SUMMARY OF MAIN FINDINGS

This thesis aimed to unravel the role of cardiac autonomic activity in the pathway from poor mental health to CVD risk, with specific focus on influences of antidepressant use, genetics, and lifestyle.

In Chapter 2, we showed within NESDA (n=2183) that persons with depression and anxiety had a hyporeactivity of HR, RMSSD, and RSA compared to controls during the n-back task, a cognitively challenging stressor. In contrast, during the psychiatric interview, a personal-emotional stressor, they showed a hyperreactivity of these measures. No significant differences were found in PEP reactivity.

In Chapter 3, findings within NESDA (n=1383) suggested that persons with depression and anxiety do not show a significantly different TWA or QTc, two measures of cardiac repolarization, compared to controls. In addition, antidepressant use was not associated with a lower TWA or a prolonged QTc.

In Chapter 4, NESDA results across a nine-year follow-up period (no. observations = 6994) indicated that cardiac autonomic dysregulation was not phenotypically or genetically associated with depression and anxiety across waves. No evidence was found for moderation by polygenic risk scores for high HR and low HRV on the relationship of cardiac autonomic activity with depression and anxiety. In contrast, a robust association was found between antidepressant use and cardiac autonomic activity across all waves. This association was not moderated by a genetic risk for high HR or low HRV.

In Chapter 5, we combined baseline (n=2618) and 2-year follow-up (n=2010) data from NESDA and showed that high physical activity, high frequency of sport activities and mild/moderate alcohol use were related to low HR. Heavy smoking was related to high HR. High frequency of sport activities was associated with high RSA and moderate smoking with longer PEP. Furthermore, 2-year change in frequency of sport activities and number of smoked cigarettes/day was accompanied by 2-year change in HR.

Chapter 6 used NESDA data from baseline (n=2379), 2- (n=2245), and 6-year (n=1876) follow-up, and indicated that temporal stability was good for HR, excellent for RSA, and moderate for PEP over 2 years. Stability decreased for a 4- and 6-year interval. The most important determinants for increase in HR were (increase in) smoking, increase in body mass index (BMI) and (starting) the use of antidepressants. Beta-blocking/antiarrhythmic drug use led to a decrease in HR. Decrease in RSA was associated with age, smoking and (starting) antidepressant use. Decrease in PEP was associated with age and (increase in) BMI.
In Chapter 7, 2- (n=1922) and 6-year (n=1616) data from NESDA suggested that higher basal HR, lower basal values of RSA and PEP, and higher RSA reactivity during cognitive challenge were cross-sectionally related to less favorable values of almost all metabolic components. Longitudinal analyses showed that higher basal HR and shorter basal PEP predicted 4-year increase in many metabolic abnormalities. Higher RSA stress reactivity during cognitive challenge predicted 4-year increase in number of metabolic components.

In Chapter 8, NESDA results from baseline (n=2823), 2-year (n=2099), and 6-year (n=1774) data, showed that higher HR and lower RSA were cross-sectionally associated with higher inflammatory levels. Higher HR predicted higher levels of C-reactive protein (CRP) and interleukin(IL)-6 at follow-up. Higher CRP levels predicted lower RSA at follow-up. NTR results confirmed that higher HR was associated with higher CRP and IL-6 levels 5 years later (n=356). In contrast to NESDA results, higher IL-6 levels predicted higher HR and lower RSA at follow-up (n=472).

Finally, in Chapter 9, we demonstrated within AHAB (n=1785) that higher Beck Depression Inventory-II (BDI-II) scores were associated with increased metabolic and inflammatory dysregulations and decreased HRV. Decreased HRV was also associated with increased metabolic and inflammatory dysregulations. Structural equation models indicated that BDI-II scores may relate to metabolic and inflammatory dysregulations via cardiac vagal activity or to vagal activity via metabolic and inflammatory dysregulations. No significant results were found for the Center for Epidemiological Studies Depression Scale (CES-D) or the State-Trait Anxiety Inventory (STAI-T).

**DISCUSSION OF MAIN FINDINGS**

**Is poor mental health associated with cardiac autonomic dysregulation?**

**Basal cardiac autonomic activity**
There has been considerable debate on the nature of the relationship between poor mental health and cardiac autonomic dysregulation. Most of these studies have been conducted with basal levels of cardiac autonomic activity measured during a rest or neutral condition, as opposed to autonomic reactivity during stressful tasks. Within nine-year longitudinal data from NESDA and multiple waves of data, we found little evidence that depressive and anxiety disorders are independently associated with basal levels of cardiac autonomic activity (Chapter 2, 4, 6). This is in line with previous studies conducted within NESDA, 1-4 as well as
with other studies.\textsuperscript{5–8} However, up until now the literature remains ambiguous, as there are also studies that suggest there is a direct association of depression and anxiety with autonomic dysregulation.\textsuperscript{9–13} These inconsistent findings are possibly due to disorder heterogeneity and discrepant control of confounding factors. That this may be the case is illustrated by one of our own studies that attempted to replicate NESDA findings within AHAB, a general population study (Chapter 9). For this study, we only found the BDI-II – but not the CES-D – to be significantly associated with HRV. We hypothesize that these scales may capture different symptom profiles. For instance, it has been suggested that the CES-D may not be specific for depression, but may be a measure of general distress.\textsuperscript{14} Additionally, in contrast to the BDI-II, the CES-D includes some idiosyncratic items, such as ‘people were unfriendly’ or ‘I felt that people disliked me’, and does not specifically address suicidal ideation.\textsuperscript{14,15} These findings stress the possibility that disorder heterogeneity and the use of different metrics may be an important factor causing the ambiguous findings in this field. Despite this possibility, we find it difficult to reconcile the contradicting findings in AHAB with NESDA results. The significant results within AHAB may be a chance finding, as only one of two depression scales was cross-sectionally associated with HRV, while within NESDA there is rather consistent longitudinal evidence that depressive and anxiety disorders are not directly related to cardiac autonomic dysregulation. In conclusion, although there might be a direct association between poor mental health and basal cardiac autonomic activity, we find more evidence suggesting that this association is negligible. Hence, we sought for other mechanisms through which depression and anxiety may be associated with the autonomic nervous system.

We did not find strong, consistent evidence for a direct association of depression and anxiety with basal levels of cardiac autonomic activity.

Cardiac autonomic stress reactivity and cardiac repolarization

It is possible that, instead of the most commonly investigated variables of basal cardiac autonomic activity, there may be other aspects of autonomic activity that are altered in depression and anxiety and in part explain the association with CVD risk, such as stress reactivity or cardiac repolarization.

Regarding stress reactivity, previous studies have suggested both hyporeactivity\textsuperscript{16–23} and hyperreactivity\textsuperscript{24–28} in depression and anxiety. When we investigated whether persons with depression and anxiety showed a differential autonomic stress reactivity compared to controls, we found that the direction of the effect depended on the type of stressor (Chapter 2). For measures of HR and
HRV, a hyporeactivity was seen for a cognitively challenging stressor, whereas a hyperreactivity was seen for a personal-emotional stressor related to the occurrence of symptoms of depression and anxiety. We hypothesize that people with depression and anxiety show disengagement from commitments to difficult to reach goals and may be less motivated to perform well in a cognitively challenging task, explaining the resulting hyporeactivity in our study. These findings are in line with research by Brinkmann and Gendolla indicating that dysphoric participants showed lower systolic blood pressure reactivity than non-dysphoric participants when facing a difficult cognitive task. In contrast, our second stressor – a psychiatric interview during which participants described depressing and anxious events – may better capture the experiences of the personal-emotional stress that especially people with depression and anxiety experience in daily life, resulting in a heightened stress reactivity in patients compared to controls. However, no significant differences were found for PEP, suggesting that sympathetic stress reactivity is less affected in depression and anxiety. In addition, the found differences in the reactivity of the other measures were rather small. More structured stress tasks eliciting a stronger response should be tested to confirm a true differential effect of personal-emotional stress in persons with depression and anxiety compared to controls. Nonetheless, our study implies that, in contrast to basal cardiac autonomic activity, a modest differential autonomic stress reactivity is associated with depression and anxiety. The direction of the altered stress reactivity seems to be dependent on the type of stressor: only the more ecologically valid stressors may evoke hyperreactivity in patients with depression and/or anxiety.

Studies investigating the association between poor mental health and cardiac autonomic activity have often neglected measures of cardiac repolarization, such as TWA and QTc. These measures are affected by sympathetic activity and associated with cardiac morbidity and mortality. Therefore, a relationship between depressive/anxiety disorder with TWA and QTc may explain part of the comorbidity between poor mental health and CVD. However, when we investigated these relationships, we did not find evidence for their existence (Chapter 3). Our findings are in line with research by Kamphuis and colleagues, indicating that there were no significant associations between QTc and depressive
symptoms, and with previous NESDA research claiming that depressive and anxiety disorders do not directly influence sympathetic activity.\(^2\) In short, depressive and anxiety disorders are likely not associated with measures of cardiac repolarization.

**Persons with depression and anxiety show no differences in cardiac repolarization compared to healthy controls.**

Confounding or moderating influences of antidepressant use, genetic risk, and lifestyle Poor mental health may not be directly associated with basal cardiac autonomic dysregulation, but there are many factors that can explain why this relationship is often found in the literature. Here we discuss our findings for confounding or moderating effects of antidepressant use, genetic risk, and lifestyle.

As mentioned before, the most important debate revolves around findings from several studies indicating that the relationship between poor mental health and cardiac autonomic activity might be attributable to antidepressant use.\(^5,6,8\) Indeed, we found antidepressant use to be robustly associated with cardiac autonomic activity across nine-year longitudinal data (Chapter 4 & 6). In line with previous NESDA studies across shorter time spans,\(^2,3\) we found a detrimental effect on cardiac autonomic activity of TCA use, followed by SNRI use, and SSRI use. The latter might even have a slightly beneficial effect on sympathetic activity. It is interesting to note that, in spite of their known sympathomimetic effects, we did not find TCA and SNRI use to be associated with measures of cardiac repolarization (Chapter 3). These findings may be explained by the biological knowledge that different mechanisms of cardiac autonomic activity are in play concerning these measures.\(^37\) Nonetheless, this thesis adds to the increasing evidence that virtually all antidepressants negatively affect cardiac autonomic activity.\(^5,38\) Although the mechanisms through which they do so are not entirely understood, it is thought that antidepressants influence relay nuclei of the parasympathetic nervous system in the brain stem,\(^39\) inhibit cardiac vagal tone by exerting anticholinergic activity,\(^40\) and/or inhibit the reuptake of norepinephrine in the heart.\(^51\)

Despite the compelling evidence that antidepressant use explains a large part of the relationship of depression and anxiety with cardiac autonomic dysregulation, this is still disputed by studies that found lowered HRV in unmedicated patients with psychiatric disorders.\(^9\)–\(^11\) Hence, we entertained the possibility of the existence of an as yet unaccounted for factor that moderates the relationship of depression and anxiety with cardiac autonomic dysregulation, such as a genetic vulnerability. An example of a gene-by-exposure interaction regarding
cardiac autonomic activity has been demonstrated by twin studies finding that genetic effects contributing to cardiac autonomic traits at rest become stronger under stress.\textsuperscript{42–44} Because depressive and anxiety disorders are associated with high levels of stress, we expected that these psychiatric disorders might also interact with genetic risk for cardiac autonomic dysregulation. The presence of such an interaction effect may explain the ambiguous findings in this field, as studies might have applied exclusion criteria that inadvertently selected samples with differential genetic risk for cardiac autonomic dysregulation. However, when investigating this, we did not find evidence for a gene-by-exposure interaction effect (Chapter 4). We conclude that genetic moderation does not explain discrepant findings in the literature regarding the association of depression and anxiety with cardiac autonomic activity.

In the absence of genetic moderation, previous findings of a significant relationship of cardiac autonomic dysregulation with depression and anxiety likely resulted from other confounding factors, including lifestyle. In the next chapters of this thesis, we investigated the association between several lifestyle factors on cardiac autonomic activity. These studies showed that high physical activity, moderate alcohol use, and non-smoking had a positive effect on cardiac autonomic activity (Chapter 5). Factors contributing to cardiac autonomic deterioration over time were older age, (increase in) smoking and BMI, and (starting) the use of antidepressants. (Starting) the use of cardiac medication, in contrast, improved cardiac autonomic activity (Chapter 6). The influences of the majority of these factors are well-established in the literature\textsuperscript{45–52} and our results stress the importance of taking them into account when researching the association of depression and anxiety with cardiac autonomic activity. This holds true especially when comparing a psychiatric sample with healthy controls, as many of these factors are known to differ between these populations.\textsuperscript{53,54}

The association of depression and anxiety with basal cardiac autonomic dysregulation is likely caused by confounding effects of antidepressant use and lifestyle, but not by genetic moderation.

Is cardiac autonomic dysregulation associated with CVD risk factors?
As NESDA includes a rather young sample, the incidence of CVD was too low to investigate the association of cardiac autonomic dysregulation with cardiovascular morbidity and mortality. However, we had access to an abundance of data on known CVD risk factors: metabolic syndrome components\textsuperscript{55} and inflammatory
In addition, we were able to support the NESDA findings with results from two independent cohorts: NTR and AHAB. Within these studies, we confirmed previous findings of a strong cross-sectional association of cardiac autonomic dysregulation with metabolic (Chapter 7 & 9) and inflammatory (Chapter 8 & 9) components. Longitudinally, higher sympathetic activity predicted increases in metabolic dysregulations over time. Unfortunately, at that time, the data was not available to test reverse associations. For inflammatory markers, we were able to investigate bidirectional prospective associations, and found that high HR predicted subsequent higher levels of CRP and IL-6. Some evidence was found that inflammatory markers might also predict future cardiac autonomic activity, but these findings were less consistent. Longitudinal studies aimed at disentangling directionality between these biological systems are scarce, but some of them suggest, in line with the current findings, that autonomic dysfunction may precede metabolic abnormalities and increased levels of systemic inflammation. It is thought that the autonomic nervous system may impact on the metabolic syndrome and systemic inflammation by modulating glucose, fat, and liver metabolism and through the cholinergic anti-inflammatory pathway: the neural mechanism that inhibits the inflammatory response by vagal acetylcholine secretion. However, according to some studies, reverse causality between autonomic activity and immune or metabolic physiology is also plausible. Or perhaps these systems may be best conceptualized as indicators of a broader, aggregate, and correlated clustering of systemic risk factors that mutually influence one another through bidirectional or feedback pathways. The findings in this thesis provide an empirical basis for future work designed to adjudicate between the above possibilities.

Cardiac autonomic dysregulations are robustly associated with and predictive for CVD risk factors over time.

Is cardiac autonomic activity in the pathway from poor mental health to CVD risk?
This question, the holy grail of this thesis, is remarkably difficult to answer. When we formally tested this question within AHAB (Chapter 9), the answer was: yes, perhaps cardiac vagal activity is a mediator in the pathway from depressive symptoms to CVD risk factors. However, these results only held for BDI-II scores in contrast to the CES-D, suggesting that these depression scales capture different symptom profiles that are differentially related to biological systems. In addition, our results suggest that the found pathway is not fully explanatory and that there may be other underlying mechanisms, as a significant direct pathway

remained between depressive symptoms and CVD risk factors. Furthermore, as cross-sectional data was used, how can we establish the temporal ordering of this pathway? Indeed, alternate statistical mediation testing also suggested that cardiovascular risk factors may be in the pathway from depressive symptoms to low cardiac vagal activity. Model fit was comparatively poorer for pathways where depressive symptoms were treated as the mediator or outcome variable. The latter finding is in contrast to results of the Whitehall II study, suggesting that cardiac autonomic dysregulations may precede onset of depression rather than the other way around. As illustrated in the previous paragraph, observational studies – even within a longitudinal design – are limited as they do not allow for a definitive statement of causality. The question of temporal ordering, however, is almost an inconsiderable issue compared to the contrary findings within NESDA, suggesting that depressive and anxiety disorders are not even cross-sectionally associated with cardiac autonomic activity. Sample heterogeneity, methodological discrepancies, and lurking confounding factors, despite the best efforts to control for them, will always affect studies of an observational nature. Future interventional and mechanistic studies are warranted to shed more light on the question whether cardiac autonomic activity is in the pathway from poor mental health to CVD risk. But to indulge the faithful reader of this thesis, who has come all this way to seek an answer, I will provide my own speculative notions: poor mental health may lead to cardiovascular disease risk, perhaps through increased cardiac autonomic stress reactivity, and/or through antidepressants and lifestyle factors that influence basal cardiac autonomic activity, and/or through other mechanisms that are not a consequence of autonomic activity.

Real knowledge is to know the extent of one’s ignorance ~ Confucius

**METHODOLOGICAL CONSIDERATIONS**

This thesis is mainly based on NESDA: one of the largest and longest followed cohorts with data on mental health, cardiac autonomic activity, and metabolic and inflammatory components. One of the strengths of NESDA is the use of DSM-IV based clinical diagnoses of depression and anxiety, allowing for the investigation of more severe mental health problems compared to general population studies based on depressive or anxious symptomatology. Another major strength is that we had data on multiple waves, enabling us to verify the consistency of our findings. Furthermore, we used two renowned studies to replicate and extend our
results within NESDA: NTR and AHAB. Despite the richness of these databases, there are some general limitations that ought to be considered.

First, methodological differences compromise the comparability between the studies. In contrast to the population-based NTR and AHAB studies, NESDA was designed to include a large sample of people with (a history of) depression and/or anxiety. In addition to sample discrepancies, there are differences in data-collection between the studies, as confounding factors have been measured differently and cardiac autonomic variables were recorded in various ways. Regarding the latter, data for NTR was collected during 24-hours of a regular day, data for NESDA was assessed during a 100-min recording of several conditions in a laboratory setting, and data for AHAB was assessed with a different device during a 5-min rest period. One could argue that the NTR data is more ecologically valid but also more prone to interpersonal variability than the NESDA data. In turn, NESDA data may be more susceptible to interpersonal variability than AHAB data, as the different laboratory conditions may have caused a different physiological response in some individuals compared to others. However, the combined findings of the three studies allowed us to investigate whether the pattern of results in this thesis holds across diverse settings.

As NESDA is the main source of data for the articles in this thesis, the following limitations are solely directed to this dataset. Cardiac autonomic measures were recorded during several conditions, but not during a true rest condition, rendering these measures prone to all sources of variability. Contrariwise, measurement of cardiac autonomic activity during rest or laboratory conditions may not be as ecologically valid as 24-hour measurements in real life, which presumably more reliably predict future disease. However, Chapter 6 showed that the average of the conditions has good temporal stability for HR and RSA, suggesting that we have assessed robust and reliable variables. PEP showed a substantially lower temporal stability, possibly due to the difficulty in the scoring of ICG wave forms and the use of different raters. These factors might have contributed to some of the null-findings for PEP.

Many of the measured variables, such as lifestyle and the presence of disease, are based on self-report, which are more prone to inaccuracy and bias than objective measures. However, data was available on cotinine levels at baseline and these levels showed strong correlations with self-reported number of smoked cigarettes/day, enhancing our confidence in the reliability and validity of the used lifestyle measures. In addition, previous research has shown that self-reported disease and general practitioners information are in good agreement.

Finally, as mentioned before, all of the studies in this thesis are based on observational data. Although prospective studies provide a better stab at causality
than cross-sectional studies, they are still imperfect. For instance, many confounders can influence a relationship and it is not possible to properly control for all of them. If these confounders affect the predictor and outcome variables at different time points in life, this can create the false impression of causality. These and other factors that may influence the results of observational studies warrant caution when making any inferences about directionality in this thesis.

**CLINICAL IMPLICATIONS**

Like many other biological measures, the effect sizes found for cardiac autonomic activity in most of our studies are rather modest and not suitable as a clinically useful marker of a dysfunctional stress system on the level of an individual. However, cardiac autonomic activity can be non-invasively and unobtrusively measured and has shown adequate validity and stability in research. On a population level, cardiac autonomic dysregulation may be considered as an early warning sign for serious somatic problems later on, and knowledge about factors that influence changes in this system are important for prevention and intervention strategies. Our research contributed to the increasing evidence that poor lifestyle and antidepressant use, both important consequences of depression and anxiety, affect cardiac autonomic dysregulation, which on its turn is associated with increased CVD risk factors. The lack of evidence for a direct relationship gives hope that depressive and anxiety disorders do not inevitably lead to cardiac autonomic dysregulations, but that this association may be broken by interventions targeting modifiable risk factors, such as lifestyle. Moreover, the robust findings of detrimental effects of antidepressant use on cardiac autonomic activity ought to be taken into account by clinicians, especially when treating patients with poor cardiovascular health. For these patients, prescription of SSRIs may be preferable to TCAs or SNRIs, and physical health should be monitored as thoroughly as mental health. Better yet, other therapy options should be explored that render less adverse side effects.

**FUTURE DIRECTIONS**

Like this thesis ‘stands on the shoulder of giants’, the hope is that future research can build upon the knowledge gathered in this thesis. A magnifying glass can be taken to look closely at disorder heterogeneity and symptomatology and their relationship with different aspects of cardiac autonomic (re)activity. More longitudinal research can be performed, investigating long-term bidirectional
effects of autonomic, metabolic, and inflammatory systems on one another. In addition, long-term studies may determine the association of these systems with incident CVD. More (replication) studies are needed to examine the role of genetic risk and genetic correlation in the relationship between poor mental health and autonomic, metabolic, and inflammatory dysregulations in larger samples. Such research will provide us with stronger empirical evidence for the (absent) causal associations considered in this thesis. A next step is to attempt to verify or falsify these observations by conducting experiments. Such experiments may include intervention studies, much like the MOod Treatment with Antidepressant or Running (MOTAR) study – currently being conducted by our research group – that compares the effect of antidepressant use versus exercise on mental and physical health, including cardiac autonomic activity. Intervention studies will be better able to answer questions of causality than observational studies. But if we really want to understand the role of the autonomic nervous system in the association between poor mental health and CVD, we will have to adopt a mechanistic approach. For instance, what are the exact effects of specific antidepressants? What effects do they exert on receptors, neurotransmitters, cells, organs, and systems? Can we deepen our understanding about the mechanisms through which the autonomic nervous system influences the metabolic and inflammatory systems and vice versa? Can we further identify genetic variants for basal autonomic tone and stress-induced reactivity and unravel their specific biological functional impact to really answer questions of causality? Such studies may proof invaluable for informing preventive interventions, pharmaceutical manufacturing, and treatment strategies that genuinely make a difference in a society burdened with mental and cardiovascular health issues.

CONCLUDING REMARKS

This thesis adds to the evidence that depressive and anxiety disorders are not associated with basal cardiac autonomic dysregulation, and that previous found associations may have been caused by confounding effects of lifestyle and antidepressant use. As robust associations are established between cardiac autonomic dysregulation and CVD risk factors, studies investigating factors that have detrimental or beneficial influences on the autonomic nervous system are paramount for clinical practice and public health.

As many a song, this thesis revolves around the heart. The autonomic nervous system is to the heart as a maestro is to the orchestra – when conducting badly, the heart will suffer, but when conducting well, it may beat in harmony.