Chapter 8

Summary and general discussion

8.1. Summary

The aim of this thesis was twofold: 1) to investigate the causes of individual differences in cardiac autonomic nervous system (re)activity in a healthy adult population using ambulatory recording, and 2) to investigate the consequences of these individual differences in (re)activity for the inflammatory and metabolic risk profile.

In **chapter 3**, the heritability of the three HRV measures that are currently used most in the fields of cardiology and physiology were researched in the largest 24-h ambulatory ANS dataset in twins to date. Moderate heritability estimates of about 50% were found for all three HRV measures across three different physical activity categories inventoried (sleep, sitting activities, and non-sitting light physical activity during the day). In addition, about 50% of the phenotypic covariance between any two HRV measures in this research could be explained by genetic factors. Interestingly, the genetic overlap between the three HRV measures turned out to be very high, especially between pvRSA and RMSSD and between RMSSD and SDNN, where genetic correlations were estimated at .94 and .89 in ambulatory sitting conditions. These findings provide us with the important message that HRV studies that assessed pvRSA, RMSSD or SDNN, can be safely pooled in future meta-analyses of genome-wide association studies because the genetic architecture is expected to be highly similar. A secondary goal of this study was to test whether heritability estimates were robust against confounding by RSA ceiling effects that may occur at low heart rates. Although 10.7% of the participants showed a quadratic IBI-RSA relationship, controlling for this ceiling effect did not lead to significant differences in the heritability estimates. This implicates that there is no pressing need to exclude recording periods (e.g. nighttime recording) or participants with low heart rates from genetic studies of HRV.

In **chapter 4**, we focused on the quantification of several rest and stress conditions in unstructured ambulatory recordings for future use in cardiovascular stress research. We used prolonged (24-h) ambulatory recordings of ANS activity during a representative weekday to research real-life stress reactivity. On the basis of the activity diaries filled out by the participants and the inbuilt accelerometer in the VU-AMS, two ambulatory rest (sleep, leisure) and four ambulatory stress (wake, work, work_sitting, work_peak) conditions were extracted for each participant. From these

conditions several ambulatory reactivity measures were defined. The usability of these real-life reactivity measures was tested by investigating their reliability, temporal stability and heritability. We found that the ambulatory reactivity measures that were employed in this study were reliable and showed moderate to high temporal stability over a period of three years ($0.36 < r < 0.91$ for IBI, $0.58 < r < 0.85$ for pvRSA, and $0.48 < r < 0.76$ for PEP). Almost every ambulatory autonomic reactivity measure showed significant heritability, ranging from 10 to 47%. Heritability of reactivity in daily life was largely due to new genetic variance emerging during real-life situations compared to that seen in the more relaxing 'baseline' conditions. It was concluded that real-life reactivity in ambulatory cardiac ANS data can be reliably assessed when recordings encompass a workday supplemented by periods of leisure time, preferably including sleep.

In **chapter 5**, we took a completely different approach to organize the 24-h ambulatory ANS data. Whereas in chapter 4 we sought to divide the day into homogeneous periods based on physical activity and psychosocial circumstances, as extracted from the combination of dairy self-report and the inbuilt accelerometer signals, chapter 5 used a physiological criterion to create homogeneity in the ambulatory signals analyzed. In this chapter the continuous ECG registrations were divided into several distinct heart rate bins, under the assumption that these bins represent different physiological states: low HR at 1 Hz (60 bpm), medium HR at 1.3 Hz (78 bpm), and high HR at 1.6 Hz (96 bpm) bins were defined using a beat binning algorithm. The heritability of four clinically relevant ECG repolarization (TpTe, QT, and TWA) and depolarization (QRS) parameters were estimated in the bins and compared to the heritability estimates of the same parameters obtained from a typical 10 sec resting ECG at 1.12 Hz (67 bpm) as commonly used in clinical practice. Results showed moderate to high heritability for all parameters (TpTe: 52 to 63%, QT: 34 to 69%, TWA: 55 to 72%, and QRS: 32 to 42%). Heritability estimates of the clinical resting ECG were generally lower compared to those of the binned ambulatory ECG. The difference reached significance for the resting QT interval and the TWA when compared to that obtained from the binned ambulatory ECG at the lowest frequency. A secondary goal of this study was to examine possible rate dependency of the genetic factors influencing the ECG parameters. For all parameters the genetic correlations among the different frequencies were very close to unity suggesting that the same genetic factor influences the parameters

at all three heart rates. Furthermore, no significantly different heritability estimates were found for TpTe and QRS at the three heart rates. For QT, heritability significantly decreased with increasing HRs and, albeit less pronounced, a similar trend was observed for the TWA. Overall, we showed that the beat binning approach may provide better endophenotypes for genetic studies of the ECG than the classical clinical resting ECG.

Finally, we investigated the genetic overlap among the three repolarization parameters. Repolarization is strongly influenced by the SNS, and ECG parameters strongly related to repolarization might be useful as indices of cardiac SNS control (van Lien et al., 2015). We found that although the phenotypic covariance between the three repolarization parameters was mostly genetically determined, the overlap between the genetic factors influencing the three repolarization parameters was only modest, which indicates that these parameters provide unique genetic information on the repolarization phase.

In **chapter 6** and **chapter 7** we focused on the potential consequences of the individual differences in autonomic regulation as measured in chapter 4. First, in chapter 6 the heritability of a set of parameters forming another cardiovascular risk cluster, inflammation, was researched. The large biobank dataset that was available allowed for extensive genetic analyses including not only twins and siblings, but also the parents of the twins and siblings. Adding parents of twins to the design enabled us to test for the presence of assortative mating and by including data of other family members than twins, the contribution of both additive and non-additive genetic effects could be estimated without the need to remove estimation of common environmental effects. The sample size in combination with the extended twin family design furthermore ensured substantial statistical power for the estimations. We found that TNF-α, IL-6, CRP and fibrinogen showed moderate heritability (39%, 21%, 45%, and 46%, respectively). A considerable part of the genetic variation in TNF-α, CRP and fibrinogen was non-additive while heritability of IL-6 was due to additive genetic effects only. Surprisingly, the environment shared by family members was not relevant for any of the inflammatory parameters. Furthermore, with the exception of a small effect for fibrinogen, no evidence was found for spousal resemblance for any of the other pro-inflammatory markers. From this study, we conclude that a clear

numerical target has been set for future genome wide screens attempting to find the actual genetic variants regulating the levels of these pro-inflammatory markers.

In the past, both a shift in autonomic balance towards sympathetic dominance and exaggerated cardiac autonomic reactivity to stress have been associated with increased cardiovascular disease risk. Previous research, mostly cross-sectional studies conducted in the laboratory, points to parallel autonomic effects on the metabolic and inflammatory profiles that are hypothesized to account in part for this risk. In **chapter 7**, the long-term bidirectional association between cardiac ANS activity and reactivity and inflammatory and metabolic risk was tested in two independent prospective studies. Metabolic and inflammatory risk scores were calculated by adding the Z-scores of several key markers of the pro-inflammatory (TNF-α, IL-6, CRP and fibrinogen) and the metabolic (waist circumference, body mass index, glucose, triglyceride, low-density cholesterol, and high-density cholesterol levels) state. Ambulatory 24-h ANS data collection took place five years before, and five years after the biobank study. On the basis of the results from the study described in **chapter 4**, IBI, RSA, and PEP sleep and leisure resting levels and the reactivity measure (work_sitting versus leisure time activity) that suffered least from confounding by posture and/or physical activity was included. It was found that a higher resting HR during leisure time paired with increased cardiac sympathetic reactivity were associated with higher inflammatory risk five years later. In addition, higher sympathetic reactivity was associated with higher metabolic risk after five years. An unfavorable metabolic or inflammatory risk profile, in turn, had no detrimental effect on ANS functioning measured at follow-up. From this we conclude that our results are most compatible with a model that has unidirectional causal effects of resting heart rate and cardiac sympathetic reactivity on inflammatory risk and additional unidirectional effects of cardiac sympathetic reactivity on metabolic risk.

8.2 General discussion

This thesis builds on a database created during two previous data collections in twin families (ANS Study 1, wave 1 and wave 2) that used the VU-AMS system developed at the VU University two decades ago. It added to this database by conducting a second study (ANS Study 2, wave 3) with a specific focus on the association between ambulatory cardiac ANS (re)activity and cardiovascular risk

factors. Due to technical improvements of the ambulatory monitoring device over time, cardiac ANS assessments for Study 2 embodied continuous recordings of the entire ECG signal (compared to R-peak registrations only for Study 1). This allowed for genetic analyses of other clinically relevant ECG components as well, such as the QT interval, the T-peak-T-end interval, QRS duration, and the T-wave amplitude. In this section, the findings will be evaluated in the light of current knowledge and directions for future research will be provided.

Individual differences in cardiac autonomic nervous system (re)activity

This thesis shows that individual differences in cardiac autonomic nervous system (re)activity in a real life setting are to a large extent caused by genetic variation, at least in a healthy adult population. These findings corroborate previous findings from ambulatory studies on twins (Busjahn et al., 1998; Kupper et al., 2004; Kupper et al., 2005; Kupper et al., 2006; Su et al., 2010) and extend them in a number of ways. First, cardiac sympathetic control has previously been operationalized by measurement of the systolic time intervals, with the PEP being the measure of first choice (de Geus et al., 2015). Recent research by our group has shown that the TWA during cardiac repolarization can be used as an additional indicator of cardiac sympathetic control (van Lien et al., 2015). Here we confirm that this measure, like the PEP, shows substantial heritability in ambulatory recordings, strengthening our confidence in the role of genetic factors in cardiac sympathetic control. Secondly, we parsed the ambulatory recordings into different conditions using two new approaches: 1) behaviorally informed conditions that can be expected to reflect states of low versus high emotional and mental engagement with the environment, and 2) physiologically informed conditions where the ECG was ensemble-averaged across fragments with similar heart rates across the day and night. In our previous studies, including chapter 3 in this thesis, conditions were created mainly by focusing on the control for posture and physical activity level.

The added value of the TWA

Although PEP is the current gold standard in clinical physiological research, PEP scoring is highly laborious especially when research moves to an epidemiological scale with ambulatory data measured

across extended time periods in thousands of participants. For this reason, the TWA has recently been put forward as a valuable addition to PEP to index sympathetic nervous system activity (van Lien et al., 2015). Earlier twin studies of the TWA estimated heritability between 34 to 72% (Haarmark et al., 2011; Mutikainen et al., 2009). The broad range in these estimates was ascribed to the lead that was used to derive the TWA. The TWA in these earlier studies was mostly assessed during quiet sitting in the laboratory. In the study described in chapter 5, heritability of the TWA was estimated between 58% and 72%, depending on the heart rate at which the TWA was measured. A remaining task is to determine the genetic underpinnings of the TWA in the same content-based way as was done for the PEP in chapter 4 and test for differences in heritability as a function of sleep, leisure and work time. Furthermore, an interesting question is to what extent the genetic factors influencing the PEP and the TWA overlap. The phenotypic correlation is significant but moderate and many different biological processes may be involved in the manifestation of cardiac contractility and ventricular repolarization. However, the PEP-TWA correlation may be due entirely to shared genetic factors which would suggest that a bivariate approach with both traits simultaneously could help detect genetic effects on cardiac SNS activity in gene finding studies.

Different ways to parse unstructured ambulatory ANS recordings into meaningful conditions

The major advantage of ANS recordings in real-life over laboratory assessments is the gain in ecological validity. Where laboratory recordings claim to capture ANS activity or responsivity to stress in general, the stress induced in the laboratory setting is actually quite artificial. Psychological challenges and mental effort experienced in real-life will correspond more closely to the chronic and repeated stress that may ultimately predispose us to adverse health conditions like cardiovascular disease. The major drawback of using real-life recordings is that they are unstructured by nature, stressful events are less demarcated and, importantly, stress during a recording on an arbitrary day is not guaranteed unless a stressful event is planned. On top of this, ambulatory measurements are more prone to confounding influences of posture, physical activity, and time of day compared to typical laboratory assessments in which measurements are typically short and recordings take place with the participants being under controlled (physical) conditions.

In this thesis, two different strategies to organize unstructured ambulatory ANS data have been explored. For one strategy, the focus was on clustering data based on the reported activities by the participants and accelerometer data obtained from the recordings (content-based, chapter 4). The other approach concerned organizing the data based on physiology and grouped ECG complexes of comparable heart rates (physiology-based, chapter 5). The latter physiology-based approach has the advantage of completely controlling for heart rate, and strongly, albeit not perfectly, reduces the confounding effects of postural change and physical activity. The disadvantage is that it cannot deal with effects of the emotional state of the subject at the time of the physiological recording. For this approach thousands of frequency-defined heart beats scattered across the entire measurement are used to constitute the different bins. As such, it is no longer possible to integrate the physiological signal with the emotion and/or activity that the participant was exposed to at a particular time during the recording. The content-based approach fares better here. However, posture and physical activity are far less stringently controlled for as compared to the physiology-based approach. Also, effects of heart rate on ECG parameters of interest cannot be as well controlled as with the physiology-based approach. Strong heart rate effects were for instance found for the TWA (van Lien et al., 2015).

From chapter 4, we concluded that all content-based rest (sleep, evening leisure time activity) and stress conditions (wake, work, work_sitting, work_peak) performed equally well from a psychometric point of view. Control for physical activity becomes particularly manifest when studying cardiovascular reactivity since this may put constraints on the pairs of conditions that can be used to define ambulatory reactivity (i.e. only make rest-and-stress-pairs of conditions that are equal in posture and physical activity). One way to overcome the issue of confounding by postural change or physical activity in the content-based approach would be to more rigorously control for these effects. A few decades ago, Blix, Stromme, & Ursin (Blix, Stromme, & Ursin, 1974) introduced the concept ‘additional heart rate’ which refers to heart rate increments that can be ascribed to (non-metabolic) task demands. This requires that metabolic demands are continuously monitored in addition to heart rate. Co-registration of heart rate and oxygen consumption (VO_2) during different levels of physical activity can establish the linear slope between heart rate and VO_2. This can be used to predict the heart rate at a given VO_2. Deviations of the actual heart rate form this predicted heart rate is known as the

'additional heart rate'. This additional heart rate method could be partly extended to PEP and RSA, as cardiac SNS and PNS activity may also scale linearly with VO2, at least at low intensity physical activity.

For heart rate, this method has already proven to be successful in the laboratory (Carroll, Turner, & Rogers, 1987; Carroll, Turner, & Hellawell, 1986; Carroll, Turner, & Prasad, 1986; Sherwood, Allen, Obrist, & Langer, 1986; Stoney, Langer, & Gelling, 1986; Turner & Carroll, 1985). Since implementation of this method in prolonged ambulatory recordings is technically not possible due to the demanding requirements for VO_2 measurements, Myrtek & Brugner (Myrtek & Brugner, 1996) introduced a new method in which continuous co-registrations of the ECG and physical activity was realized by means of accelerometery. In this method, heart rate and accelerometer values at a given time point (during an event) were compared to the moving average of the previous minutes. When the difference in heart rate between these periods was at least 3 beats and the concurrent change in physical activity was minimal, an event was considered 'emotional'. Higher levels of physical activity were dealt with by an algorithm that is explained in detail in Myrtek (Myrtek, 2004). Although additional heart rate was succesfully captured by this method in several studies, associations with the emotional appraisal of the events have not been consistently found in the ambulatory situation (Ebner-Priemer et al., 2007; Myrtek, Aschenbrenner, & Brugner, 2005; Myrtek & Brugner, 1996). This may in part be due to the fact that postural changes, with a large impact on heart rate through venous pooling effects on stroke volume, are not taken into account in their approach. The increased precision of accelerometer data and the improved algorithms for both posture and metabolic demands detection from such signals may well make it worthwhile to revive this method.

Gene by environment interaction

The most striking pattern observed in the ambulatory setting was the increase in genetic variance during psychologically engaging events. This increase, which reflects gene-environment interaction, had already been noted in previous laboratory stress research (de Geus et al., 2007; Wang et al., 2009). Stress-specific genetic effects were generally more evident in the ambulatory setting (up to 40%) compared to those found in the standardized part of our study (4% to 11%) and previous laboratory

studies (7% to 23%) (de Geus et al., 2007; Wang et al., 2009). This can probably be ascribed to the difference in the appraisal of the stressors encountered in the different studies. Whereas de Geus et al. (2007) included typical short mental stress tasks in their study, Wang et al. (2009) used tasks that more closely approached stress in real-life, and the ambulatory part of our study described in chapter 4 solely comprised of real-life work-related demands. The latter are more likely to be motivationally relevant for the individual and invoke more subjective stress. Unfortunately, we did not confirm this by adequate measurements of subjective stress, as an added set of items to the paper diary was considered to increase the already high burden of ambulatory monitoring for the subject. Current day smartphone-based ecological momentary assessment was not yet in reach when Study 1 was started. In retrospect, our data show that it is not necessary to include pre-planned stressors in the measurement protocol to induce sufficient reactivity. The choice of measurement days will depend on the research question. The inclusion of a work day with social engagement paired to mental effort seems important, but other stressors like child care or prolonged caregiving for elderly/diseased family members may also yield substantial psychophysiological reactivity.

Because the studies described in this thesis are larger than any previous study and have the added advantage of being performed under ecologically valid conditions they provide the research community with the best estimates of the genetic architecture of cardiac autonomic control to date. As it stands, we conclude that individual differences in sympathetic and parasympathetic influences on cardiac electrical activity are best explained by invoking only two sources of variance, additive genetic influences and unique environmental influences. The additive genetic variance varies between different markers, across the different times of day, and across the different pursuits of daily life, but the average of all reported ambulatory RSA heritabilities throughout this thesis was 49% and for PEP it was 38%.

The long-term association between ANS (re)activity and inflammatory and metabolic risk

Inflammatory processes and metabolic disturbances have both been associated with cardiovascular disease, and both have been associated with ANS functioning. Although evidence for a deleterious role of ANS functioning characterized by a shift in sympathovagal balance to sympathetic dominance

in increased inflammatory and metabolic risk has previously been found in both directions, bidirectional influences between ANS functioning and both risk profiles has never been researched in one study before. The study described in chapter 7 was also the first to include reactivity to real-life stress, captured as work-related ANS activity versus leisure time ANS activity in the evening, as a factor.

Our results point to an unidirectional association of higher ambulatory resting heart rate paired to exaggerated cardiac sympathetic reactivity with higher inflammatory risk at follow-up. Exaggerated cardiac sympathetic reactivity did also precede higher metabolic risk measured 4.6 years later. Conversely, inflammatory and metabolic risk were not associated with ANS functioning measured 5.4 years later. With this study we show that both higher baseline heart rate and large sympathetic responsivity to work-related challenges can make one prone to develop cardiovascular problems in future. Multiple short-term stress experiences or stress that is experienced chronically may both be at the basis of these associations. Since the reactivity measure that was incorporated in this study pertains to work and recovery from work-related activity (evening leisure time), it may be noteworthy that considerable variation in the experience of work stress between individuals exists. Vrijkotte, van Doornen, & de Geus (Vrijkotte et al., 2000) studied this topic and found that individuals that experienced high imbalance at work (a combination of high effort and low reward) had higher heart rates at work and directly after work, a higher systolic blood pressure during work and leisure time, and lower 24-h vagal tone on work days and a non-workday. Individuals that experienced high overcommitment (inability to unwind from work), on the other hand, showed a general pattern of shorter PEP during work and leisure time periods across workdays and the non-workday. In addition, smaller absolute sleep-wake differences and decreased PEP variability were observed in individuals experiencing high overcommitment, possibly pointing to down-regulation of cardiac beta-receptors by chronic cardiac SNS activity (Vrijkotte et al., 2004). These results illustrate that certain coping styles or personality profiles may give rise to a particular ANS reactivity profiles that may compromise physical health in the long run.

Future directions

Having established that the regulation of cardiac autonomic control can largely be explained by genetic variation, it becomes important to characterize the actual causal variants responsible for this heritability. As already outlined in the introductory chapter, the first steps have been undertaken towards this aim. Many candidate gene association studies have been conducted and even a few single-cohort GWA studies. The yield of these attempts has, perhaps not surprisingly, been modest. Cardiac autonomic activity is a complex phenotype, and only a very small percentage of the total genetic variance is expected to be explained by a single genetic variant. Instead, lots of variants with very small effects may be at play. In addition, structural variation and rare variants could be of importance to ANS traits in comparable ways as they are to other traits (Eichler et al., 2010; Manolio et al., 2009). For future research, the key lies in expanding the sample sizes of the gene finding efforts, preferably by pooling across many studies in meta-analysis and by taking a hypothesis-free stance, i.e. by focusing on a GWA approach rather than a candidate gene approach. This is exactly what is currently being done by the Genetic Variance in Heart Rate Variability (V_gHRV) consortium, in an attempt to detect the genetic variants causing individual differences in resting RSA. A major concern that this consortium is facing is that the various studies to be pooled did not always use the same metric to quantify RSA. In that regard, our finding that future gene finding studies can safely pool studies that have assessed RSA and RMSSD or RMSSD and SDNN to gain power is very reassuring.

A major component missing from the current ANS data set is self-reported emotional state during the ambulatory recording. There are clear links between ANS functioning and emotion although a one-to-one mapping of emotional state on ANS remains very complex (Kreibig, 2010). Previous research found that people that have a genetic vulnerability to develop anxiety and/or depression showed more negative affect in response to stress in daily life compared to people who did not have this genetic vulnerability (Gunthert et al., 2007; Wichers et al., 2007; Wichers et al., 2008). These findings point to gene-environment interaction effects that may also be reflected in ANS functioning.

Emotional state can be assessed in several ways and at several levels. It was only until few decades ago that emotional state was solely assessed by means of classical questionnaires, the focus of which is on past experience and current beliefs built on that experience. This makes the outcomes of

questionnaires inevitably prone to retrospective bias. Although this may be a good thing for some purposes, for instance in clinical practice where focus lies on the participants' beliefs or mental representations of certain experiences, it may not be a good thing in other instances when one is interested in co-occurring states of multiple modes (i.e. mood, physiology, and behavior). For this reason, emotional state is increasingly measured by Ecological Momentary Assessment (EMA) procedures that repeatedly assess e.g. mood at various (random) times of the day using tablets or smartphones (Conner & Barrett, 2012; Fahrenberg et al., 2007). The idea that two different constructs are measured by EMA and by questionnaires is emphasized even more by studies that found only moderate correlations between outcomes of real-time mood assessment and retrospective questionnaires measuring the same phenomena (Ebner-Priemer & Trull, 2009b).

This discrepancy between momentary and recalled symptoms may also explain why research linking the physiological state to mood or personality assessed by retrospective questionnaire has been rather inconclusive (Conner & Barrett, 2012). Emotional states assessed by EMA in real-life settings may be more strongly correlated to ANS activity than questionnaire-based measures of anxiety and depression symptoms to laboratory based physiology. Support for this notion comes from studies that found ambulatory levels of cardiovascular parameters (BP, HR, HRV) to be more strongly associated to momentary self-report measures compared with the classical retrospective self-reports (Bhattacharyya, Whitehead, Rakhit, & Steptoe, 2008; Kamarck et al., 2005). In ANS Study 1 and 2 we used a paper-and-pencil diary to mainly record posture and (physical) activity at the expense of detailed emotional state measurement. The current-day more advanced assessment methods were simply not available. We deliberately kept the protocol identical in Study 2 as we needed to balance the comparability of data across waves against the advantages of the newly available technology. Future research, however, would do well to replace the paper dairies for applications on a smart phone. Such *apps* can accommodate many types of self-reported data collection, including the assessment of momentary mood (Trull & Ebner-Priemer, 2013). Data obtained from these assessments is 'on the spot' and has great potential to help us gain more insight in the complex interplay between our ambulatory behavioral state and our ambulatory ANS functioning. This thesis clearly confirmed that ambulatory ANS functioning plays a major role in our long-term health.