having an edge to a neighbor for time windows of 2+2, 5+5 and 10+10 neighbors, in the ADHD and control groups. The largest group difference in this probability further elucidated which time window best illustrated group differences, (D) Differences in bridges between the ADHD and control groups for time windows of 2+2, 5+5, and 10+10 neighbors, providing additional information about inter-relatedness, (E) Bivariate correlations of ADHD-RS scores and significant IBI indices from the main analyses, investigating if ADHD symptom severity correlated with the indices, and (F) HRV analyses of five-minute IBI series, to see if this yielded comparable results to analysis of the first 237 IBIs of the series.

3 | RESULTS

3.1 | Preliminary analyses

There were no outliers for values obtained by time- and frequency domain analyses or the similarity graph algorithm, or for physical activity levels, HF peak, number of comorbidities, ADHD-RS or STAI-T scores. The exception was one outlier for missing edges (in the ADHD group), and that the cliques displayed a skewed distribution in both groups. There was one outlier in the BMI values (in the ADHD group).

The adolescents with ADHD reported significantly higher STAI-T scores than the control group (for

TABLE 4 IBI analyses based on the similarity graph algorithm. 2+2 neighbors

	ADHD (n=37)	Controls (n = 36)	p	d	CI
Edges	.70 ± .38	.54 ± .26	.037*	.50	[.030, .96]
Components	162.43 ± 35.68	177.03 ± 26.54	.052	.46	[.00, .93]
Missing edges	189.14 ± 24.59	198.00 ± 17.81	.083	.41	[-.050, .88]
Max edges	3.24 ± .72	2.69 ± .86	.0040**	.69	[.23, 1.16]
Zero edges	120.14 ± 41.21	137.25 ± 35.26	.061	.45	[-.021, .91]
Cliques	7.78 ± 9.58	3.31 ± 4.07	.0080*	.61	[.14, 1.07]
Edges10+10/edges2+2	4.52 ± .88	5.57 ± 1.20	<.0010**	1.00	[0.54, 1.47]

Note: Data were analyzed by the independent samples t tests, except for cliques, where the Mann-Whitney U test was applied. CI: 95% confidence interval of d. Data are given as (mean ± SD).
*$p \le .050$;; **$p \le .010$.

descriptive statistics for the ADHD and control group, see Table 1). The ADHD group also had higher parent-rated ADHD-RS scores than the controls, and a higher number of comorbidities. Further, controls reported higher levels of physical activity than their ADHD counterparts. There were no significant differences in age, sex, BMI or HF peak between adolescents with ADHD and controls. The mean ECG-derived respiratory frequency in the sample corresponded to .26 Hz (SD .050; Supporting Information Table S1).

3.2 | IBI assessment with the similarity graph algorithm

3.2.1 | Systematically varying criteria of similarity

First, we systematically varied the criterion of similarity from 1% to 5% (Supporting Information Figure S2) and 5–50 ms (Supporting Information Figure S3), respectively, to investigate which setting best illustrated differences in IBI organization between the ADHD and control group. The only significant differences in the number of edges between adolescents with ADHD and controls were found applying the 1.5% criterion and the 10 msec criterion. For both these approaches, we found significant group differences in the number of edges in a time window of 2+2 neighbors but not 10+10 neighbors. Further, group differences in edges10+10/edges2+2 were significant for both approaches (Supporting Information Table S2). The largest effect size for both approaches was found for edges10+10/edges2+2. The effect size was larger using the 1.5%-based approach compared to the 10 ms-based approach. Therefore, 1.5% was chosen as the criterion of similarity for the remainder of the analyses.

3.2.2 | IBI assessment in different time windows

Second, in order to investigate differences in interrelatedness between adolescents with ADHD and controls, and which time window best illustrated such differences, we ran the similarity graph algorithm in three different time windows. Applying a time window of 2+2 neighbors, we found a significantly higher number of edges, maximum edges and cliques, and a lower edges10+10/edges2+2 in the ADHD group compared to controls as analyzed by the independent samples t test (Table 4). Differences in components, missing edges, and zero edges were non-significant.

Analyzing a time window of 5+5 neighbors, the independent samples t test found no significant differences in the number of edges, components, missing edges, maximum edges, zero edges, cliques or edges10+10/edges5+5 between the ADHD and control groups (Table 5).

Analysis of a time window of 10+10 neighbors detected no significant differences between the ADHD and control groups regarding number of edges, components, missing edges, maximum edges, zero edges or cliques (Table 6).

As a time window of 2+2 neighbors best distinguished differences in IBI organization in the ADHD and control group, we performed post-hoc analyses investing group differences in time windows of 1+1 and 3+3 neighbors to see if these yielded even larger effect sizes (Supporting Information Table S3). Independent samples t-tests detected a significantly lower edges10+10/edges1+1 and edges10+10/edges 3+3 in the ADHD group compared to controls. However, effect sizes were smaller compared to what was found for edges10+10/edges2+2. The differences in number of edges were non-significant for both time windows.

3.3 | Time- and frequency domain analyses

Third, applying time- and frequency domain analyses to investigate differences in HRV between the ADHD and control groups, independent samples t-tests showed a significantly higher LF/HF ratio in the ADHD group compared to controls. There were no significant group differences in SDNN, RMSSD, LF-HRV or HF-HRV (Table 7).

3.4 | Follow-up analyses

Fourth, we adjusted for effects of possible confounding factors. We performed ANCOVAs with the significantly different IBI indices, respectively, as dependent variables, and diagnostic group status (ADHD/controls) as the independent variable. As covariates, we included (A) Age, sex, physical activity levels, BMI, and HF peak. HF peak predicted edges10+10/edges2+2 ($R^2 = .29$, $F = 4.43$, $p = .033$). Age ($R^2 = .26$, $F = 8.12$, $p = .0060$) and physical activity levels ($R^2 = .26$, $F = 5.53$, $p = .022$) predicted the LF/HF ratio. Neither sex nor BMI significantly predicted any of the IBI indices. Thus, HF peak, age, and physical activity levels were included as covariates in a follow-up ANCOVA on significant results. In this final model, the effect of diagnostic status was still significant for edges, maximum edges, cliques, and edges10+10/edges2+2, but not for the LF/HF ratio (Table 8, Figure 4). HF peak significantly predicted edges10+10/edges2+2, but no other indices. Age and physical activity levels covaried with the LF/HF ratio, but no other indices.

(B) The number of comorbidities. The effect of diagnostic status on maximum edges and edges10+10/edges2+2 remained significant (Supporting Information Table S4). The effect of diagnostic status on edges, cliques, and the LF/HF ratio was no longer significant. The number of comorbidities covaried significantly with the LF/HF ratio, but not with any of the graph theory-based indices, and (C) STAI-T scores. The effect of diagnostic status on maximum edges, cliques, and edges10+10/edges 2+2 remained significant (Supporting Information Table S5). The effect of diagnostic status on the number of edges and the LF/HF ratio was no longer significant. STAI-T scores covaried significantly with the LF/HF ratio, but not with any of the graph theory-based indices.

3.5 | Supplemental analyses

Fifth, we performed supplemental analyses. These analyses found that (A) Comparing our method to established nonlinear approaches, there were no significant differences between the ADHD and control groups by applying RQA (Supporting Information Table S6) or SampEn, (B) Aiding in the interpretation of the graph theory-based ratios, edges10+10/edges2+2 and edges10+10/edges5+5 correlated positively with each other, RMSSD and HF-HRV, and negatively with the LF/HF ratio. The ratios did not covary with SDNN or LF-HRV (Supporting Information Table S7), (C) Aiding in the question of which time window best illustrated differences in IBI organization between adolescents with ADHD and controls, the largest group difference in the probability of an index node having an edge to one of its neighbors was found for a time window of 2+2 neighbors (Supporting Information Table S8), (D) As an additional index of inter-relatedness, we did not find any significant differences in the number of bridges between the ADHD and control groups (Supporting Information Table S9), (E) Higher ADHD-RS scores correlated significantly with a lower edges10+10/edges2+2 ($\rho = -.37$, $p = .0010$) and a higher number of maximum edges ($\rho = -25$, $p = .030$). The LF/HF ratio, number of edges or cliques did not correlate significantly with the ADHD-RS scores, and (F) In order to compare

TABLE 5 IBI analyses based on the similarity graph algorithm. 5+5 neighbors

	ADHD ($n = 37$)	Controls ($n = 36$)	p	d	CI
Edges	1.64 ± .89	1.50 ± .65	.44	.18	[−.29, .65]
Components	105.24 ± 41.25	106.14 ± 37.83	.92	.023	[−.44, .49]
Missing edges	190.30 ± 24.16	198.83 ± 17.37	.088	.40	[−.062, 0.87]
Max edges	5.62 ± 1.53	5.22 ± 1.27	.23	.28	[−.18, .75]
Zero edges	67.95 ± 30.36	67.19 ± 30.69	.92	.025	[−.44, .49]
Cliques	73.51 ± 85.19	52.19 ± 49.39	.58	.31	[−.16, .77]
Edges 10+10/edges 5+5	1.88 ± .12	1.91 ± .14	.32	.24	[−.23, .70]

Note: Data were analyzed by the independent samples *t* tests, except for cliques, where the Mann-Whitney U test was applied. CI: 95% confidence interval of *d*; Data are given as (mean ± *SD*).

*$p \leq .050$;; **$p \leq .010$.

TABLE 6 IBI analyses based on the similarity graph algorithm. 10+10 neighbors

	ADHD ($n = 37$)	Controls ($n = 36$)	p	d	CI
Edges	3.06 ± 1.61	2.83 ± 1.18	.50	.16	[−.31, .62]
Components	66.05 ± 29.98	62.44 ± 30.29	.61	.12	[−.35, .59]
Missing edges	192.35 ± 23.16	200.72 ± 16.18	.078	.42	[−.049, .88]
Max edges	8.92 ± 2.95	8.42 ± 2.37	.43	.19	[−.28, .65]
Zero edges	46.78 ± 17.11	44.47 ± 17.37	.57	.13	[−.33, .60]
Cliques	310.30 ± 340.48	231.61 ± 209.91	.81	.28	[−.019, .74]

Note: Data were analyzed by the independent samples t tests, except for cliques, where the Mann-Whitney U test was applied. CI: 95% confidence interval of d. Data are given as (mean ± SD).

$^{*}p \leq .050$;; $^{**}p \leq .010$.

TABLE 7 Time- and frequency domain analyses of HRV

	ADHD ($n = 37$)	Controls ($n = 36$)	p	d	CI
SDNN	69.75 (30.33)	68.34 (27.27)	.84	.049	[−.42, .52]
RMSSD	69.93 (42.74)	75.27 (36.23)	.57	.13	[−.33, .60]
LF (ms^2)	6.67 (1.05)	6.51 (1.05)	.51	.16	[−.31, .62]
HF (ms^2)	7.19 (1.28)	7.46 (1.08)	.33	.23	[−.24, .70]
LF/HF	.94 (.11)	.88 (.11)	.018*	.54	[.079, 1.01]

Note: CI: 95% confidence interval of d. HRV frequency bands were naturally log transformed to approximate a normal distribution. Data are given as (mean, SD).

Abbreviations: HF (ms^2), high frequency-HRV given in ms^2; HRV, heart rate variability; LF (ms^2), low frequency-HRV given in ms^2; LF/HF, low frequency/high frequency ratio; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal IBIs.

$^{*}p \leq .050$;; $^{**}p \leq .010$.

results from analyses of the first 237 of the IBI series to analyses of the conventional recording length, we did not detect any group differences in time- and frequency domain analyses when analyzing five-minute ECG recordings (Supporting Information Table S10).

4 | DISCUSSION

The purpose of the current study was to assess IBI organization with a nonlinear, graph theory-based method not hitherto applied to IBIs: the *similarity graph algorithm*. We investigated whether the algorithm could detect group differences in IBI organization as exemplified in a sample of adolescents with ADHD compared to a control group. As hypothesized, we found higher inter-relatedness (i.e., a higher number of similar IBIs) in a time window of 2–5 s in the ADHD group compared to controls. This might suggest altered vagal activity in the ADHD group. As expected, traditional HRV analyses detected a higher LF/HF ratio in the ADHD group. However, this effect was nonsignificant after controlling for possible confounding factors. In contrast, the graph theory-based indices were in large part unaffected by such confounding factors. Other nonlinear approaches (RQA and SampEn) did not detect any significant IBI differences between the ADHD and control groups. Altogether, our findings suggest that the similarity graph algorithm might provide additional information to other methods for IBI analysis as a proxy for ANS functioning.

Using the similarity graph algorithm, we detected the largest effect size for differences in IBI organization between the ADHD and control groups when applying a 1.5% criterion for defining IBIs as similar. This was in line with our hypothesis based on previous work with the SampEn method (Hauge et al., 2011; Richman & Moorman, 2000), where it is customary to use a threshold of 20% of the SD for defining two points as similar. The mean SD of the IBI time series in the current study was approximately 70 ms, and the mean IBI approximately 900 ms, giving 1.5% of the mean as a reasonable threshold for similarity ($0.20*SD/\text{mean} = 14/900 \approx 1.5\%$).

Applying the 1.5% criterion of similarity in the similarity graph algorithm, several differences between the adolescents with ADHD and controls were detected in the graph theory-based IBI indices. The ADHD group displayed a higher number of edges, maximum edges, and cliques. These indices reflect higher inter-relatedness, as a result of a higher number of similar IBIs. Contrary to our expectations, the difference in components was

TABLE 8 Prediction of diagnostic status on selected IBI indices, controlling for potential confounders

Predictor	R^2	*df*	*F*	*p*
Edges				
ADHD	.13	4	4.32	.041*
HF peak			3.62	.061
Age			2.51	.12
Physical activity levels			.46	.50
Maximum edges				
ADHD	.14	4	7.46	.0080**
HF peak			.11	.75
Age			2.22	.14
Physical activity levels			.12	.73
Cliques				
ADHD	.11	4	5.32	.024*
HF peak			1.37	.25
Age			.28	.60
Physical activity levels			.24	.63
Edges10+10/edges2+2				
ADHD	.27	4	11.32	<.0010**
HF peak			4.20	.044*
Age			.27	.61
Physical activity levels			.41	.53
LF/HF				
ADHD	.22	4	2.81	.098
HF peak			.53	.47
Age			5.45	.023*
Physical activity levels			4.06	.048*

Note: Time window applied: 2+2 neighbors .

Abbreviations: ADHD, ADHD diagnostic status; LF/HF, Low frequency/high frequency ratio; HF peak, Peak of high frequency heart rate variability.

*$p \leq .050$;; **$p \leq .010$.

non-significant, although this index also reflects higher inter-relatedness. This non-finding could be due to lack of statistical power in our study. Still, as expected, the indices reflecting inter-relatedness generally appeared to be more sensitive than the indices representing lack of inter-relatedness (i.e., missing edges and zero edges), which were non-significant. The similarity graph algorithm further detected a significantly lower edges10+10/edges2+2 in the ADHD group compared to controls. As expected, this index was inversely related to the LF/HF ratio calculated by HRV analyses. Although the interpretation is debated (Billman, 2013), a higher LF/HF ratio is frequently considered to index sympathetic dominance of the ANS, and a lower edges10+10/edges2+2 might represent a comparable construct. Our results therefore seem to be in line with previous findings of higher LF/HF ratios in ADHD samples (Griffiths et al., 2017; Tonhajzerova et al., 2009).

Differences between the ADHD and control groups in our sample were most prominent in analyses of time windows of 2–5 s. In line with our hypothesis, this suggests that the IBI organization occurring over a few seconds is most affected in ADHD. This could be due to altered functioning of the vagus nerve, which normally has a rapid course of action compared to the sympathetic nervous system (Nunan et al., 2010). As a result of altered vagal functioning, HR changes are not induced as rapidly as with optimal functioning, resulting in more similar IBIs. This could be reflected in higher inter-relatedness and relatively more sympathetic regulation of IBIs—in line with the aforementioned results from analyses of the graph theory-based indices in the ADHD group. As the control group, on the other hand, displayed lower inter-relatedness, our findings are in line with expectations of higher vagally medicated HRV in controls compared to individuals with ADHD (Rash & Aguirre-Camacho, 2012). There were no significant differences between adolescents with ADHD and controls in the time windows of 6–13 or 12–25 s, respectively. In line with our hypothesis, we suggest that these time windows provide less refined indices of vagal activity compared to the time window of 2-5 seconds. As vagally mediated HRV is considered to mark self-regulatory abilities and general psychopathology (Beauchaine, 2015; Beauchaine & Thayer, 2015; Holzman & Bridgett, 2017), we suggest that our indices of inter-relatedness in a time window of 2–5 s, as well as edges10+10/2+2, could represent similar constructs. This is supported by ADHD symptom severity correlating negatively with edges10+10/edges2+2 and positively with maximum edges. Interestingly, maximum edges and edges10+10/edges2+2 appeared to be the most robust of the graph theory-based indices when controlling for comorbidities and trait anxiety symptoms, and therefore seem to be most sensitive to ADHD-specific IBI alterations.

It is crucial to address the question of whether our study is sufficiently powered in terms of the ability to detect group differences in IBI organization, as we have investigated a method not previously applied to IBI analysis. Performing an *a priori* power analysis was challenging due to no previous studies applying a similar method to ours in an ADHD sample. We could therefore not calculate appropriate estimates of expected effect sizes. However, we will suggest that the similarity graph algorithm might provide larger power than traditional methods as the algorithm systematically compares every IBI in a given time window to every other IBI in the time window. This gives a substantially higher number of data points compared to traditional analyses of whole IBI series. It is important to note in this regard that our analyses were performed in

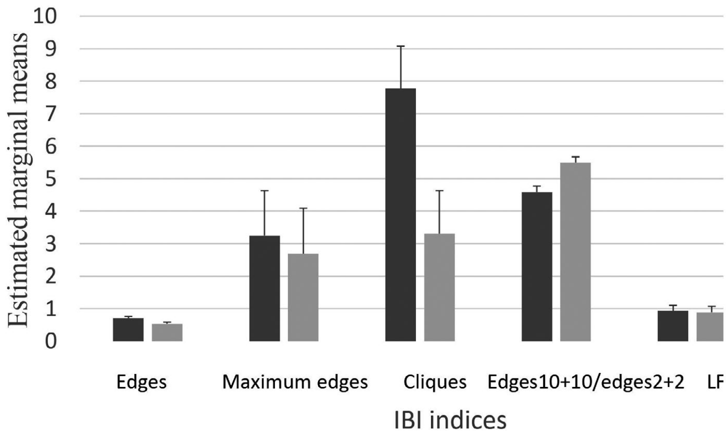


FIGURE 4 Estimated marginal means for inter-beat interval indices that show significant group differences in adolescents in ADHD and controls. The means were adjusted for the effect of HF peak as a proxy of respiration, age, and physical activity levels. Error bars represent standard errors. Abbreviations: LF/HF: low frequency/high frequency ratio.

a relatively small sample, and we therefore cannot rule out that some of the statistically significant graph theory-based differences have occurred due to multiple testing or sampling error. With regard to the traditional HRV analyses, we did not find group differences in SDNN or LF-HRV, contrary to expectations. This could be due to a lack of statistical power in these analyses. Further, also of relevance to the statistical power is the IBI recording length. Often, 30,000 data points are used to validate novel methods that assess indices of complexity (see Costa et al., 2002; Yang et al., 2020). Our investigation of 237 IBIs does not provide as many data points. However, the reason that a longer data series is often required is to gain enough complexity for the method to detect group differences (Costa et al., 2002; Yang et al., 2020). Although we had a shorter data length than often required, the statistically significant group differences detected by the similarity graph algorithm were in line with our *a priori* hypotheses. This supports the notion that our findings did not appear by coincidence, along with the fact that several of the detected group differences appeared to be robust also when controlling for confounding factors.

The current study has several strengths and limitations. Among several strengths, we assessed IBIs approximately at the same time of day for all participants, controlling for circadian influences. In addition, we assessed physical activity levels and BMI, which are not regularly investigated in IBI studies despite influencing IBIs (Gutin et al., 2005; Koenig et al., 2014). Further, the ADHD and control groups were matched on age and sex, and there were no significant group differences in BMI, reducing potential confounding effects of these factors on IBIs. Individuals with ADHD generally have higher BMI than controls (Cortese et al., 2008; Fliers et al., 2013); however, our finding of a non-significant BMI difference could be due to a substantial number of adolescent girls with ADHD in our sample, who seem to be at lower risk of being overweight (Fliers et al., 2013). However, the control group had higher physical activity levels than the ADHD group, and the adolescents with ADHD had higher symptoms of trait anxiety and number of comorbidities, in line with expectations (Cook et al., 2015; Gnanavel et al., 2019; Sharma et al., 2011). Regarding limitations and threats-to-validity, in addition to the already discussed aspects related to the power of the current study, possible effects of medication on IBIs cannot be ruled out. Still, short-term effects were reduced by almost all participants conducting a washout period. Also important to note is that ADHD medication tend to shift IBI indices toward control values (see Buchhorn et al., 2012; Kim et al., 2015; Negrao et al., 2011), and have probably not contributed to any false group differences in the current study. Yet, a participant in the control group used other types of medication that were not subjected to a washout period, which could have influenced their IBIs. The graph theory-based indices have further not been investigated in relation to ANS functioning, and our interpretation of them is of an explorative nature. In addition, IBI organization investigated in short time windows might not be reproducible for a given individual to the same extent as the traditionally used HRV indices. Future studies investigating the reliability of the graph theory-based indices from the similarity graph algorithm, and their sensitivity and specificity as "trait" markers of self-regulatory abilities are called for.

5 | CONCLUSION

Our study suggests that the similarity graph algorithm can provide complementary information to other analyses of IBI organization, which has potentially important theoretical and clinical implications. The indices computed by the algorithm seem to detect complex features of the IBI series that reveal spontaneous or transient ANS alterations and might thus be sensitive markers of psychopathology. As the graph theory-based indices were largely unaffected by comorbidities or trait anxiety symptoms, the indices might represent more disorder-specific patterns of vagal alterations compared to traditional HRV indices – which have been suggested as transdiagnostic markers of psychopathology (Beauchaine & Thayer, 2015). This might have further implications for the etiological understanding and treatment of various disorders. These implications are, however, largely hypothetical as of now, and further research is needed to investigate them.

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CONFLICT OF INTEREST

During the past three years, JH has received lecture honoraria as part of continuing medical education programs sponsored by Shire, Takeda, Medice and Biocodex. Otherwise, all authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Elisabet Kvadsheim: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; visualization; writing – original draft; writing – review and editing. **Ole Bernt Fasmer:** Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; writing – original draft; writing – review and editing. **Erlend Eindride Fasmer:** Conceptualization; data curation; formal analysis; methodology; software; writing – original draft; writing – review and editing. **Erik R. Hauge:** Conceptualization; investigation; methodology; writing – original draft; writing – review and editing. **Julian F. Thayer:** Methodology; writing – review and editing. **Berge Osnes:** Data curation; writing – review and editing. **Jan Haavik:** Supervision; writing – review and editing. **Julian Koenig:** Conceptualization; methodology; supervision; writing – review and editing. **Steinunn Adolfsdottir:** Investigation; writing – review and editing. **Kerstin Jessica Plessen:** Funding acquisition; project administration; writing – review and editing. **Lin Sørensen:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; writing – original draft; writing – review and editing.

ORCID

Elisabet Kvadsheim https://orcid.org/0000-0002-4319-2861
Julian Koenig https://orcid.org/0000-0003-1009-9625

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

FIGURE S1 Flow chart of inter-beat interval preparation and analysis. ECG, electrocardiogram; IBI, inter-beat interval; Msec, milliseconds

FIGURE S2 Effect sizes for diagnostic group differences in number of edges detected with a systematically varied percentage-based approach to criterion of similarity. Time window: 2+2 neighbors. *$p \leq .050$; **$p \leq .010$

FIGURE S3 Effect sizes for diagnostic group differences in number of edges detected with a systematically varied msec-based approach to criterion of similarity. Time window: 2+2 neighbors. Msec, milliseconds. *$p \leq .050$; **$p \leq .010$

TABLE S1 EDR values in the total sample and diagnostic groups

TABLE S2 Number of edges detected with a 1.5%- and 10msec-based approach**,** respectively, to criterion of similarity

TABLE S3 Number of edges as analyzed with time windows of 1+1 and 3+3 neighbors

TABLE S4 Effect of comorbidities on selected IBI indices

TABLE S5 Effect of STAI-T scores on selected IBI indices

TABLE S6 Reccurence quantification analyses

TABLE S7 Bivariate correlations among selected HR organization indices

TABLE S8 Probability of the index node having an edge to one of its neighbors, dependent on size of time window

TABLE S9 Group differences in bridges for varying numbers of neighbors

TABLE S10 Time- and frequency domain analyses of HRV based on 5-min recordings

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