LIFESTYLE AND HEART RATE VARIABILITY IN THE GENERAL POPULATION

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Knowing is not enough - we must apply
Willing is not enough - we must do

Johann Wolfgang von Goethe

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ABBREVIATIONS

AHA = American Heart Association

AHI = Apnea-hypopnea index

ANS = Autonomic nervous system

ARIC study = Atherosclerosis Risk in Communities Study

BMI = Body mass index

CI = Confidence interval

CVD = Cardiovascular disease

ECG = Electrocardiogram

GAPP study= genetic and phenotypic determinants of blood pressure and other cardiovascular risk factor study

GCP = Good clinical practice

h = Hour

HbA_{1c} = Glycated hemoglobin A_{1c}

HDL-C = High-density lipoprotein cholesterol

HF = High frequency

HR = Heart Rate

HRV = Heart rate variability

Hs-CRP = High- sensitivity C-reactive protein

Hz = Hertz

i.e. = id est

IL-6 = Interleukin-6

IPAQ = International physical activity questionnaire

LC = Leukocytes

LDL-C = Low-density lipoprotein cholesterol

LF = Low frequency

ms = Milliseconds

ms^2 = Milliseconds squared

NHANES = National health and nutrition examination survey

ODI = Oxygen desaturation index

p = P-value

RR = Beat-to-beat interval

SAPALDIA = Swiss study on air pollution and lung disease in adults

SD = Standard deviation

SDNN = Standard deviation of all normal RR intervals

SOP = Standard operating procedure

T2DM = Type 2 diabetes mellitus

TP = Total power

WHR = Waist-to-hip ratio

FIGURE AND TABLE LEGEND

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Stefanie Aeschbacher

SUMMARY

Background: Cardiovascular diseases (CVD) are among the main causes of death worldwide. An unhealthy lifestyle, sleep-related breathing disorders and inflammation are all significantly and independently associated with an increased risk of cardiovascular events.

The autonomic nervous system (ANS) is an important player within the cardiovascular system. Over the last years, heart rate variability (HRV) has become a validated measure of the autonomic function and was found to be associated with several cardiovascular risk factors, disease outcomes and mortality. To date, several aspects related to HRV among young adults remain unclear.

Based on those gaps of knowledge, the general aim of this PhD thesis was to assess the relationships of HRV with lifestyle, sleep-related breathing disorders and inflammatory biomarkers among young and healthy adults from the general population. The specific aims were 1) to evaluate the prevalence of a healthy lifestyle and assess its relationship with HRV, 2) to assess the prevalence of sleep-related breathing disorders and investigate its association with HRV and 3) to assess the interrelationships between HRV and blood markers of inflammation.

Methods: This PhD thesis is based on data from the *Genetic and Phenotypic Determinants of Blood Pressure and other Cardiovascular Risk Factors* (GAPP) study, a prospective population based cohort study. Overall, 2170 inhabitants of the Principality of Liechtenstein, aged between 25-41 years, without established CVD and a BMI ≤35kg/m^2 were included in this study. Study participants obtained a 24-hour (h) Holter electrocardiogram (ECG), and careful post-processing was applied. Time- and frequency domain HRV variables and ambulatory heart rate (HR) were exported. The standard deviation of all normal RR intervals (SDNN) was pre-specified as the main HRV variable for all analyses. Personal, medical, lifestyle and nutritional information were assessed using standardized questionnaires. A fasting venous blood sample was taken to determine biomarkers. For assessment of sleep-related breathing disorders, a nocturnal pulse oximetry with additional nasal airflow recording was performed. Resting HR was recorded using a 10-second resting ECG. Multivariable linear regression models were constructed using HRV related parameters as the outcome variables.

Results: Overall, 2170 participants (47% male) with a median age of 37 years were included. We found that only 11% of our population adopted a healthy lifestyle defined as a lifestyle-score of 6 or 7, whereas 5% had a very unhealthy lifestyle defined as a score of 0 or 1. In general, women had a higher lifestyle-score compared to men. Having a healthy

lifestyle was significantly associated with SDNN, with a β-coefficient (95% confidence interval (CI)) of 0.14 (0.11; 0.17), p=0.0001 per one point increase in the lifestyle-score. This result was attenuated but remained significant after additional adjustment for either resting or ambulatory HR. In the second analysis we found that 9.6% of the population had an apnea-hypopnea index (AHI) ≥5, which is one important component for the diagnosis of an obstructive sleep apnea syndrome. After comprehensive multivariable adjustment, SDNN was inversely associated with categories of AHI and oxygen desaturation index (ODI). These relationships were strongly weakened after the additional adjustment for resting and 24-h HR and most of these relationships lost significance. Resting and ambulatory HR by itself were positively associated with increasing levels of AHI and ODI categories. However, only the relationships with ambulatory HR remained significant after the adjustment for HRV. In the final analysis, we found a close and independent link between HRV and inflammatory biomarkers. Inverse associations of SDNN with all available inflammatory biomarkers were found, with β-coefficients (95%CI) of -0.11 (-0.16;-0.07), p<0.0001 for high-sensitivity C-reactive protein, -0.13 (-0.17;-0.09), p<0.0001 for total leukocyte count, -0.12 (-0.16;-0.08), p<0.0001 for neutrophils, -0.04 (-0.09;0.00), p=0.05 for lymphocytes and -0.08 (-0.09;-0.02), p=0.005 for monocytes. These associations were strongly attenuated after additional adjustment for ambulatory HR and partly lost significance. Ambulatory HR by itself was positively associated with all inflammatory biomarkers, except lymphocytes.

Conclusion: In this young and healthy population, HRV was significantly related to a comprehensive healthy lifestyle, sleep-related breathing disorders and inflammatory biomarkers, suggesting an interrelationship between the ANS and these entities. However, most of the information seems to be contained in HR, and the incremental information of HRV parameters was modest in most analyses. These data may allow some insights in the pathophysiology of CVD occurrence. Finally, the adoption of a healthy lifestyle was rather low in this population, underscoring the importance of healthy lifestyle promotion in the society.

Outlook: More data are needed on the role of the autonomic function in the development of CVD outcomes and on the independent role of HRV in the prediction of cardiovascular risk factor progression or outcome occurrence.

ZUSAMMENFASSUNG

Hintergrund: Kardiovaskuläre Erkrankungen gehören weltweit zu den häufigsten Ursachen für Morbidität und Mortalität. Ein ungesunder Lebensstil, schlafassoziierte Atemstörungen und Entzündung sind unabhängig mit einem erhöhten Risiko für kardiovaskuläre Ereignisse assoziiert.

Das autonome Nervensystem (ANS) spielt eine wichtige Rolle in verschiedenen Prozessen des kardiovaskulären Systems. Die Herzfrequenzvariabilität (HRV) gilt als valides Mass der autonomen Funktion und ist mit verschiedenen kardiovaskulären Risikofaktoren und Mortalität assoziiert. Jedoch sind gerade in einer jungen Population noch einige Aspekte der HRV unklar.

Basierend auf diesen Wissenslücken, war das generelle Ziel dieser Arbeit herauszufinden, ob es in einer jungen und gesunden Population einen Zusammenhang zwischen der HRV und dem Lebensstil, schlafassoziierten Atemstörungen und Entzündungswerten gibt. Die spezifischen Ziele waren 1) die Prävalenz eines gesunden Lebensstils zu berechnen und den Zusammenhang zwischen der HRV und dem Lebensstil zu quantifizieren, 2) die Prävalenz von schlafassoziierten Atemstörungen in einer gesunden Population zu berechnen und die Beziehung mit der HRV herzustellen und 3) die Wechselwirkung zwischen der HRV und den verschiedenen Entzündungsmarkern herzustellen.

Methoden: Diese PhD Arbeit basiert auf den Daten der *Genetic and Phenotypic Determinants of Blood Pressure and other Cardiovascular Risk Factors* (GAPP) Studie, einer populations-basierten Kohortenstudie. Insgesamt konten 2170 im Fürstentum Liechtenstein wohnhafte Personen im Alter zwischen 25 und 41 Jahren, ohne kardiovaskuläre Vorerkrankungen und einem body mass index (BMI) ≤35kg/m^2 vor Ort in die Studie eingeschlossen werden. Bei allen Studienteilnehmern wurde ein 24-Stunden Elektrokardiogramm (EKG) aufgezeichnet und im Anschluss manuell nachbearbeitet. Die zeit- und frequenzbasierten HRV Variablen, sowie die Herzfrequenz (HR) wurden exportiert. Die Standardabweichung aller RR Intervalle (SDNN) haben wir als primäre HRV Variable vorgängig bestimmt. Informationen zur Person, der medizinischen Vorgeschichte und den Lebensstilfaktoren wurden mittels standardisierter Fragebögen erhoben. Zur Bestimmung wichtiger Biomarker erfolgte eine Nüchtern-Blutentnahme. Um schlafassoziierte Atemstörungen detektieren zu können, wurde eine nächtliche Pulsoxymetrie mit zusätzlicher Atemflussmessung durchgeführt. Die Ruhe HR wurde mit einem 12-Kanal Ruhe-EKG bestimmt. Mittels multivariaten Regressionsmodellen wurden die Zusammenhänge zwischen der HRV als abhängige Variable und den verschiedenen Prädiktoren berechnet.

Resultate: Insgesamt wurden 2170 Studienprobanden (47% männlich) mit einem mittleren Alter von 37 Jahren in die Studie eingeschlossen. Der Lebensstil-Score ergab bei 11% der Studienpopulation einen Wert von 6 oder 7 (sehr gesund) wohingegen 5% einen sehr ungesunden Lebensstil mit einem Score von 0 oder 1 hatten. Im Vergleich zu Männern zeigte sich bei Frauen ein höherer Lebensstil-Score. Ein gesunder Lebensstil war positiv assoziiert mit der HRV, mit einem β-Koeffizienten (95% Konfidenzinterval (CI)) von 0.14 (0.11; 0.17), p=0.0001 pro Punktanstieg im Lebensstil-Score. Die zusätzliche Adjustierung für die Ruhe- und 24-h HR hat den Zusammenhang abgeschwächt, jedoch blieb der Zusammenhang signifikant. In einer zweiten Analyse zeigte sich eine Prävalenz von 9.6% für schlafassoziierte Atemstörungen (apnea-hypopnea index (AHI) ≥5). Auch nach einer multivariaten Korrektur gab es signifikante, inverse Zusammenhänge zwischen der SDNN und dem AHI und ODI (oxygen desaturation index), welche jedoch nach zusätzlicher Adjustierung durch die Ruhe- und 24-h HR nur noch teilweise signifikant waren. In der dritten Analyse haben wir signifikante und unabhängige Zusammenhänge zwischen der HRV und allen verfügbaren Entzündungsparametern gefunden. Die SDNN war invers assoziiert mit allen verfügbaren Entzündungsmarkern mit einem β-Koeffizienten (95%CI) von -0.11 (-0.16;-0.07), p<0.0001 für hoch-sensitives C-reaktives Protein, -0.13 (-0.17;-0.09), p<0.0001 für Leukozyten, -0.12 (-0.16;-0.08), p<0.0001 für neutrophile Granulozyten, -0.04 (-0.09;0.00), p=0.05 für Lymphozyten und -0.08 (-0.09;-0.02), p=0.005 für Monozyten. Die zusätzliche Korrektur für HR, welche auch unabhängig mit den Entzündungsmarkern assoziiert war, hat die Wechselbeziehung zwischen der HRV und den Entzündungsmarkern deutlich abgeschwächt.

Schlussfolgerungen: In dieser jungen und gesunden Population war die HRV signifikant assoziiert mit einem gesunden Lebensstil, schlafassoziierten Atemstörungen und Entzündungswerten. Deshalb kann von einer Wechselwirkung zwischen der autonomen Funktion und den drei Entitäten ausgegangen werden. Jedoch scheint die HR den Grossteil der Information zu enthalten und die zusätzliche Information der HRV zeigte in den meisten Analysen nur moderate Auswirkungen. Diese Resultate können Einblicke in die Pathophysiologie der Entstehung kardiovaskulärer Erkrankungen ermöglichen. Ausserdem war die Prävalenz eines gesunden Lebensstils eher gering, was die Wichtigkeit für strukturierte Präventionsprogramme aufzeigt.

Ausblick: Weitere Daten sind notwendig um einerseits die Rolle der autonomen Funktion in der Entwicklung von kardiovaskulären Erkrankungen zu verstehen und andererseits die Rolle der HRV für die Prädiktion von kardiovaskulären Events besser untersuchen zu können.

1.BACKGROUND

1.1 Cardiovascular disease – public health impact and risk factors

Cardiovascular diseases (CVD) are among the main causes of death worldwide, causing approximately 48% of all deaths due to non-communicable diseases.[1-4] The global burden of CVD is expected to further increase in the upcoming decades, which will have an additional impact on the health care systems.[3] Cerebrovascular and ischemic heart diseases have the highest impact on CVD mortality among men and women.[1] Age and other cardiovascular risk factors are additively increasing lifetime-risk for death from CVD *(Figure 1)*.[5] Consequently, a reduction in CVD related morbidity and mortality largely depends on avoidance and optimal management of its modifiable risk factors. Accordingly, ideal cardiovascular health metrics are strongly recommended by professional societies.[6,7]

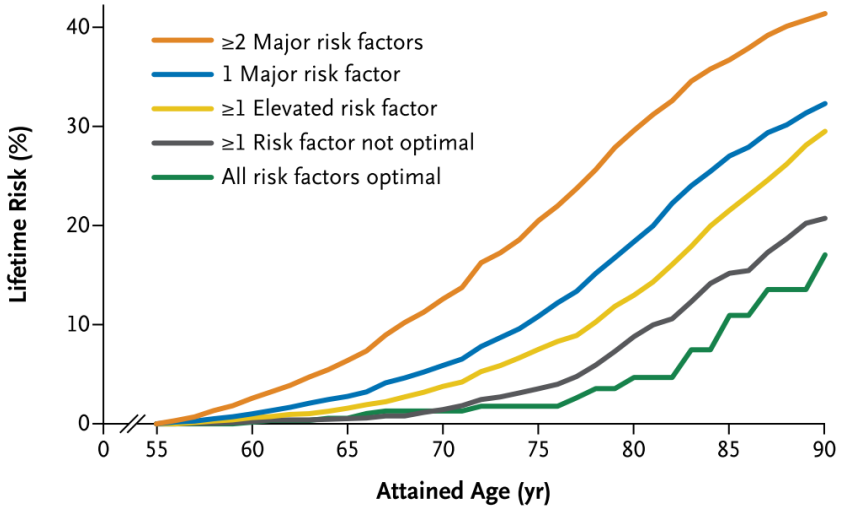

Figure 1 Lifetime risk of death from cardiovascular disease among men at 55 years of age, according to the burden of risk factors (Berry et al., N Engl J Med, 2012)

1.1.1 Lifestyle – and cardiovascular risk factors

A healthy lifestyle represents a key component to improve cardiovascular risk factors, and to reduce the development of CVD. The Global Burden of Disease Study in 2013 showed that the population attributable risk for death from CVD of known cardiovascular risk factors was 88.5%, suggesting that avoiding these factors in the population would greatly reduce the occurrence of CVD.[4] Other studies also highlighted, that the number of ideal lifestyle factors and cardiovascular health metrics is strongly associated with a lower rate of CVD and all-cause mortality in general population,[8,9] which further highlights the importance of adopting a healthy lifestyle.

Previous studies found an association of diet, physical activity, body mass index (BMI) and smoking status with the risk for cardiovascular events.[10-12] Compared to women with a normal BMI, obese women had a 57% increased risk for cardiovascular events. Additionally, being physically active resulted in a 41% reduced risk for such events.[11] Among elderly adults, higher walking pace and walking distance were associated with a 40 to 50% risk reduction for CVD.[13] Based on data of the Nurses' Health Study, Chomistek et al. have indicated that having a normal BMI and being physically active were the most important components to avoid the development of coronary heart disease.[12] According to these results, accomplishing either one of those components may prevent around one fifth of all newly diagnosed cases of coronary heart disease.[12] Adopting an unhealthy lifestyle is associated with most major cardiovascular risk factors, including hypertension, Type 2 Diabetes mellitus (T2DM) or dyslipidemia.[12] Taken together, having ideal lifestyle factors is a key component in CVD prevention.

1.1.2 Sleep-related breathing disorders

Sleep-related breathing disorders include multiple entities,[14] while the obstructive sleep apnea (OSA) syndrome is the most prevalent in the general population.[15] OSA is characterized by repetitive partial or complete obstructions during sleep caused by collapsing airways with following arousals.[16] Sleep-related breathing disorders are highly prevalent and remain often undiagnosed.[17] 20 years ago, the estimated prevalence of sleep-related breathing disorders among women and men was 9 and 24%, respectively.[17] In a recently published population-based study of 2100 individuals with performed polysomnography, Heinzer et al. found a prevalence of moderate to severe sleep-related breathing disorders of 23% in women and 50% in men.[15] OSA is independently associated with an increased risk of hypertension,[18-20] coronary artery disease,[21] cerebrovascular events[22] and sudden cardiac death.[23] Studies showed that adequate treatment of overt OSA is associated with a lower risk of cardiovascular outcomes.[21,24] Obesity represents the main risk factor for the development of an OSA. A study including middle-aged adults also highlighted that age and male sex are independent predictors for incident sleep-related breathing disorders.[25] BMI and sex seem to become less important predictors with increasing age.[25] In addition to those predictors, others found associations of OSA with T2DM, cholesterol, metabolic syndrome and depression, even after the adjustment for BMI, waist-to-hip ratio (WHR) or neck circumference.[15,26] Since overweight and obesity are epidemic, an increasing prevalence of sleep-related breathing disorders in the general population is expected in the future.

The main measures of sleep-related breathing disorders are the apnea-hypopnea index (AHI) and the oxygen desaturation index (ODI). AHI is defined as the average number of apnea and hypopnea episodes per hour of sleep. An apnea/hypopnea event is characterized

as a complete cessation or a fading reduction in breathing over at least 10 seconds[14] The ODI is corresponding to the number of oxygen desaturations of ≥4% per hour of recording. According to official guidelines, a diagnosis of an OSA consists of having an AHI ≥5 and additional symptoms, such as excessive daytime sleepiness, recurrent awakenings form sleep, snoring or markedly impaired concentration during daytime.[14] As a definition of sleep-related breathing disorders, an AHI ≥5 is most commonly used in literature.[15]

1.1.3 Inflammation

Inflammation consists of different biological reactions protecting the body against pathogens. Over recent years, inflammation has shown to be strongly involved in the pathogenesis of atherosclerosis.[27] Lowering inflammation could therefore be a potential therapeutic goal, which is currently topic of intense research activities.[27]

Several inflammatory biomarkers are highly related to the development of CVD.[28] Especially, the relationship of high-sensitivity C-reactive protein (hs-CRP) with cardiovascular outcomes is well studied, showing a positive association between hs-CRP levels and death from CVD.[29-31] A meta-analysis including 31 studies showed a linear relationship between hs-CRP and the risk for coronary heart disease, ischemic stroke and vascular deaths.[32] Additionally, hs-CRP and fibrinogen were strongly associated with the development of atrial fibrillation.[33,34] Finally, Interleukin-6 (IL-6), an important cytokine with a broad range of immune effects, is associated with the risk of myocardial infarction.[35]

Inflammatory biomarkers are strongly influenced by different lifestyle factors. BMI was positively associated with inflammation, assuming a low-grade systemic inflammation in obese individuals. Moreover a high WHR, indicative for a high amount of visceral body fat, was independently associated with inflammation among men and women.[36] Adipose tissue is releasing cytokines and may therefore explain the relationship between obesity and systemic inflammation. Another study showed a negative dose-response relationship between physical activity and inflammation that was independent of BMI.[37] This anti-inflammatory effect of regular physical activity was confirmed by other studies and is summarized in a systematic review.[38] Past and current smoking was positively associated with hs-CRP, leukocytes (LC), IL-6 and fibrinogen.[39,40]

1.2 Heart rate variability and the autonomic nervous system

The autonomic nervous system (ANS) plays an important role in the regulation and function of the cardiovascular system and helps to adapt the cardiovascular system to environmental demands.[41] The ANS consists of two major branches, the sympathetic and parasympathetic nervous system, which should be well-balanced. With regard to heart rate (HR) and rhythm, the sinus node is directly regulated via efferent sympathetic and parasympathetic nerve activities. The sympathetic nervous system has a positive chrono-, dromo- and inotrope effect, which is corresponding to an increase in HR, a faster conduction speed in the AV-node and a higher myocardial contractibility. The parasympathetic system has the inverse effect. Chronic sympathetic overactivity is associated with the development of CVD.[42] Thus a well-balanced ANS is important to prevent the development of CVD events.

The heart rate variability (HRV) represents the beat-to-beat variation of the HR, a component strongly regulated by autonomic inputs to the sinus node *(Figure 2)*. Those variations can be quantified using HRV analysis, which has become a validated measure of the autonomic modulation.[43,44] HRV can be easily assessed using a short-term ECG, or alternatively measured using a 24-hour (h) ECG, while individuals perform their daily activities, and which represents the current gold standard. Based on all normal RR intervals, HRV can be analyzed by either time- or frequency domain. Most important HRV variables are presented in the *Table* below.

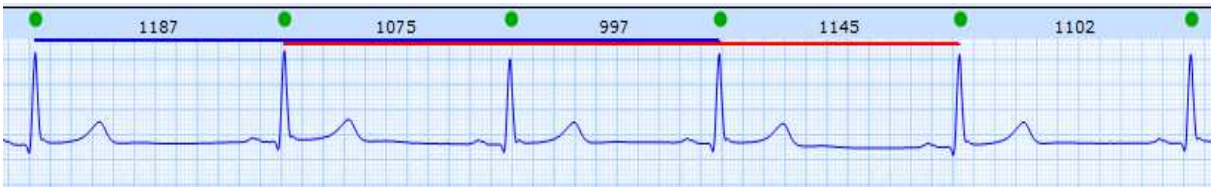

Figure 2 Variance in RR intervals

Table Time- and frequency domain HRV indices and their significance

Variable	Frequency band (Hertz)	Description
SDNN	-	Standard deviation of all normal RR intervals, reflects total variability, including circadian differences
HF	0.15-0.40	High frequency component, mainly controlled by parasympathetic activity
Normalized HF	-	HF/(TP-VLF); HF independent of total power
LF	0.04-0.15	Low frequency component, controlled by sympathetic and parasympathetic activity
Normalized LF	-	LF/(TP-VLF); LF independent of total power
VLF	0.0033-0.04	Very low frequency component, controlled by sympathetic and parasympathetic activity
TP	≤0.40	Variance of all RR intervals

Referring to European to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. SDNN = standard deviation of normal RR intervals; HF = high frequency; LF = low frequency; VLF = very low frequency; TP = total power

Time domain heart rate variability

All normal RR intervals are measured over the recording time and different measures can be derived based on those intervals. The standard deviation of all normal RR intervals (SDNN) is one of the most commonly used HRV variables for research proposes and, in contrast to frequency-domain variables, relatively easy to understand. SDNN is depending on the length of the ECG recording, hence it is important to be attentive regarding the comparability of absolute HRV values with other studies. Therefore it is an advantage to use standardized 24-h ECG monitorings.[43]

Frequency domain heart rate variability

Frequency domain HRV indices, measured in ms^2, contain information about the amount of variance in HR.[45,46] Differences in HR (RR intervals) are underlying certain oscillatory patterns. According to the frequency of these oscillations *(Figure 3, left)*, they are grouped in different frequency bands, such as high or low frequency. Mainly Fast Fourier Transformation is used to convert these signals to a spectral analysis, showing the power of all individual frequency bands *(Figure 3, right)*. The high frequency (HF) band is corresponding to a frequency of 0.15-0.40 Hertz (Hz) (i.e. 2.5 - 7 seconds), which is mainly modulated by parasympathetic activity. In contrast the low frequency (LF) band is defined as a frequency of

0.04-0.15 Hz (i.e. 7 - 25 seconds) and modulated by both, sympathetic and parasympathetic activity. Total power (TP) is corresponding to the total variance of all RR intervals.[43] LF and HF may be normalized in order to have values independent of TP.

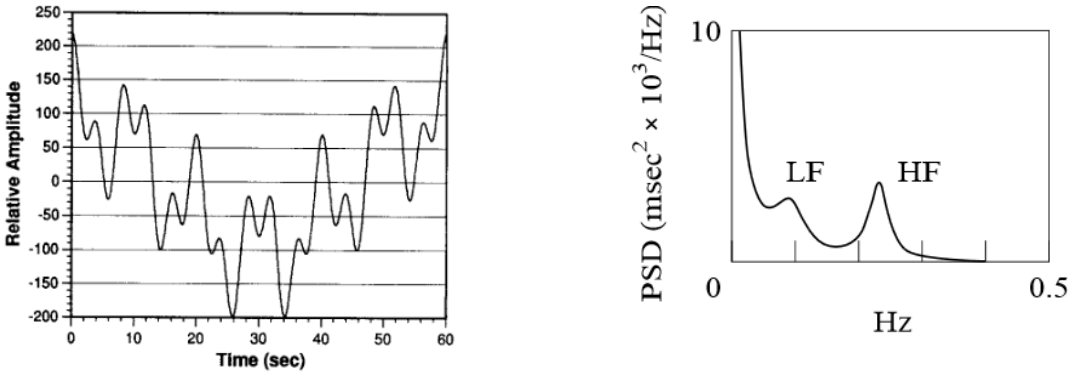

Figure 3 Periodic oscillations based on differences of the RR intervals (left) and spectral analysis of RR interval variability (right) (Stein et al., Am Heart J, 1994 and Task Force of the European Society of Cardiology, Circulation, 1996)

1.2.1 Heart rate variability and cardiovascular outcomes

Several studies found a relationship between short- and long-term HRV and adverse cardiovascular outcomes. Lower SDNN was significantly associated with an increased risk for adverse cardiac events in the general population.[47-50] Among patients with a history of myocardial infarction, having a SDNN <70ms was associated with an increased risk for future cardiovascular events.[51,52] Data from the ARIC (Atherosclerosis Risk in Communities) study indicated an increased risk for incident myocardial infarction and coronary heart disease among diabetic patients.[53] Moreover, HRV was associated with a higher incidence of heart failure.[54] However, even though there is a growing literature about HRV and cardiovascular outcomes, the precise underlying mechanisms of the relationship between the autonomic function and those outcomes are not fully understood. In addition, it remains unclear, to what extent HRV measures contain information independent of HR, as highlighted in a recent publication.[55]

1.2.2 Heart rate variability and healthy lifestyle

HRV linearly decreases with age.[56,57] Recent studies found associations of HRV with several cardiovascular risk and lifestyle factors.[58] Specifically, HRV was associated with smoking cigarettes,[59] physical activity,[60-62] T2DM,[63,64] hypertension[65,66] and BMI.[60] Using data of the ARIC study, Schroeder et al. found a 24% increased risk of hypertension among participants in the lowest SDNN quartile compared to participants in the highest SDNN quartile.[66] In the same cohort, patients with an impaired fasting glucose or a manifest T2DM had a significantly reduced HRV compared to participants with normal glucose levels.[63] These

results were adjusted in multivariable models and therefore independent of other known confounders. According to data of the SAPALDIA (Swiss Study on Air Pollution and Lung Disease in Adults) study, current smokers had a 4.5% lower SDNN compared to never smokers. Moreover, per 1-point increase in BMI, SDNN is lowered by 0.7%. In contrast, individuals performing regular physical activity had a 2% higher SDNN compared to physically inactive individuals.[58] This is in line with another study, showing a positive association between the time spent for physical activity and SDNN.[61]

Adopting a healthy lifestyle is highly promoted by associations and governments. In order to better target future campaigns, a better knowledge of the prevalence of healthy lifestyle adoption in a contemporary western European population is needed. Moreover, while some individual parameters have been related to autonomic function, it is currently unknown whether these cardiovascular health metrics in combination have an incremental effect on autonomic function. Finally, most prior studies have not taken into account the effect of HR, and it is therefore unknown whether HRV carries any incremental information beyond HR alone in this context.

1.2.3 Heart rate variability and sleep-related breathing disorders

Clinical studies assessing the relationship between sleep-related breathing disorders and HRV in general population are scarce. In a very small study, OSA patients had a significantly worse HRV profile and a higher HR compared to healthy controls.[67] Moreover, patients with moderate to severe OSA had significantly higher normalized LF and lower normalized HF compared to controls.[68,69] Another small study presented a negative association between normalized HF and the number of respiratory disturbances.[70]

Taken together, only few studies showed associations of HRV with overt OSA and population-based studies regarding this topic are rare. The relationship of HRV with preliminary stages of sleep-related breathing disorders is uncertain. Moreover, the prevalence of sleep-related breathing disorders in a young and healthy population is unknown. Again, the incremental information of HRV beyond resting HR is also unknown in this context.

1.2.4 Heart rate variability and inflammation

Up to now, some studies have assessed the relationship of mainly hs-CRP with HRV or HR mainly in elderly populations. In a previous study, hs-CRP was inversely associated with SDNN. Moreover, SDNN was a significant predictor for having high CRP levels, defined as the upper third of this middle-aged population.[71] These results were confirmed by another study, showing inverse associations of hs-CRP with LF, TP and SDNN.[72] The association between HRV and hs-CRP was not only found among healthy populations but also among

patients with coronary heart disease.[73] Lampert et al. found a significant correlation between HRV and IL-6, however this relationship did not persist using multivariable regression models.[74] Finally, there was a significant inverse correlation between time-domain HRV variables and total LC count.[75]

Inflammation is complex and consists of different inflammatory pathways and biomarkers. Regarding the relationship with HRV, information on inflammatory biomarkers other than hs-CRP is scarce. Inflammation is highly related to other cardiovascular risk factors. Therefore, an appropriate adjustment in well-characterized cohorts is needed. As well, the association of HRV and inflammatory biomarkers should be adjusted for HR to evaluate potential incremental information of HRV over HR.

1.2.5 Heart rate

Previous studies found strong associations of resting and ambulatory HR with an increased risk for cardiovascular outcomes.[76,77] Moreover, HR was found to be associated with some individual cardiovascular health metrics or lifestyle factors, individual inflammatory biomarkers and OSA.[68,78-80] Monfredi et al. have recently presented a tight inverse, but nonlinear link between HR and time-domain HRV *(Figure 4)*.[55] Based on data of the Framingham study, Tsuji et al. showed years ago similar inverse relationships with frequency-domain variables, such as LF and HF.[81] Based on this tight link, it is suggested to take HR into account when investigating associations with HRV, to assess relationships independent of HR. To date, this additional adjustment for HR was only done in very few studies and should therefore be better elaborated.

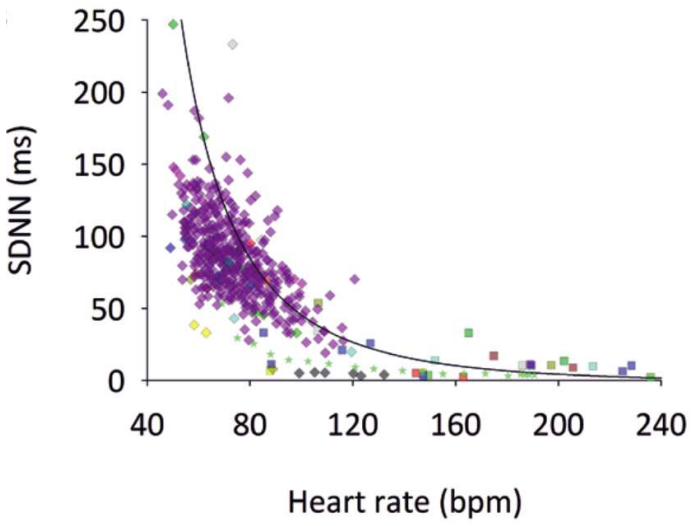

Figure 4 Relationship between SDNN and heart rate (Monfredi et al., Circulation, 2015)

2.AIMS AND OBJECTIVES

Based on the previously elucidated gaps in knowledge, the general aim of this study was to assess the relationship of HRV with lifestyle and lifestyle-related factors in young and healthy adults from the general population.

2.1 Healthy lifestyle and heart rate variability

The aim of this study was to assess the relationship of HRV with a validated lifestyle-score among young and healthy adults from the general population.

Objectives

1. To build a validated lifestyle-score based on seven lifestyle factors and health metrics.
2. To investigate the prevalence of a healthy lifestyle in the general population.
3. To assess the relationship of HR and HRV related parameters with a validated lifestyle-score.
4. To assess the incremental information of HRV with a validated lifestyle-score after adjustment for resting and ambulatory HR.
5. To investigate the relationship of every individual lifestyle-score component with the main HRV variable (i.e. SDNN).

Specific research questions

1. What is the prevalence of a healthy lifestyle among young men and women from a western European general population sample?
2. Is there a relationship between different HRV variables and a healthy lifestyle?
3. Is there a relationship between different HRV variables and a healthy lifestyle that is independent of resting or ambulatory HR?
4. Which components of the lifestyle-score are independently associated with HRV?

The results are presented in the manuscript ***Healthy lifestyle and heart rate variability in young adults,*** which has been published in the European Journal of Preventive Cardiology (see manuscript in Chapter 4 on page 17). This study was also presented at the Meeting of the European Society of Cardiology in 2015 in the Young Investigator Award Session for Population Science.

2.2 Heart rate variability and sleep-related breathing disorders

The aim of this study was to evaluate the association of HRV and sleep-related breathing disorders in a young population without known sleep apnea syndrome.

Objectives

1. To assess the prevalence of sleep-related breathing disorders in the general population without known sleep apnea syndrome.
2. To assess the relationship of HR and HRV related parameters with sleep-related breathing disorders.
3. To assess the relationship of ambulatory HR with AHI and ODI.
4. To assess the incremental information of HRV with AHI, ODI and sleep-related breathing disorders after the additional adjustment for either resting and ambulatory HR.

Specific research questions

1. What is the prevalence of sleep-related breathing disorders in young and healthy adults from the general population?
2. Is there an association of different HRV variables with sleep-related breathing disorders?
3. Is resting and ambulatory HR associated with sleep-related breathing disorders?
4. Is there a relationship between different HRV variables and sleep-related breathing disorders that is independent of resting or ambulatory HR?

The results are presented in the manuscript ***Heart rate variability and sleep-related breathing disorders in the general population***, which has been submitted to the "American Journal of Cardiology". This analysis was presented as Poster at the Meeting of the American Heart Association (AHA) 2015 in Orlando, USA. Please find this manuscript in chapter 5 on page 33.

2.3 Heart rate, heart rate variability and inflammatory biomarkers

The aim of this study was to evaluate the association between the HRV and inflammatory biomarkers in a young and healthy population.

Objectives

1. To assess the relationships of HR and HRV related variables with a panel of different inflammatory biomarkers.
2. To assess the incremental information of HRV with inflammatory biomarkers after the adjustment for ambulatory HR.

Specific research questions

1. Is there a relationship of different HRV variables with hs-CRP, LC, neutrophils, lymphocytes and monocytes?
2. Is there a relationship between ambulatory HR and inflammatory biomarkers?
3. Is there a relationship between different HRV variables and inflammatory biomarkers that is independent of HR?

The results of this analysis are presented in the manuscript ***Heart rate, heart rate variability and inflammatory biomarkers among young and healthy adults***, which has been submitted to the journal “Annals of Medicine”. Please find the manuscript in chapter 6 on page 57.

3.STUDY DESIGN AND METHODS

3.1 Design and Participants

This thesis is based on data of the *Genetic and Phenotypic Determinants of Blood Pressure and other Cardiovascular Risk Factor* (GAPP) study, a prospective, population-based cohort study. Between 2010 and 2013, all (n=6887) inhabitants of the Principality of Liechtenstein aged 25 to 41 years were invited to participate in this study. Based on cooperation with the health department in the Principality of Liechtenstein we had the possibility to write to all inhabitants in our age range. Of them, 84% were eligible and could be contacted by phone. Overall, 2170 individuals agreed to participate and were included in GAPP. Study recruitment is presented in *Figure 5*. The local ethics committee approved the study protocol. A written informed consent was obtained from every participant. We have published the detailed study methodology previously.[82]

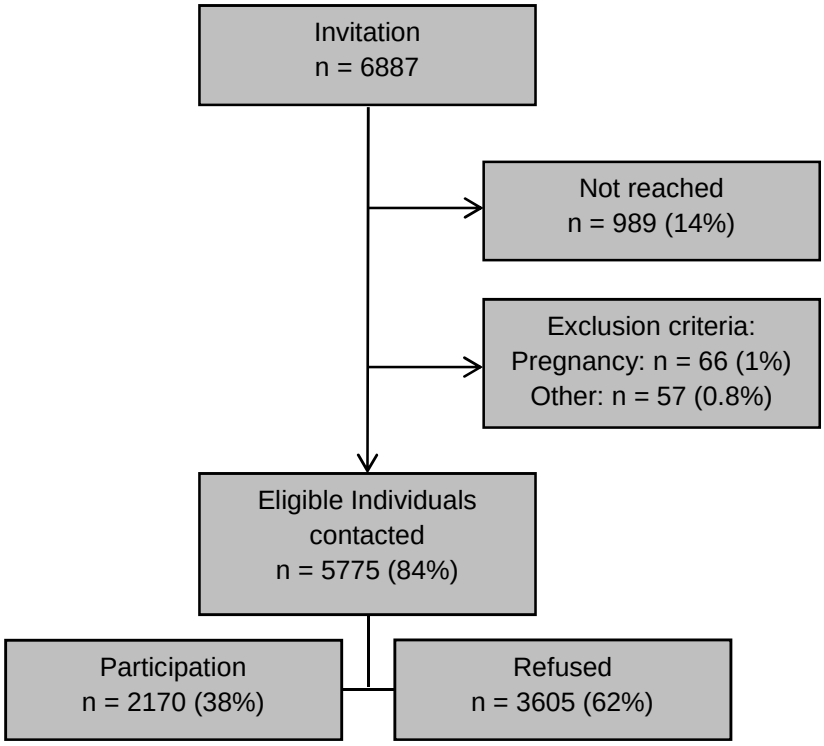

Figure 5 Recruitment of the GAPP-study

Inclusion criteria:

- Inhabitants of the Principality of Liechtenstein
- Aged between 25 to 41 years

Exclusion criteria:

- Any established CVD, such as coronary artery disease, peripheral artery disease, congestive heart failure, significant arrhythmia or history of stroke
- Known OSA
- Renal disease or renal failure
- BMI>35 kg/m^2
- Current intake of antidiabetic drugs or insulin
- Regular intake of steroids, nonsteroidal anti-inflammatory drugs and sympathomimetic drugs
- Current pregnancy
- Intention to leave the Principality of Liechtenstein on a permanent basis

3.2 Study Procedures

Study enrollment was managed by trained study nurses in the local study center in Schaan, Principality of Liechtenstein. All study related procedures were performed in a highly standardized manner.

3.2.1 24-hour electrocardiogram

24-h ECG was performed in every participant using a validated three-channel device (AR12plus, Schiller AG, Baar, Switzerland). The device was started in the morning at the end of the study investigation. If the recording quality was low or the recording duration was <80% of the maximal duration (24 hours), participants were asked to repeat the ECG recording. Data were imported into a dedicated software (MedilogDARWIN V2, Schiller AG, Baar, Switzerland) and systematically edited in order to remove artefacts and redefine premature atrial or ventricular beats. Ambulatory HR was calculated automatically over 24 hours. Time- and frequency domain variables that were used for this study included SDNN, TP, LF and HF.

3.2.2 Assessment of lifestyle factors and other variables

Personal, medical, lifestyle and nutritional factors were assessed using standardized questionnaires. Age was calculated as the difference between inclusion date and birthday. Highest educational level achieved or current educational training was assessed. Smoking status was self-reported and categorized into current, former or never smoking. Physical activity was defined using the International Physical Activity Questionnaire (IPAQ)[83] and dietary habits were assessed using the official questionnaire of the Federal Office of Public Health (Swiss health survey, 2007). The frequency of alcohol consumption was self-reported from a validated questionnaire. Weight and height were measured in a standardized way with validated devices (Seca, Hamburg, Germany). Conventional systolic and diastolic blood pressure were measured three times in a sitting position after five minutes of rest before and with one minute intervals between the measurements using a validated device (Microlife, BP3AG1, Taipei, Taiwan). Resting HR was obtained using a 12-channel Resting ECG over 10 seconds. Bioelectrical impedance analysis was used to assess body composition (BIA egofit, Eggstätt, Germany).

3.2.3 Blood sampling

Fasting venous blood samples were obtained from every participant by venipuncture and immediately processed in an accredited laboratory. High-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine and hs-CRP were assayed on a Roche Cobas (Roche, Basel, Switzerland) analyzer and glycated hemoglobin A_{1c} (HbA_{1c}) was quantified using high performance liquid chromatography (Bio-Rad D-10, Bio-Rad Laboratories AG, Cressier, Switzerland). White blood cell count, such as leukocytes, neutrophils, lymphocytes and monocytes were quantified using a validated method (Sysmex XE 5000, Sysmex, Japan).

3.2.4 Nighttime pulse oximetry and nasal airflow measurement

Participants obtained a nocturnal pulse oximetry with nasal airflow measurement using a validated device (Apnea Link, Resmed, San Diego, USA). Recording was started by the participant before falling asleep and was stopped when waking up in the morning. Recording had to be at least >180minutes for both pulse oximetry and nasal airflow measurement, otherwise the recording was repeated whenever possible. Oxygen saturation was measured on the forefinger using a pulse oximetry device and nasal airflow was detected with a nasal cannula. AHI and ODI were defined according official guidelines.[14] AHI was defined as the average number of apnea and hypopnea episodes per hour of sleep. An apnea was defined as a nasal airflow reduction of at least 80% during ≥10 seconds. A hypopnea was defined as a nasal airflow reduction of ≥30% with a concomitant decrease in oxygen saturation of ≥4%.

ODI was defined as the mean number of oxygen desaturations of ≥4% per hour of recording.[14]

3.3 Statistical analysis

For all analyses, distribution of continuous variables was checked using skewness, kurtosis and visual inspection of the histogram. Baseline characteristics were presented as means ± standard deviations (SD), medians (interquartile ranges) or numbers (percentages), as appropriate. According the distribution of continuous variables, group comparisons were done using unpaired t-tests or Wilcoxon rank sum tests. Dichotomous variables were compared using Chi-square tests.

The relationships of HRV with lifestyle, sleep-related breathing disorders and inflammation were assessed using HRV variables as the outcome variables. All relationships were checked for linearity using categories of the independent variables. P for trend was calculated using category-specific medians. To have a better comparability of the estimates, HRV variables were transformed to z-scores, calculating (HRV – HRV population mean)/ SD. All linear regression models were adjusted for a broad set of potential confounders. Separate regression models were constructed with additional adjustment for HR. Analysis-specific statistical methods are explained in the manuscripts (chapter 3-6).

A p-value of <0.05 was pre-specified as level of statistical significance. All statistical analyses were performed using the software SAS version 9.4 (SAS Institute Inc, Cary, North Carolina, USA).

4.MANUSCRIPT 1 – Healthy lifestyle and HRV

Healthy lifestyle and heart rate variability in young adults

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Healthy lifestyle and heart rate variability in young adults

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Abstract

Background: We aimed to determine the association of a comprehensive healthy lifestyle with heart rate variability (HRV), a validated measure of autonomic function.

Design: This was a prospective cohort study.

Methods: A population-based sample of 2079 individuals aged 25–41 years without prevalent cardiovascular disease was investigated. The standard deviation of all normal RR intervals (SDNN) during 24-hour electrocardiography was used as main HRV marker. Healthy lifestyle metrics were summed to a validated lifestyle-score ranging from 0 = most unhealthy to 7 = most healthy. One point was given for each of the following items: never smoking cigarettes; consuming a healthy diet; performing moderate (≥150 min/week) or vigorous (≥75 min/week) physical activity; body mass index (BMI) <25 kg/m^2; total cholesterol <200 mg/dl; glycated haemoglobin A1c <5.7%; and blood pressure <120 (systolic) and <80 mm Hg (diastolic).

Results: Median age of the participants (47% males) was 37 years. Mean SDNN was 153 ms and median lifestyle-score was four. A score of 0/1 or 6/7 was found in 5.2% and 11.0%, respectively. In multivariable linear regression analysis with SDNN as the outcome variable, the β-estimate (95% confidence interval (CI)) for a one-point increase of the lifestyle-score was 0.14 (0.11–0.17), $p < 0.0001$. This relationship was attenuated but remained significant after additional adjustment for resting heart rate (HR) (β-estimate (95% CI) 0.07 (0.07–0.10), $p < 0.0001$) or 24-hour HR (0.04 (0.01–0.07), $p = 0.003$).

Conclusions: Few individuals adopted a healthy lifestyle in this large contemporary cohort of young adults from the general population. Adopting a healthy lifestyle has an important effect on autonomic function.

Keywords
Heart rate, heart rate variability, lifestyle, population-based

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Introduction

Cardiovascular risk factors and their consequences are among the leading causes of disability and death around the world.[1] A healthy lifestyle is a key component of effort to improve cardiovascular risk factors and to reduce the occurrence of cardiovascular events,[2–4] such that ideal health metrics are strongly recommended by governments and professional societies.[5,6]

The autonomic nervous system plays an important role in the physiological regulation of the

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cardiovascular system.[7] Heart rate variability (HRV) has become an easily measurable and validated marker to measure cardiac autonomic function and to quantify imbalances of the autonomic nervous system.[8] Several studies have shown that a reduced HRV is associated with an increased risk of developing cardiovascular events.[9–12]

Thus, autonomic function may be an important mediator of the beneficial effects of a healthy lifestyle. HRV is associated with several individual cardiovascular risk factors.[13] Specifically, prior studies have shown that smoking cigarettes,[14] reduced physical activity,[15,16] type 2 diabetes mellitus[17] and hypertension[18] are associated with a lower HRV. However, it is currently unknown whether these factors have an incremental effect on the autonomic nervous system. The incremental effect of every additional healthy lifestyle factor on cardiovascular outcomes highlights the relevance of a composite score.[2] Finally, it is unclear to what extent the prognostic information of HRV is reflected by heart rate (HR) alone, which by itself is an independent cardiovascular risk factor[19,20] and tightly linked to HRV.[21]

In order to address these issues, we assessed the prevalence of a healthy lifestyle and its relationships with 24-hour HRV using a previously validated lifestyle-score, which is based on seven lifestyle factors and cardiovascular health metrics.[2] This analysis was realised in a large contemporary cohort of young and healthy adults from the general population.

Methods

Study participants

All 5898 inhabitants of the Principality of Liechtenstein aged between 25–41 years were contacted to participate in the 'Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors' (GAPP) study. Of these, 3605 (61%) refused to participate, 66 (1%) women were excluded due to pregnancy and 57 (1%) potentially interested individuals had at least one exclusion criterion, such that 2170 (37%) participants could be enrolled in this study. The exact study methodology has been published previously.[22] Main exclusion criteria were a history of cardiovascular disease, sleep apnoea syndrome, renal failure, current intake of antidiabetic drugs, other severe co-morbidities, or a body mass index (BMI)>35 kg/m^2. Of the 2170 participants that were included in GAPP, we excluded 91 participants from this analysis due to missing 24-hour Holter electrocardiogram (ECG) recordings ($n=20$), Holter ECG monitoring duration<80% ($n=22$), missing health metrics ($n=26$), missing covariates ($n=21$) and intake of beta-blockers ($n=2$), leaving 2079 participants for the current analysis. The study protocol was approved by the local ethics committee, and informed written consent was obtained from each participant.

Assessment of HR variability

Holter ECG monitoring during 24 hours was obtained in every participant using a validated three-channel device (AR12plus, Schiller AG, Switzerland). If the monitoring time was <80% of the target time (i.e. <19.2 h) or recording quality was low, the Holter study was repeated whenever possible. All Holter ECG studies were post-processed by a trained study collaborator using dedicated software (Medilog Darwin V2, Schiller AG, Switzerland), including complete removal of all artefacts and exact definition of ventricular and atrial premature beats. The mean ambulatory 24-hour HR was automatically calculated. All heart beats defined as normal were used to quantify HRV. For the present analysis we used the standard deviation of all normal RR intervals (SDNN) as a time-domain variable, as well as the frequency domain variables high frequency (HF, 0.15–0.40 Hz) and low frequency (LF, 0.04–0.15 Hz).

Assessment of other study variables

Information about personal, medical, nutritional and lifestyle factors were obtained using standardised questionnaires.[22] Smoking status was self-reported as current, former or never smoker. Nutritional factors and dietary habits were obtained using the Swiss health survey questionnaire from 2007. Sodium excretion was quantified from fasting morning urinary samples and sodium intake was estimated using the Kawasaki-formula.[23] The frequency and amount of alcohol consumption was assessed and transformed into grams of alcohol consumed per day. Highest educational level achieved was classified into three groups (high school, college degree and university degree). Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ).[24] Weight and height were directly measured in a standardised manner by trained study nurses and BMI calculated as body weight (kg) divided by height (m^2). Systolic and diastolic blood pressures were measured three times on the non-dominant arm in a sitting position with one minute of rest in between (Microlife BP3AG1, Switzerland). The mean of the second and third measurement was used for analysis. Resting HR was obtained from a 12-lead ECG conducted during the same study visit. Glycated haemoglobin A_{1c} (HbA_{1c}) and total cholesterol levels were directly measured from fasting venous blood samples as previously described.[22]

Lifestyle score

We used a validated score consisting of seven lifestyle factors and cardiovascular health metrics. This score has previously been shown to strongly predict cardiovascular and all-cause mortality in the general population.[2] Using the same definitions and cut-off points, we summed healthy lifestyle habits to a lifestyle score with a scale from 0 = most unhealthy to 7 = most healthy.

One point was given for the following characteristics: never smoking cigarettes, consuming a healthy diet, performing moderate (≥150 min/week) or vigorous (≥75 min/week) physical activity, BMI<25 kg/m^2, total cholesterol <200 mg/dl, HbA_{1c}<5.7% and systolic and diastolic blood pressure<120 and 80 mm Hg, respectively, without using antihypertensive treatment. Because of the importance of low-density lipoprotein cholesterol (LDL-C) as a cardiovascular risk factor,[25] a modified lifestyle score was constructed by substituting total cholesterol with LDL-C, using a cut point of 160 mg/dl. A healthy diet was defined as accomplishing at least two out of the three following components: consuming ≥5 servings of fruits and vegetables per day, consuming ≥2 servings of fish per week, or consuming <1500 mg of sodium per day.

Statistical analysis

Baseline characteristics were stratified by sex. Skewness, kurtosis and visual inspection of the histogram were used to evaluate the distribution of continuous variables. Normally distributed variables were presented as mean ± standard deviation, skewed variables as median (interquartile range). Count data are given as numbers (percentages).

To assess the relationship between HRV and lifestyle-score categories, separate multivariable linear regression models were constructed using SDNN, HF and LF as the outcome variables. Due to low numbers in the extreme lifestyle-score categories, we collapsed participants with either 0–1 points or 6–7 points in single categories. Due to group size, participants with a lifestyle score of four were chosen as the reference group. HF and LF variables were log-transformed for analysis to improve the normality of their distributions. In order to improve comparability of the associations across the three HRV variables, these variables were transformed into z-scores. Due to the approximately linear relationships between HRV variables and lifestyle-score categories, we constructed another series of multivariable regression models using the lifestyle score as a continuous variable. As sensitivity analyses, these regression analyses were repeated using the modified lifestyle score explained above. All regression models were first adjusted for age, sex, educational status, alcohol consumption and family history of cardiovascular disease. We then additionally adjusted these models for either resting or ambulatory HR in separate steps.

Pre-specified subgroup analyses were done across strata of age, sex, educational status and each individual lifestyle component. Differences across strata were assessed using multiplicative interaction terms in the non-stratified models. We also assessed the relationships of HRV parameters with each individual component of the lifestyle score in a combined model. Finally, we evaluated whether resting and ambulatory HR were associated with the lifestyle score using the same series of linear regression models detailed above. SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) was used for all statistical analysis. A p-value < 0.05 was pre-specified to indicate statistical significance.

Results

Baseline characteristics stratified by sex are presented in Table 1. Compared to women, men had a higher BMI (26.0 versus 23.4 kg/m^2), were more often current smokers (25.0% versus 18.8%) and had higher systolic and diastolic blood pressure values (all p-values < 0.0001). Resting and ambulatory HRs were significantly higher in women compared to men (64 versus 60 bpm, $p < 0.0001$, and 78 versus 73 bpm, $p < 0.0001$, respectively). SDNN and LF among men and women were 160 versus 147 ms and 1337 versus 884 ms^2, respectively (all p-values < 0.0001). HF did not differ between men and women (289 versus 274 ms^2, $p = 0.18$).

The prevalence of individual lifestyle-score components is also shown in Table 1. Among men and women, a BMI<25 kg/m^2 was found in 43% and 73%, $p < 0.0001$, respectively. Men exercised more often (83% versus 79%, $p = 0.009$), but women had a higher prevalence of ideal cholesterol and blood pressure levels and a lower prevalence of prediabetes (all p-values < 0.0003). The distribution of participants across lifestyle-score categories is presented in Figure 1. Overall, 107 participants (88% male) had a lifestyle score of 0 or 1 and 229 participants (13% male) had a lifestyle score of 6 or 7. The median lifestyle score was four (interquartile range 3–5).

Relationship of lifestyle score with HRV and HR

Results of the multivariable regression models assessing the associations between HRV parameters and lifestyle-score categories are presented in Table 2. We observed a strong positive relationship that was similar for all three HRV parameters assessed (each p for trend < 0.0001). Including resting HR in the models attenuated these relationships, but all remained statistically

Table 1. Baseline characteristics stratified by sex.

	Male $n = 972$ (46.8%)	Female $n = 1107$ (53.2%)	p-Value[a]
Age, years	37 (31–40)	37 (31–40)	0.20
Body mass index, kg/m^2	26.0 ± 3.3	23.4 ± 3.8	<0.0001
Smoking, %			0.0003
Current	243 (25.0)	208 (18.8)	
Past	238 (24.5)	249 (22.5)	
Never	491 (50.5)	650 (58.7)	
Systolic BP, mm Hg	127.8 ± 11.2	113.5 ± 10.2	<0.0001
Diastolic BP, mm Hg	82.4 ± 8.2	74.9 ± 7.9	<0.0001
LDL-C, mmol/l	3.28 ± 0.9	2.72 ± 0.7	<0.0001
HDL-C, mmol/l	1.32 ± 0.3	1.72 ± 0.4	<0.0001
HbA_{1c}, %	5.43 ± 0.4	5.38 ± 0.4	0.008
Education, %			<0.0001
High school	64 (6.6)	106 (9.6)	
College	484 (49.8)	648 (58.5)	
University	424 (43.6)	353 (31.9)	
Heart rate variability and heart rate			
SDNN (ms)	160 ± 40	147 ± 36	<0.0001
High frequency (ms^2)	289 (164–511)	274 (159–475)	0.18
Low frequency (ms^2)	1337 (914–1921)	884 (604–1285)	<0.0001
Heart rate, resting (bpm)	60.2 ± 9.1	63.6 ± 9.2	<0.0001
Heart rate, ambulatory (bpm)	72.7 ± 8.8	77.9 ± 7.4	<0.0001
Health metrics for lifestyle score			
BMI<25 kg/m^2 (%)	413 (42.5)	813 (73.4)	<0.0001
Never smoking (%)	491 (50.5)	650 (58.7)	0.0002
Regular physical activity (%)	808 (83.1)	870 (78.6)	0.009
Healthy diet (%)	34 (3.5)	53 (4.8)	0.14
Healthy cholesterol level (%)	538 (55.4)	825 (74.5)	<0.0001
Healthy blood pressure level (%)	174 (17.9)	730 (65.9)	<0.0001
Healthy HbA_{1c} (%)	705 (72.5%)	878 (79.3)	0.0003

BMI: body mass index; BP: blood pressure; HbA_{1c}: glycated hemoglobin A1c; HDL-C: high density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SDNN: standard deviation of all normal RR-intervals.

Data are means ± standard deviations, medians (interquartile range) or numbers (percentage). Regular physical activity: moderate physical activity ≥75 or vigorous physical activity ≥150 min per week. Healthy diet≥5 fruit or vegetable servings per day + ≥2 servings of fish per week + salt consumption. Healthy cholesterol level<200 mg/dl. Healthy blood pressure: systolic and diastolic blood pressure<120 mm Hg and 80 mm Hg without treatment. Prediabetes: HbA_{1c}>5.6%.

[a]p-Value based on Students t-test, Wilcoxon rank sum test or Chi square test.

significant. Further attenuation was observed when the models were adjusted for ambulatory instead of resting HR, as shown in Table 2. In analyses using the lifestyle score as a continuous variable, β coefficients (95% confidence interval (CI)) per one point increase in lifestyle score were 0.14 (0.11–0.17) for SDNN, 0.12 (0.08–0.15) for log-transformed HF and 0.12 (0.09–0.15) for log-transformed LF (all $p < 0.0001$). These results were again attenuated after additional adjustment for resting or ambulatory HR, as shown in Supplementary Material, Figure S1. Similar results were observed when LDL-C was used as a lifestyle-score component instead of total cholesterol (Supplementary Material, Table S1). Subgroup analyses for the relationship between SDNN and lifestyle score showed consistent results across all strata (Supplementary Material, Table S2).

Resting and ambulatory HR were significantly associated with the lifestyle score, as shown in Table 3. In multivariable adjusted models, β coefficients for resting and ambulatory HR per one point increase in the score were −1.43 (−1.74–−1.11), $p < 0.0001$ and −1.22 (−1.47–−0.98), $p < 0.0001$, respectively.

The relationships between individual lifestyle components and HRV as measured by SDNN are shown in Supplementary Material, Table S3. Statistically

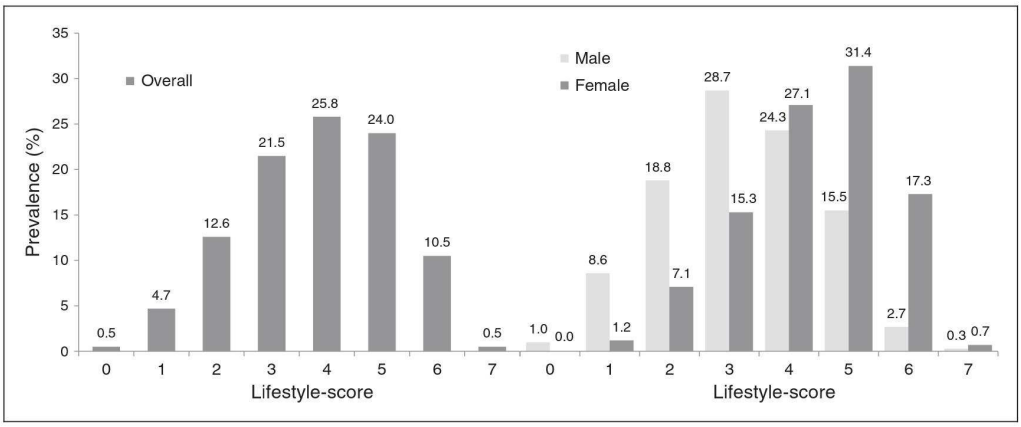

Figure 1. Prevalence of individual lifestyle-score categories.

Table 2. Relationship between variables of the heart rate variability and lifestyle score.

$n = 2079$	Score = 0–1 ($n = 107$)	Score = 2 ($n = 261$)	Score = 3 ($n = 448$)	Score = 4 ($n = 536$)	Score = 5 ($n = 498$)	Score = 6–7 ($n = 229$)	p for trend
	SDNN (ms)						
Model 1	−0.31 (−0.51– −0.11)	−0.28 (−0.42–−0.14)	−0.09 (−0.21–0.03)	Ref.	0.22 (0.11–0.34)	0.29 (0.14–0.44)	<0.0001
Model 2	−0.10 (−0.28–0.08)	−0.18 (−0.31–−0.05)	−0.05 (−0.15–0.06)		0.11 (0.003–0.21)	0.15 (0.02–0.29)	<0.0001
Model 3	0.05 (−0.12–0.22)	−0.13 (−0.25–−0.01)	−0.03 (−0.13–0.07)		0.14 (0.04–0.24)	0.10 (−0.03–0.23)	0.003
	High frequency (ms^2)						
Model 1	−0.35 (−0.54– −0.15)	−0.18 (−0.32–−0.04)	−0.13 (−0.25– −0.01)	Ref.	0.14 (0.03–0.26)	0.28 (0.13–0.43)	<0.0001
Model 2	−0.16 (−0.34– −0.02)	−0.09 (−0.22–0.04)	−0.09 (−0.20–0.01)		0.04 (−0.06–0.14)	0.16 (0.02–0.29)	0.0001
Model 3	0.03 (−0.14–0.19)	−0.03 (−0.15–0.09)	−0.07 (−0.17–0.03)		0.06 (−0.04–0.15)	0.08 (−0.04–0.21)	0.05
	Low frequency (ms^2)						
Model 1	−0.36 (−0.55– −0.17)	−0.21 (−0.34–−0.08)	−0.16 (−0.28 −0.05)	Ref.	0.13 (0.02–0.24)	0.26 (0.12–0.40)	<0.0001
Model 2	−0.17 (−0.35– −0.02)	−0.12 (−0.24–0.00)	−0.13 (−0.23– −0.02)		0.02 (−0.08–0.12)	0.14 (0.01–0.26)	<0.0001
Model 3	−0.03 (−0.20–0.13)	−0.08 (−0.19–0.04)	−0.11 (−0.21– −0.01)		0.05 (−0.04–0.15)	0.09 (−0.04–0.21)	0.003

ms: millisecond; Ref.: reference; SDNN: standard deviation of all normal RR intervals.
Values are β coefficient (95% confidence interval). SDNN, high and low frequency were transformed to z-scores. Model 1: adjusted for age, sex, educational status, alcohol consumption and family history for cardiovascular disease. Model 2: additionally adjusted for resting heart rate. Model 3: additionally adjusted for ambulatory heart rate instead of resting heart rate.

significant associations were observed for normal BMI (β for SDNN 0.25 (0.16–0.34), $p < 0.0001$), never smoking cigarettes (β for SDNN 0.13 (0.04–0.21), $p = 0.003$) and regular physical activity (β for SDNN 0.30 (0.20–0.41), $p < 0.0001$).

Discussion

In this large population-based study of young and healthy adults, a healthy lifestyle was strongly and consistently associated with a higher HRV. This relationship persisted after comprehensive multivariable adjustment, but was attenuated when resting or ambulatory HR was added to the models. These findings suggest that a healthy lifestyle may have an important positive effect on the autonomic nervous system and that this effect can already be observed in young and healthy individuals. We suggest that this may be one mechanism by which a healthy lifestyle reduces the occurrence of cardiovascular events. We are not aware of other large scale population-based studies that have comprehensively assessed the potential effect of a healthy lifestyle on the autonomic nervous system.

Unfortunately, a comprehensive healthy lifestyle was adopted by only a small minority of participants. As shown in Figure 1, only 3% of men and 18% of women had a lifestyle score of six or seven. These findings are consistent with earlier studies from the USA, including the fact that women had a higher lifestyle score compared to men.[2,3] While our study population has a very low short-term cardiovascular risk, the high penetrance

Table 3. Relationship of heart rate with lifestyle-score categories.

$n = 2079$	Score = 0–1 ($n = 107$)	Score = 2 ($n = 261$)	Score = 3 ($n = 448$)	Score = 4 ($n = 536$)	Score = 5 ($n = 498$)	Score = 6–7 ($n = 229$)	p for trend
	Ambulatory heart rate						
Model 1	6.03 (4.27–7.79)	2.43 (1.19–3.67)	0.98 (−0.06–2.03)	Ref.	−1.41 (−2.43– −0.40)	−3.16 (−4.47– −1.85)	<0.0001
Model 2	4.58 (3.09–6.08)	1.14 (0.08–2.20)	0.56 (−0.33–1.45)		−0.38 (−1.24–0.49)	−1.81 (−2.93– −0.70)	<0.0001
	Resting heart rate						
Model 1	4.32 (2.41–6.23)	2.24 (0.89–3.59)	0.81 (−0.33–1.94)	Ref.	−2.44 (−3.54– −1.33)	−2.81 (−4.24– −1.39)	<0.0001
Model 2	2.95 (1.25–4.65)	0.98 (−0.23–2.19)	0.43 (−0.59–1.44)		−1.46 (–2.44– −0.47)	−1.54 (−2.81– −0.27)	<0.0001

Ref: Reference.

Values are β coefficients (95% confidence interval) adjusted for age, sex, educational status, alcohol consumption and family history for cardiovascular disease. Model 1 was adjusted for age, sex, education, family history of cardiovascular disease and alcohol consumption. Model 2 was additionally adjusted for the standard deviation of all normal RR intervals (SDNN).

of an unhealthy lifestyle may be one explanation for the very high lifetime risk of cardiovascular disease in the population.[26,27] Thus, our results imply that promoting a healthy lifestyle should remain a key issue on the agenda of professional societies and governments, and that these efforts should also target young individuals.

The HRV displays the beat-to-beat variation of the sinus node, which is modulated by the autonomous nervous system. An experimental study has shown that HF is predominantly modulated by vagal activity,[28] whereas LF is influenced by both systems.[8,28] The positive relationships with HF, LF and SDNN found in our study may therefore suggest increased vagal and decreased sympathetic activity among participants adopting a healthy lifestyle. However, prospective and interventional studies are needed to test this hypothesis.

Thus, the exact mechanisms that connect an unhealthy lifestyle with the autonomic nervous system remain unclear. Our study suggests that smoking, physical activity and obesity may be particularly important, given the individual associations of these factors with HRV. Prior studies suggest that regular cigarette consumption leads to an impaired baroreceptor sensitivity, which may result in autonomic dysfunction due to a deficient negative feedback-loop.[29] Further mechanisms related to oxidative stress and nicotinic acetylcholine receptors have also been suggested.[30] One reason for the favourable effect of regular physical activity on autonomic function[15] may include exercise-induced structural and functional adaptions of the cardiovascular system.[31] In general, HRV is lower in overweight and obese people compared to normal weight people. There is some evidence that body fat mass, especially visceral fat mass is independently associated with HRV,[32,33] but the precise underlying mechanisms remain unclear.

Similar to HRV, both resting and 24-hour ambulatory HR were significantly associated with lifestyle-score categories. Adjusting the HRV-relationship for HR attenuated but did not abolish the relationship with lifestyle. Similar to HRV, HR is also a well-established marker of autonomic function, and an increased HR is strongly associated with a higher incidence of cardiovascular events and death.[19,20] Our findings suggest that HR captures some but not all information contained in HRV. A recent study showed that the HRV is indivisibly linked to HR, meaning that decreasing HR is automatically linked with increasing HRV.[21] Future studies are needed to assess whether the incremental value of HRV over HR might be smaller than initially thought.

Strengths and limitations

Strengths of this study included the large sample size, the population-based design and the well-characterised young and healthy population. In addition, 24-hour ECG monitoring was available in all participants, allowing for reliable quantification of HRV. Several potential limitations should also be taken into account in the interpretation of our findings. First, the study population was mainly white and the generalisability of the study results is uncertain. Second, this was a cross-sectional analysis and it is not possible to draw any conclusions about the causality of the results. Third, a BMI>35 kg/m^2 was an exclusion criterion for GAPP. Assuming an unhealthy lifestyle in strongly obese individuals, the prevalence of an unhealthy lifestyle may therefore be underestimated in this study. Moreover, the association between the lifestyle and HRV might even be stronger without BMI restrictions. Fourth, assessing an individual's diet is challenging. Although we used a validated questionnaire, we cannot exclude the presence of measurement error. In addition, not all diet criteria of the previously validated lifestyle score were available in our study, such that we had to slightly modify the healthy diet criterion.

However, we expect that if anything these two factors may have biased our findings towards the null hypothesis.

Conclusion

Our study shows that a comprehensive healthy lifestyle is poorly adopted in a large contemporary cohort of young adults from the general population. In this study, adopting a healthy lifestyle was associated with a greater HRV. These findings are in agreement with the importance of promoting a healthy lifestyle. We also found that additional adjustment for ambulatory HR strongly attenuated these relationships, suggesting that HR might explain a substantial part of the association between a healthy lifestyle and HRV.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplement

Healthy lifestyle and heart rate variability in young adults

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Figure legend

Figure S1: Relationship between heart rate variability and lifestyle-score

Data are β coefficients (95% confidence interval) per 1-category increase in the lifestyle-score. SDNN, High and low frequency were transformed to z-scores. Model 1: adjusted for age, sex, educational status, alcohol consumption and family history for cardiovascular disease. Model 2: additionally adjusted for resting heart rate. Model 3: additionally adjusted for ambulatory heart rate instead of resting heart rate. SDNN = standard deviation of all normal RR intervals; ms = millisecond;

Table S1 Relationship between variables of the heart rate variability and a modified lifestyle-score

N=2079	Score = 0-1 (n=62)	Score = 2 (n=198)	Score = 3 (n= 382)	Score = 4 (n= 585)	Score = 5 (n= 571)	Score = 6-7 (n= 281)	p for trend
			SDNN (ms)				
Model 1	-0.40 (-0.65; -0.15)	-0.20 (-0.35; -0.05)	-0.10 (-0.22; 0.02)		0.24 (0.13; 0.35)	0.35 (0.21; 0.48)	<0.0001
Model 2	-0.16 (-0.39; 0.06)	-0.08 (-0.22; 0.06)	-0.03 (-0.14; 0.08)	Ref.	0.15 (0.05; 0.25)	0.20 (0.08; 0.32)	<0.0001
Model 3	0.07 (-0.14; 0.30)	-0.03 (-0.16; 0.10)	-0.02 (-0.12; 0.09)		0.16 (0.06; 0.24)	0.16 (0.04; 0.27)	0.001
			High Frequency (ms^2)				
Model 1	-0.51 (-0.75; -0.26)	-0.11 (-0.26; 0.04)	-0.12 (-0.24; -0.00)		0.18 (0.07; 0.29)	0.27 (0.14; 0.41)	<0.0001
Model 2	-0.30 (-0.52; -0.07)	0.003 (-0.13; 0.14)	-0.06 (-0.17; 0.05)	Ref.	0.10 (-0.00; 0.20)	0.15 (0.02; 0.27)	0.0002
Model 3	0.03 (-0.23; 0.18)	0.06 (-0.06; 0.19)	-0.04 (-0.14; 0.06)		0.09 (-0.00; 0.18)	0.08 (-0.04; 0.19)	0.12
			Low Frequency (ms^2)				
Model 1	-0.58 (-0.81; -0.34)	-0.12 (-0.26; 0.03)	-0.15 (-0.27; -0.03)		0.18 (0.07; 0.28)	0.26 (0.13; 0.40)	<0.0001
Model 2	-0.37 (-0.59; -0.16)	-0.009 (-0.14; 0.12)	-0.09 (-0.20; 0.01)	Ref.	0.10 (0.002; 0.19)	0.14 (0.02; 0.25)	<0.0001
Model 3	-0.16 (-0.36; 0.05)	0.03 (-0.09; 0.16)	-0.08 (-0.18; 0.02)		0.10 (0.01; 0.19)	0.09 (-0.02; 0.21)	0.003

Values are β coefficient (95% confidence interval). SDNN, High and low frequency were transformed to z-scores. Low-density lipoprotein cholesterol was added to the score instead of total cholesterol. Model 1: adjusted for age, sex, educational status, alcohol consumption and family history for cardiovascular disease. Model 2: additionally adjusted for resting heart rate. Model 3: additionally adjusted for ambulatory heart rate instead of resting heart rate. SDNN = standard deviation of all normal RR intervals; Ms = millisecond; Ref. = reference.

Table S2 Subgroup analyses for the relationship between SDNN and the lifestyle-score

		number	β-coefficient (95% CI)	p for interaction
Sex	Men	n=972	0.14 (0.09; 0.19)	0.35
	Women	n=1107	0.13 (0.09; 0.18)	
Age (years)	<35	n=882	0.11 (0.05; 0.17)	0.06
	≥ 35	n=1197	0.16 (0.12; 0.20)	
BMI (kg/m^2)	<25	n=1226	0.14 (0.08; 0.20)	0.26
	≥ 25	n=853	0.08 (0.02; 0.14)	
Smoking	never	n=1141	0.16 (0.11; 0.21)	0.67
	ever	n=938	0.11 (0.06; 0.17)	
Regular physical activity (moderate and vigorous)	< 150 or <75min	n=401	0.10 (0.03; 0.17)	0.60
	≥ 150 or ≥75min	n=1678	0.12 (0.08; 0.16)	
Systolic and diastolic Blood pressure (mmHg)	< 120 and 80 without trt	n=904	0.12 (0.06; 0.18)	0.24
	≥ 120 or 80 or trt	n=1175	0.15 (0.10; 0.20)	
Cholesterol level (mg/dl)	< 200	n=1363	0.15 (0.10; 0.20)	0.86
	≥ 200	n=716	0.13 (0.06; 0.19)	
HbA_{1c} level (%)	< 5.7	n=1583	0.17 (0.12; 0.21)	0.96
	≥ 5.7	n=496	0.18 (0.10; 0.25)	
Healthy diet (items)	<2	n=1992	0.14 (0.11; 0.18)	0.68
	≥2	n=87	0.18 (0.01; 0.34)	
Education	high school	n=170	0.10 (-0.03; 0.22)	0.88
	college	n=1132	0.14 (0.09; 0.18)	
	university	n=777	0.15 (0.09; 0.20)	

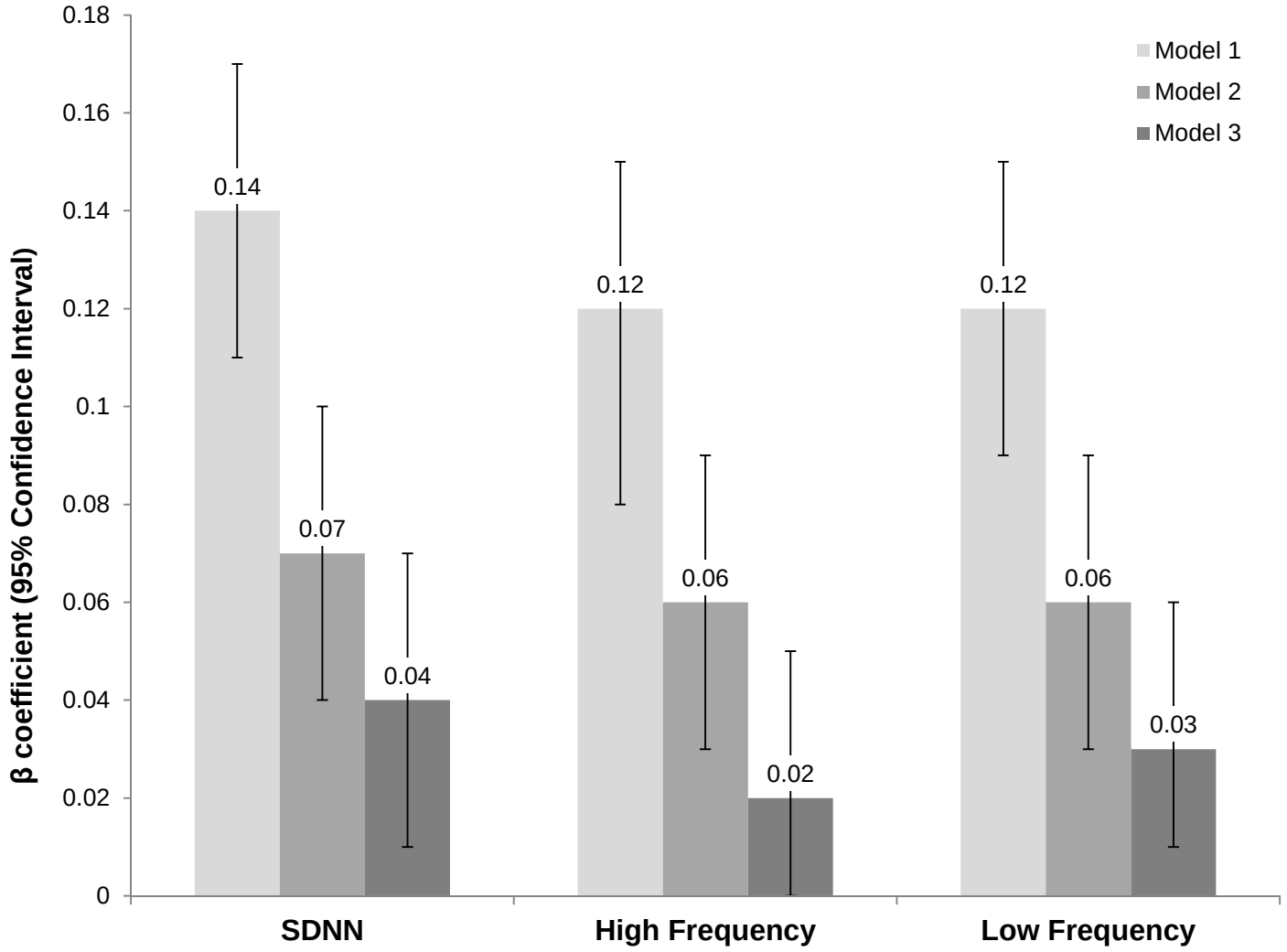
SDNN was transformed to z-score. Data are β coefficients (95% confidence Interval) adjusted for sex, age, alcohol consumption, educational status, family history of cardiovascular disease. SDNN = standard deviation of all normal RR intervals; BMI = body mass index; CI = confidence interval; Trt = treatment.

Table S3 Relationship between HRV and individual components of the lifestyle-score

n=2079	SDNN	High frequency	Low Frequency
Never smoking	0.13 (0.04; 0.21), p=0.003	0.12 (0.04; 0.20), p=0.004	0.18 (0.10; 0.26), p<0.0001
BMI <25kg/m^2	0.25 (0.16; 0.34), p<0.0001	0.15 (0.06; 0.24), p=0.001	0.17 (0.08; 0.25), p=0.0001
Regular physical activity	0.30 (0.20; 0.41), p<0.0001	0.09 (-0.008; 0.20), p=0.07	0.09 (-0.01; 0.19), p=0.08
Healthy diet	-0.005 (-0.21; 0.20), p=0.96	-0.07 (-0.27; 0.13), p=0.50	-0.16 (-0.35; 0.04), p=0.11
Healthy blood pressure level	0.11 (0.02; 0.21), p=0.02	0.18 (0.09; 0.28), p=0.0002	0.12 (0.03; 0.22), p=0.007
Healthy cholesterol level	0.11 (0.02; 0.20), p=0.02	0.11 (0.02; 0.20), p=0.02	0.10 (0.02; 0.19), p=0.02
Healthy HbA_{1c}	-0.04 (-0.13; 0.06), p=0.47	0.05 (-0.04; 0.15), p=0.29	0.08 (-0.01; 0.17), p=0.08

SDNN, high and low frequency were transformed to z-scores. Data are β coefficients (95% confidence Interval) per 1-unit increase of the respective variable and adjusted for sex, age, alcohol consumption, educational status, family history of cardiovascular disease. SDNN = standard deviation of all normal RR intervals; BMI = body mass index; Regular physical activity = ≥75 or 150 minutes per week of vigorous or moderate physical activity; Healthy diet = ≥2 diet components achieved; Healthy blood pressure level = systolic and diastolic blood pressure <120 and 80mmHg without treatment; Healthy cholesterol level = total cholesterol <200mg/dl; Healthy HbA_{1c} = glycated hemoglobin A_{1c} <5.7%.

Figure S1 Relationship between heart rate variability and lifestyle-score



5.MANUSCRIPT 2 – HRV and sleep-related breathing disorders

Heart rate variability and sleep-related breathing disorders in the general population

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CURRENT STANDING: IN REVISION

Heart rate variability and sleep-related breathing disorders in the general population

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Abstract

Background: Obstructive sleep apnea seems to have an important influence on the autonomic nervous system. In this study, we assessed the relationships of sleep apnea related parameters with 24-hour (h) heart rate variability (HRV) in a large population of young and healthy adults.

Methods: Participants aged 25-41 years with a BMI <35kg/m^2 and without known obstructive sleep apnea were included in a prospective population-based cohort study. HRV was assessed using 24-h ECG monitoring. The standard deviation of all normal RR intervals (SDNN) was used as main HRV variable. Apnea-hypopnea index (AHI) and oxygen desaturation index (ODI) were obtained from nighttime pulse oximetry with nasal airflow measurements. We defined sleep-related breathing disorders as an AHI≥5 or an ODI≥5. Multivariable regression models were constructed to assess the relationship of HRV with either AHI or ODI.

Results: Median age of the 1255 participants was 37 years, 47% were male and 9.6% had an AHI≥5. Linear inverse association of SDNN across AHI and ODI groups were found (p for trend=0.006 and 0.0004, respectively). The β-coefficient (95% confidence interval) for the relationship between SDNN and elevated AHI was -0.20 (-0.40;-0.11), p=0.04 and -0.29 (-0.47;-0.11), p=0.002 for elevated ODI. After adjustment for 24-h heart rate (HR), the same β-coefficients (95%CI) were -0.06 (-0.22; 0.11), p=0.51 and -0.14 (-0.30; 0.01), p=0.07, respectively.

Conclusion: Even early stages of sleep-related breathing disorders are inversely associated with HRV in young and healthy adults, suggesting that they are tightly linked with autonomic dysfunction. However, the incremental information beyond HR is minimal.

Keywords: Heart rate variability, sleep-related breathing disorders, population-based, general population

Introduction

Sleep-related breathing disorders, such as obstructive sleep apnea (OSA), are highly prevalent and remain often undiagnosed.[1-3] OSA is independently associated with an increased risk of hypertension, coronary artery disease, and sudden death.[4-10] Treatment of sleep-related breathing disorders can effectively lower the risk for adverse cardiovascular outcomes.[11,12]

The autonomic nervous system is involved in numerous physiologic processes of the cardiovascular system.[13] Heart rate variability (HRV) is a validated and easily obtainable measure of the influence of autonomic function on the heart and by itself associated with cardiovascular risk factors and mortality.[14-16] An experimental study showed markedly higher sympathetic nerve activity among OSA patients without treatment compared to treated patients.[17] Another small study has found a worse HRV profile and an increased heart rate (HR) directly after an apnea.[18] An unbalanced autonomic function among patients with manifest OSA could therefore be one potential reason for their increased cardiovascular risk.

Population-based studies assessing the relationship of the autonomic nervous function with OSA are scarce and the shape of the relationship is currently unknown. In addition, it is unclear whether autonomic function is altered influenced by sleep-related breathing disorders among otherwise asymptomatic individuals with subclinical sleep-related breathing disorders. Finally, HR by itself is an independent risk factor for cardiovascular events,[19] seems to be modified among OSA patients,[18] and is tightly linked with HRV.[20] In this context, it is unknown whether HRV contains any additional information beyond HR.

In order to address some of these issues, we evaluated the association of HRV with several sleep-apnea related parameters in a large cohort of young and healthy adults from the general population.

Methods

Study population

Between 2010 and 2013 inhabitants of the Principality of Liechtenstein aged between 25 to 41 years were invited to participate in the *'genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors'* (GAPP) study, a prospective population-based cohort study. Study design and methodology have been published previously.[21] Study exclusion criteria were known OSA, renal failure, current intake of antidiabetic drugs, a body mass index (BMI) >35kg/m^2, established cardiovascular disease or other severe illnesses. Of the 2170 participants enrolled, 1410 participants performed a nighttime pulse oximetry and nasal flow measurement. Of these, participants were excluded if the sleep study duration was <180 minutes (n=114) or if they had missing or incomplete 24-hour (h) electrocardiogram (ECG) recordings (n=19) or other missing covariates (n=22), leaving 1255 participants for the current analysis. Written informed consent was obtained from every participant and the study protocol was approved by the local ethics committee.

24-hour electrocardiogram

Every participant underwent a 24-h three-channel Holter ECG recording with a validated device (AR12plus, Schiller AG, Switzerland). When the ECG monitoring duration was <80% of the target time (i.e. <19.2 hours), the study was repeated whenever possible. ECG devices were started in the morning after the study examination. All Holter studies were post-processed using a dedicated Software (MediLog Darwin V2, Schiller AG, Switzerland) to remove artefacts and redefine premature ventricular and atrial beats. Time- and frequency domain HRV and mean HR were automatically calculated by the software. The standard deviation of all normal RR intervals (SDNN) was pre-defined to be the main HRV variable. In addition, low frequency (LF), high frequency (HF) and total power (TP) were used for this analysis. LF and HF were normalized, by calculating LF/(TP-VLF)*100 and HF/(TP-VLF)*100, respectively.

Nighttime pulse oximetry with nasal flow measurement

Nighttime pulse oximetry with nasal flow measurement was performed in every participant using a validated device (ApneaLink, ResMed, USA).[22] Participants were instructed to place the nasal flow cannula and the finger pulse oximetry probe, to start the device before falling asleep and to stop the measurement when waking up in the morning. Recording length had to be at least 180 minutes for both nasal airflow measurement and pulse oximetry, otherwise participants were asked to repeat the measurement.

Apnea-hypopnea index (AHI) was defined as the average number of apnea and hypopnea episodes per hour of sleep. An apnea was defined as a nasal airflow reduction of at least

80% during ≥10 seconds.[23] A hypopnea was defined as a nasal airflow reduction of ≥30% with a concomitant fall in oxygen saturation of ≥4%. Oxygen desaturation index (ODI) was defined as the mean number of oxygen desaturations of ≥4% per hour of recording.[23] Sleep-related breathing disorders were defined as an AHI ≥5 or an ODI ≥5.

Assessment of other study variables

Questionnaires were used to obtain information about personal, medical, lifestyle and nutritional factors. Smoking status was self-assessed and classified as never, past or current smoker. Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ)[24] and regular physical activity was defined as ≥150 minutes of moderate activity or ≥75 minutes of vigorous activity per week. Information about fruit and vegetable consumption was dichotomized into ≥5 versus <5 servings per day. Highest educational status achieved was self-reported and classified into the categories high school, college and university degree. Daytime sleepiness was assessed using the Epworth Sleepiness Scale (ESS) questionnaire.[25] Height and weight were measured in a standardized way by trained study nurses. BMI was calculated as body weight in kilogram divided by height in meters squared. Office blood pressure was measured in a sitting position after 5 minutes of rest. Three blood pressure measurements were performed. For the current analysis the mean of the second and third measurement was used. Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glycated hemoglobin A_{1c} (HbA_{1c}), creatinine and copeptin were measured from a fasting venous blood sample immediately after the blood draw according to standard methodology.[21] Estimated glomerular filtration rate was calculated using the creatinine based chronic kidney disease epidemiology collaboration (CKD-EPI) formula.[26]

Statistical analyses

Baseline characteristics were stratified according the presence or absence of an AHI ≥5. Distribution of continuous variables was checked using skewness, kurtosis and visual inspection of the histogram. Continuous variables were presented as medians (interquartile ranges) and categorical variables as numbers (percentages). Group comparisons were done using Wilcoxon rank sum tests or Chi-square tests, as appropriate.

To evaluate the relationship of AHI or ODI with HRV, separate multivariable linear regression models were constructed using different HRV variables as the dependent variable. To assess the linearity of the relationships with HRV, AHI and ODI were categorized into 0 (reference category), 1, 2, 3-4 and ≥5 episodes per hour. LF, HF and TP were log-transformed. All HRV variables were converted into z-scores to improve comparability. All models were adjusted for a predefined set of covariates, including sex, age, BMI, current

smoking, LDL-C, HDL-C, copeptin, prediabetes, systolic blood pressure, eGFR, regular physical activity, fruit and vegetable consumption and educational status.

We also evaluated the association of HR with sleep-related breathing disorders in similar models described above. In order to evaluate the incremental information of HRV beyond HR, all HRV based models were additionally adjusted for resting and 24-h heart rate in a separate step.

To assess potential effect modification of the relationship of HRV with sleep-related breathing disorders, pre-specified subgroup analyses for sex, smoking, BMI, age and physical activity were performed. Multiplicative interaction terms were entered in the non-stratified models to evaluate significant subgroup effects. A p-value <0.05 was pre-specified to indicate statistical significance. SAS version 9.4 (SAS Institute Inc, Cary, North Carolina, USA) was used for all analyses.

Results

Baseline characteristics stratified by the presence or absence of an AHI≥5 are shown in *Table 1*. Overall 120 (9.6%) participants had an AHI≥5, 83% of them were male. Compared to participants with a normal AHI, participants with an AHI≥5 had a higher BMI (27 versus 24 kg/m^2, p<0.0001), were more often current smokers (29 versus 21%, p=0.04) and had a higher blood pressure (128 versus 120 mmHg for systolic, and 85 versus 78 mmHg for diastolic blood pressure, p<0.0001 for both comparisons). The ESS was not significantly different among both groups (median score 7 versus 7, p=0.38). SDNN and HF were lower among participants with an AHI ≥5 (147 versus 151 ms, p=0.04 and 12.7 versus 15.5 ms^2, p=0.002, respectively). Normalized LF and TP did not differ between the two groups.

Relationship of heart rate variability with sleep apnea related parameters

The relationships between HRV categories and AHI are shown in *Table 2*. After multivariable adjustment SDNN significantly decreased across increasing AHI categories (p for trend=0.006). There was no significant relationship of AHI with normalized LF, normalized HF and TP. Additional adjustment for resting and 24-h HR attenuated these relationships. Similar findings were obtained when AHI categories were replaced by ODI categories, as shown in *Table 3*. After multivariable adjustment we found significant inverse relationships for SDNN and TP (all p for trend <0.05) and a positive association with normalized LF (p for trend =0.02). After additional adjustment for either resting of 24-h HR, only SDNN remained linearly associated with ODI (p for trend = 0.006 and 0.03, respectively) *(Table 3)*. Similar findings were also obtained, when the AHI or ODI were entered into the models as dichotomous variable (≥5 versus <5), as shown in *Table 4*.

Subgroup analyses are presented in *Table S1.* These analyses suggest that the relationships of HRV with both AHI and ODI are stronger among current smokers compared with non-current smokers. None of the other p values for interaction were statistically significant.

Relationship of heart rate with sleep apnea related parameters

The results for the relationships of either resting or 24-h HR with AHI and ODI are presented in *Table S2* and *S3*. AHI and ODI categories were significantly related to resting and 24-h HR after multivariable adjustment. Additional adjustment for SDNN attenuated these relationships, however the associations for both AHI and ODI with ambulatory HR remained statistically significant *(Tables S2 and S3)*.

Discussion

In this large population based cohort of young and healthy adults we found significant relationships of HRV with both AHI and ODI after comprehensive multivariable adjustment. These associations were found to be linear without evidence of a threshold. Our data therefore suggest a link between subclinical sleep-related breathing disorders and autonomic dysfunction, which may contribute to the unfavorable outcome of patients with overt OSA. As an additional finding we observed that the relationships of sleep-related breathing disorder with SDNN became strongly attenuated after additional adjustment for resting and mainly 24-h HR. Thus, HRV parameters do not seem to provide a significant amount of additional information beyond HR.[20] This is an important finding, as the quantification of HR, in particular resting HR, is much easier than the quantification of 24-h HRV.

Overall, around 10% of this young and healthy population without overt OSA had an AHI or ODI ≥5, and men were more often affected compared to women. This is in line with at least one prior population-based study showing an OSA prevalence of around 20% in a middle-aged population.[1] In our population, the ESS was similar among both groups, suggesting that affected individuals in our study may be at an early, still asymptomatic stage of the disorder. Nevertheless, our findings suggest that autonomic dysfunction could be an early phenomenon already present among otherwise healthy individuals with subclinical disease. Prospective follow-up of the GAPP cohort will shed more light on the course of this potential public health problem. At the current stage, lifestyle modification in affected individuals seems prudent, given the strong correlation between BMI and sleep-related breathing disorders and the stronger relationship with HRV observed among current smokers. [27,28]

Our results showed linear associations of AHI and ODI with markers of the HRV, such as the SDNN and TP, which are corresponding to total variability.[16] Those variables might detect early effects of sleep-related breathing disorders on the autonomic function. Normalized LF, partly modulated by sympathetic activity, was linearly increasing with increasing ODI but not AHI. These results are supported by another study, showing higher sympathetic nerve activity among untreated compared to treated OSA patients.[17] According to our results, normalized HF, which is controlled by parasympathetic activity, was not significantly associated with sleep apnea-related variables. Our results might therefore reflect increased sympathetic but not reduced parasympathetic activity among participants with a higher number of apneas and desaturations.

Similar to HRV we found strong relationships of resting and 24-h HR with AHI and ODI, with a stronger association for 24-h HR compared with resting HR. Even though HR is strongly influenced by various factors, HR is also linked to the autonomic nervous system.[29] A recent study has confirmed the tight link between HR and HRV, suggesting that these variables

should not be assessed independent of each other.[20] Further studies are needed to evaluate the effect of HRV that is independent of HR.

Strengths and limitations:

Strengths of this study were the population-based study design, the well-characterized young population and the availability of 24-h HRV in the entire study population. However several potential limitations have to be taken into account in the interpretation of our results. First, mainly white people were included in the GAPP study, and the generalizability of our results to other population samples is unclear. Second, this is a cross-sectional analysis and therefore the directionality of our associations as well as their causality remain unknown. Finally, polysomnography as a gold standard for diagnosing OSA was not available in our population. However, previous studies have shown excellent sensitivity and specificity of nighttime pulse oximetry with nasal flow measurement for this purpose.[22]

Conclusion:

Almost 10% of this young and healthy population without known OSA had increased AHI or ODI levels. Increasing levels of AHI and ODI were significantly associated with a decreasing HRV. Our results therefore suggest that even subclinical stages of sleep-related breathing disorders are associated with autonomic dysfunction. This relationship was particularly strong among smokers. Our results also show that the incremental information of HRV parameters beyond 24-h HR is minimal. Further studies are needed to better assess the role of autonomic dysfunction in the development of cardiovascular disease among patients with clinical and subclinical sleep-related breathing disorders.

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Table 1 Baseline Characteristics stratified by apnea-hypopnea index

n=1255	AHI < 5 n=1135 (90.4%)	AHI ≥ 5 n=120 (9.6%)	p-value
Age, years	35 (30; 39)	38 (33; 40)	0.001
Male sex, %	492 (43.4)	100 (83.3)	<0.0001
Smoking, %			0.08
Current	238 (21.0)	35 (29.2)	
Past	260 (22.9)	29 (24.1)	
never	637 (56.1)	56 (46.7)	
BMI, kg/m^2	23.9 (21.9; 26.5)	27.4 (24.5; 30.9)	<0.0001
Systolic BP, mmHg	120 (111; 127)	128 (120; 134)	<0.0001
Diastolic BP, mmHg	78 (73; 84)	85 (79; 89)	<0.0001
Hypertension, %	142 (12.5)	33 (27.5)	<0.0001

LDL-C, mmol/l	2.85 (2.33; 3.44)	3.34 (2.75; 3.92)	<0.0001
HDL-C, mmol/l	1.53 (1.27; 1.79)	1.22 (1.02; 1.48)	<0.0001
Prediabetes, %	244 (21.5)	38 (31.7)	<0.0001
Hs-CRP, mmol/l	1.0 (0.5; 2.0)	1.5 (0.6; 3.2)	0.002
Education, %			0.01
High school	81 (7.1)	18 (15.0)	
College	629 (55.4)	60 (50.0)	
University degree	425 (37.4)	42 (35.0)	
AHI	1 (0; 2)	8 (6; 12)	<0.0001
ODI	1 (0; 2)	9 (6; 13)	<0.0001
ODI≥5	36 (3.2)	105 (87.5)	<0.0001
Epworth Sleepiness Scale	7 (5;10)	7 (5; 11)	0.38
Epworth Sleepiness Scale ≥11	221 (19.5)	30 (25.0)	0.15

Heart Rate Variability			
Resting heart rate, bpm	61 (56; 67)	63 (58; 68)	0.04
Ambulatory heart rate, bpm	74 (69; 80)	76 (71; 82)	0.09
SDNN, ms	151.3 (128.3; 177.7)	147.1 (118.2; 170.4)	0.04
High frequency nu, ms/s^2	15.5 (11.4; 19.9)	12.7 (9.1; 17.4)	0.002
Low frequency nu, ms/s^2	53.6 (47.5; 59.0)	56.8 (51.2; 61.9)	0.55
Total power	3625 (2502; 5058)	3459 (2119; 4887)	0.12

Data are median (interquartile range) or number (percentage). P-value was calculated using Wilcoxon Rank Sum Test or Chi-square Test, as appropriate. Hypertension was defined as a systolic or diastolic blood pressure ≥140 or ≥90mmHg or intake of antihypertensive treatment. Prediabetes was defined as a glycated hemoglobin A_{1c} >5.6%. AHI = Apnea-hypopnea index; ODI = oxygen desaturation index; BMI = body mass index; BP = blood pressure; LDL-C = low densitiy lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; hs-CRP = high sensitivity C - reactive protein; SDNN = standard deviation of all normal RR intervals.

Table 2 Relationship of heart rate variability with categories of the apnea-hypopnea index

		AHI 0 n= 388	AHI 1 n= 397	AHI 2 n= 204	AHI 3-4 n= 146	AHI≥5 n= 120	p-value
SDNN	Model 1	Ref.	-0.06 (-0.19; 0.08)	-0.07 (-0.24; 0.10)	-0.22 (-0.41; -0.02)	-0.29 (-0.50; -0.07)	0.006
	Model 2		-0.06 (-0.18; 0.06)	-0.06 (-0.21; 0.09)	-0.16 (-0.33; 0.02)	-0.17 (-0.37; 0.02)	0.04
	Model 3		-0.03 (-0.15; 0.09)	0.00 (-0.15; 0.15)	-0.11 (-0.28; 0.05)	-0.09 (-0.28; 0.10)	0.23
LFnu	Model 1	Ref.	0.08 (-0.05; 0.21)	0.18 (0.01; 0.34)	0.24 (0.05; 0.43)	0.14 (-0.07; 0.36)	0.17
	Model 2		0.08 (-0.05; 0.21)	0.17 (0.01; 0.34)	0.22 (0.03; 0.40)	0.10 (-0.11; 0.31)	0.05
	Model 3		0.07 (-0.06; 0.20)	0.14 (-0.02; 0.30)	0.19 (-0.00; 0.37)	0.04 (-0.17; 0.25)	0.17
HFnu	Model 1	Ref.	-0.03 (-0.17; 0.10)	-0.13 (-0.30; 0.03)	0.02 (-0.17; 0.21)	-0.05 (-0.27; 0.16)	0.63
	Model 2		-0.03 (-0.16; 0.10)	-0.13 (-0.29; 0.04)	0.05 (-0.14; 0.23)	-0.005 (-0.21; 0.20)	0.88
	Model 3		-0.01 (-0.14; 0.11)	-0.09 (-0.24; 0.07)	0.09 (-0.10; 0.27)	0.08 (-0.13; 0.28)	0.44
TP	Model 1	Ref.	0.04 (-0.09; 0.17)	-0.06 (-0.22; 0.10)	-0.05 (-0.24; 0.13)	-0.19 (-0.40; 0.01)	0.06
	Model 2		0.04 (-0.07; 0.15)	-0.05 (-0.18; 0.09)	0.01 (-0.14; 0.17)	-0.07 (-0.25; 0.10)	0.31
	Model 3		0.07 (-0.03; 0.17)	0.02 (-0.10; 0.14)	0.07 (-0.07; 0.21)	0.04 (-0.11; 0.20)	0.75

Data are β-coefficients (95%CI). Model 1 was adjusted for sex, age, body mass index, current smoking, low densitiy lipoprotein, high densitiy lipoprotein, prediabetes, systolic blood pressure, glomerular filtration rate, physical activity, fruit and vegetable intake and copeptin. Model 2 was additionally adjusted for resting heart rate. Model 3 was additionally adjusted for ambulatory HR instead of resting HR. HRV variables were transfomed to z-scores. SDNN = standard deviation of all normal RR intervals, LFnu = low frequency normalized units; HFnu = high frequency normalized units; TP = total power; Ref= Reference; AHI = Apnea-hypopnea index. n=1255.

Table 3 Relationship of heart rate variability with categories of the oxygen desaturation index

		ODI 0 n= 377	ODI 1 n= 360	ODI 2 n= 195	ODI 3-4 n= 182	ODI ≥5 n= 141	p-value
SDNN	Model 1	Ref.	-0.003 (-0.14; 0.14)	-0.06 (-0.24; 0.11)	-0.20 (-0.39; -0.01)	-0.37 (-0.58; -0.16)	0.0004
	Model 2		-0.003 (-0.12; 0.12)	-0.05 (-0.20; 0.11)	-0.15 (-0.31; 0.02)	-0.25 (-0.44; -0.06)	0.006
	Model 3		-0.009 (-0.13; 0.11)	-0.05 (-0.20; 0.11)	-0.12 (-0.28; 0.04)	-0.19 (-0.38; -0.009)	0.03
LFnu	Model 1	Ref.	0.11 (-0.02; 0.25)	0.06 (-0.11; 0.23)	0.17 (-0.01; 0.36)	0.26 (0.05; 0.47)	0.02
	Model 2		0.11 (-0.02; 0.24)	0.06 (-0.11; 0.23)	0.15 (-0.02; 0.34)	0.22 (0.01; 0.43)	0.06
	Model 3		0.12 (-0.01; 0.25)	0.05 (-0.11; 0.22)	0.13 (-0.05; 0.31)	0.17 (-0.04; 0.37)	0.17
HFnu	Model 1	Ref.	-0.06 (-0.19; 0.08)	-0.09 (-0.26; 0.08)	0.008 (-0.18; 0.19)	-0.17 (-0.38; 0.04)	0.14
	Model 2		-0.06 (-0.19; 0.08)	-0.08 (-0.25 ; 0.09)	0.03 (-0.15; 0.21)	-0.12 (-0.33; 0.08)	0.33
	Model 3		-0.06 (-0.19; 0.07)	-0.08 (-0.24; 0.09)	0.06 (-0.11; 0.24)	-0.06 (-0.26; 0.14)	0.99
TP	Model 1	Ref.	0.06 (-0.07; 0.19)	0.04 (-0.13; 0.20)	0.01 (-0.17; 0.19)	-0.17 (-0.37; 0.04)	0.05
	Model 2		0.06 (-0.05; 0.17)	0.05 (-0.08; 0.19)	0.07 (-0.08; 0.22)	-0.04 (-0.21; 0.13)	0.48
	Model 3		0.05 (-0.05; 0.15)	0.06 (-0.07; 0.19)	0.11 (-0.02; 0.25)	0.05 (-0.11; 0.21)	0.62

Data are β-coefficients (95%CI). Model 1 was adjusted for sex, age, body mass index, current smoking, low densitiy lipoprotein, high densitiy lipoprotein, prediabetes, systolic blood pressure, glomerular filtration rate, physical activity, fruit and vegetable intake and copeptin. Model 2 was additionally adjusted for resting heart rate. Model 3 was additionally adjusted for ambulatory HR instead of resting HR. HRV variables were transfomed to z-scores. SDNN = standard deviation of all normal RR intervals, LFnu = low frequency normalized units; HFnu = high frequency normalized units; TP = total power; Ref= Reference; ODI = oxygen desaturation index. n=1255.

Table 4 Relationship between heart rate variability and apnea-hypopnea index or oxygen desaturation index

		AHI≥5	p-value	ODI≥5	p-value
SDNN	Model 1	-0.20 (-0.40; -0.11)	0.04	-0.29 (-0.47; -0.11)	0.002
	Model 2	-0.11 (-0.28; 0.06)	0.21	-0.19 (-0.35; -0.03)	0.02
	Model 3	-0.06 (-0.22; 0.11)	0.51	-0.14 (-0.30; 0.01)	0.07
LFnu	Model 1	0.02 (-0.17; 0.20)	0.85	0.16 (-0.02; 0.33)	0.08
	Model 2	-0.02 (-0.20; 0.17)	0.85	0.12 (-0.05; 0.30)	0.18
	Model 3	-0.06 (-0.24; 0.12)	0.52	0.08 (-0.09; 0.25)	0.37
HFnu	Model 1	-0.01 (-0.20; 0.17)	0.89	-0.14 (-0.31; 0.04)	0.13
	Model 2	0.03 (-0.16; 0.21)	0.77	-0.10 (-0.27; 0.08)	0.28
	Model 3	0.08 (-0.09; 0.26)	0.36	-0.04 (-0.21; 0.13)	0.63
TP	Model 1	-0.18 (-0.36; -0.002)	0.05	-0.20 (-0.37; -0.03)	0.02
	Model 2	-0.08 (-0.23; 0.07)	0.31	-0.09 (-0.24; 0.05)	0.21
	Model 3	-0.003 (-0.14; 0.14)	0.97	-0.02 (-0.15; 0.12)	0.81

Data are β-coefficients (95%CI). Model 1 was adjusted for sex, age, body mass index, current smoking, low densitiy lipoprotein, high densitiy lipoprotein, prediabetes, systolic blood pressure, glomerular filtration rate, physical activity, fruit and vegetable intake and copeptin. Model 2 was additionally adjusted for resting heart rate. Model 3 was additionally adjusted for ambulatory HR instead of resting HR. HRV variables were transfomed to z-scores. SDNN = standard deviation of all normal RR intervals, LFnu = low frequency normalized units; HFnu = high frequency normalized units; TP = total power; Ref= Reference; AHI = Apnea-Hypopnea Index. n=1255.

Supplement

Heart rate variability and sleep-related breathing disorders in the general population

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Table S1 Subgroup analyses of the relationship between the Apnea Hypopnea Index and the SDNN

SDNN		n	AHI pathologic	p-value for interaction	ODI pathologic	p-value for interaction
Sex	Men	592	-0.16 (-0.37; 0.06)	0.28	-0.24 (-0.44; -0.04)	0.42
	Women	663	-0.06 (-0.48; 0.36)		-0.21 (-0.63; 0.21)	
Smoking	Current	273	-0.35 (-0.70; -0.003)	0.01	-0.66 (-1.01; -0.31)	0.0009
	Past/never	982	-0.06 (-0.28; 0.16)		-0.11 (-0.32; 0.10)	
BMI	<25	729	-0.32 (-0.66; 0.02)	0.52	-0.30 (-0.64; 0.05)	0.86
	>= 25	526	-0.13 (-0.36; 0.09)		-0.27 (-0.47; -0.06)	
Age	<35	628	-0.35 (-0.66; -0.03)	0.59	-0.40 (-0.70; -0.10)	0.62
	>=35	627	-0.07 (-0.30; 0.16)		-0.18 (-0.34; 0.05)	
Regular physical activity	Yes	876	-0.15 (-0.39; 0.09)	0.14	-0.32 (-0.54; -0.10)	0.68
	No	379	-0.37 (-0.67; -0.07)		-0.33 (-0.63; -0.02)	

Data are presented as β-coefficients (95% confidence interval). Multivariable model was adjusted for sex, age, body mass index, smoking status, low densitiy lipoprotein, high densitiy lipoprotein, prediabetes systolic blood pressure, glomerular filtration rate, physical activity, fruit and vegetable intake and copeptin. HRV variables were transformed to z-scores. SDNN= standard deviation of all normal RR intervals. Regular physical activity was defined as vigorous activity ≥75 minutes/week or moderate activity ≥150 minutes per week.

Table S2 Relationship of heart rate with apnea-hypopnea Index

	AHI ≥5	AHI 0 n= 388	AHI 1 n= 397	AHI 2 n= 204	AHI 3-4 n= 146	AHI≥5 n= 120	p-value
				Resting Heart Rate			
Model 1	0.21 (0.02; 0.40), p=0.03	Ref.	-0.00 (-0.14; 0.14)	0.03 (-0.14; 0.20)	0.14 (-0.06; 0.33)	0.24 (0.03; 0.46)	0.01
Model 2	0.12 (-0.05; 0.29), p=0.18		-0.03 (-0.15; 0.10)	-0.002 (-0.16; 0.10)	0.04 (-0.14; 0.21)	0.11 (-0.08; 0.31)	0.18
				Ambulatory Heart Rate			
Model 1	0.28 (0.10; 0.46), p=0.002	Ref.	0.05 (-0.08; 0.18)	0.13 (-0.03; 0.30)	0.20 (0.008; 0.38)	0.38 (0.17; 0.58)	0.0002
Model 2	0.19 (0.03; 0.34), p=0.02		0.02 (-0.09; 0.14)	0.10 (-0.04; 0.24)	0.09 (-0.07; 0.26)	0.24 (0.06; 0.42)	0.007

Data are β-coefficients (95%CI). Model 1 was adjusted for sex, age, body mass index, current smoking, low density lipoprotein, high density lipoprotein, prediabetes systolic blood pressure, glomerular filtration rate, physical activity, fruit and vegetable intake and copeptin. Model 2 was additionally adjusted for the standard deviation of all normal RR intervals. HR was transformed to z-scores. AHI = Apnea-hypopnea index

Table S3 Relationship of heart rate with oxygen desaturation index

	ODI ≥5	ODI 0 n= 377	ODI 1 n= 360	ODI 2 n= 195	ODI 3-4 n= 182	ODI ≥5 n= 141	p-value
			Resting Heart Rate				
Model 1	0.21 (0.03; 0.40), p=0.02	Ref.	-0.00 (-0.14; 0.14)	0.03 (-0.14; 0.21)	0.12 (-0.07; 0.21)	0.26 (0.04; 0.48)	0.03
Model 2	0.08 (-0.08; 0.24), p=0.33		-0.002 (-0.13; 0.12)	0.005 (-0.15; 0.16)	0.03 (-0.14; 0.20)	-0.09 (-0.10; 0.28)	0.32
			Ambulatory Heart Rate				
Model 1	0.29 (0.11; 0.46), p=0.001	Ref.	-0.01 (-0.15; 0.12)	0.04 (-0.13; 0.21)	0.16 (-0.02; 0.34)	0.34 (0.14; 0.54)	0.0003
Model 2	0.15 (-0.00; 0.30), p=0.05		-0.01 (-0.13; 0.10)	0.007 (-0.14; 0.15)	0.06 (-0.09; 0.22)	0.17 (-0.01; 0.35)	0.04

Data are β-coefficients (95%CI). Model 1 was adjusted for sex, age, body mass index, current smoking, low densitiy lipoprotein, high densitiy lipoprotein, prediabetes systolic blood pressure, glomerular filtration rate, physical activity, fruit and vegetable intake and copeptin. Model 2 was additionally adjusted for the standard deviation of all normal RR intervals. HR was transformed to z-scores. ODI = oxygen desaturation index.

6.MANUSCRIPT 3 – HRV, HR and inflammation

HEART RATE, HEART RATE VARIABILITY AND INFLAMMATORY BIOMARKERS AMONG YOUNG AND HEALTHY ADULTS

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CURRENT STANDING: IN REVISION

Heart rate, heart rate variability and inflammatory biomarkers among young and healthy adults

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Abstract

Background: Heart rate (HR), heart rate variability (HRV) and inflammation are all associated with cardiovascular morbidity and mortality. The aim of this study was to assess potential interrelationships between these parameters in a population of young and healthy adults.

Methods and Results: Individuals aged 25-41 years without established cardiovascular disease were included in a prospective population-based study. All participants underwent 24-hour electrocardiography using a validated device. The standard deviation of all normal RR intervals (SDNN) was pre-defined as the main HRV outcome variable. High-sensitivity C-reactive protein (hs-CRP), total leukocyte (LC) count and LC subtypes were obtained from venous blood samples. Multivariable linear regression models were constructed to assess the relationships of inflammatory biomarkers with HRV and HR. Overall, 2064 participants (47% men, median age 37years) were included in this analysis. In multivariable linear regression analyses using SDNN as the outcome variable, β-coefficients (95% confidence intervals) per 1 standard deviation (SD) increase on the log-scale were -0.11 (-0.16;-0.07), p<0.0001 for hs-CRP, -0.13 (-0.17;-0.09), p<0.0001 for total LC count, -0.12 (-0.16;-0.08), p<0.0001 for neutrophils, -0.04 (-0.09;0.00), p=0.05 for lymphocytes and -0.08 (-0.09;-0.02), p=0.005 for monocytes. Multivariable adjusted β-coefficients (95%CI) for 24-hour HR were 0.68 (0.30;1.07), p=0.0005 for hs-CRP, 1.21 (0.83;1.58), p<0.0001 for LC, 1.38 (1.01;1.75), p<0.0001 for neutrophils, -0.19 (-0.57;0.19), p=0.32 for lymphocytes and 0.66 (0.28;1.04), p=0.0007 for monocytes.

Conclusion: In this large cohort of young and healthy adults, inflammatory parameters were strongly associated with increased HR and decreased HRV, suggesting an important interaction between inflammatory pathways and the autonomic nervous system.

Keywords: heart rate variability, heart rate, inflammation, high-sensitivity C-reactive protein, leukocytes

Introduction

Inflammation plays a key role in the pathogenesis and progression of atherosclerosis.[1] Accordingly, several inflammatory biomarkers have been prospectively associated with all-cause mortality[2-4] and a broad set of cardiovascular outcomes including atrial fibrillation, stroke and myocardial infarction.[5-9]

The autonomic nervous system (ANS) is strongly involved in different mechanisms of the cardiovascular system. Heart rate variability (HRV) has become a validated marker of the autonomic function.[10,11] A reduced HRV is associated with an increased risk for cardiovascular and all-cause mortality.[12,13] Interestingly, experimental studies have suggested direct relationships between inflammation and the ANS.[14,15] Thus, autonomic dysfunction might be one mechanism why individuals with elevated inflammatory biomarkers have an increased cardiovascular risk. Up to now, some studies in rather small populations have evaluated the relationship between inflammation and HRV, and mainly inverse relationships have been described.[16-19] However, information on inflammatory biomarkers other than high-sensitivity C-reactive protein (hs-CRP) is scarce.[20] In addition, inflammatory biomarkers have recently been associated with heart rate (HR),[21] which by itself is an independent risk factor for cardiovascular outcomes.[22] Given the tight link between HRV and HR,[23] it is currently unclear whether HRV contains any additional information over and above HR.

In order to get a deeper understanding of these interrelationships between inflammation and the ANS, we assessed the relationships of 24-hour (h) HR and HRV with several inflammatory biomarkers in a large population based cohort of young and healthy adults from the general population.

Methods

All inhabitants of the Principality of Liechtenstein aged between 25 to 41 years were invited to participate in the *Genotypic and Phenotypic Determinants of Blood Pressure and other Cardiovascular Risk Factors (GAPP)* study, a prospective population-based cohort study. Study design and methodology have been published previously.[24] Main exclusion criteria were established cardiovascular disease, known renal failure, current intake of antidiabetic drugs, a body mass index (BMI) >35kg/m^2 or any other severe illness. Overall, 2170 participants were successfully enrolled in GAPP. For the present study we excluded 106 participants for the following reasons: missing 24-h electrocardiogram (ECG) or recording time <80% of the target time (n=39), regular intake of beta blockers (n=2), missing laboratory values (n=10) and other missing covariates (n=55), leaving 2064 participants for the current analysis. The study protocol was approved by the local ethics committee and all study participants have signed a written informed consent.

24-hour electrocardiogram

Participants underwent 24-h Holter ECG monitoring using a validated three-channel device (Schiller AG, Baar, Switzerland). The 24-h ECG was attached by trained study nurses and started in the morning immediately after the study examination. Recordings with a duration of less than 80% of the target time (i.e. <19.2 hours) or of low quality were repeated whenever possible. Every 24-h ECG study was post-processed using a dedicated software to remove artefacts and redefine premature ventricular and atrial beats (Medilog Darwin, Schiller AG, Baar, Switzerland). Mean 24-h ambulatory HR was automatically calculated by the software. All heart beats defined as normal were used to calculate HRV. For this analysis, we used the standard deviation of all normal RR intervals (SDNN), total power (TP), low frequency (LF) and high frequency (HF) to quantify HRV. SDNN is measured in milliseconds (ms) whereas TP, LF and HF are measured in ms^2.The low and high frequency band is between 0.04-0.15 and 0.15-0.40Hz, respectively. LF and HF were normalized by calculating LF/(TP-VLF)*100 and HF /(TP-VLF)*100, respectively, to receive values independent of total power (TP). SDNN was pre-defined as the main HRV outcome variable.

Blood sampling

Fasting venous blood samples were collected of every participant and immediately processed. Total number of leukocytes (LC), neutrophils, lymphocytes and monocytes were quantified using a validated method (Sysmex XE 5000, Japan). Hs-CRP, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were assayed on a Roche Cobas 6000 analyzer (Roche, Switzerland). Glycated hemoglobin A_{1c} (HbA_{1c}) was measured using high performance liquid chromatography (Bio-Rad D-10, Bio-

Rad Laboratories AG, Switzerland). Prediabetes was defined as an HbA_{1c} between 5.6 and 6.4%.[25]

Assessment of other study variables

Information about personal, medical, lifestyle and nutritional factors were self-assessed using standardized questionnaires.[24] Smoking status was classified as never, former or current smoking. Highest educational status achieved was divided into three categories: high school, college or university degree. Fruit/vegetable consumption was dichotomized into ≥5 and <5 servings per day. Alcohol consumption was dichotomized into drinkers and non-drinkers. Moderate and vigorous physical activity was estimated using the International Physical Activity Questionnaire (IPAQ).[26] Regular physical activity was defined as moderate activity ≥150 or vigorous activity ≥75 minutes per week, respectively. Height and weight were directly measured in a standardized way. BMI was calculated as weight in kilograms divided by height in meters squared. Body fat was assessed by bioelectrical impedance analysis using a validated device (BIA egofit, 2010, Eggstätt, Germany). Conventional blood pressure was measured three times in a sitting position after five minutes of rest. The mean of the second and third measurement was used for the current analysis.

Statistical analysis

Baseline characteristics were stratified by sex. Data are presented as medians (interquartile ranges) for continuous variables and numbers (percentages) for dichotomous variables. Group comparisons were done using Wilcoxon rank sum tests or Chi-square tests as appropriate. Distribution of continuous variables was checked using skewness, kurtosis and visual inspection of the histogram.

In order to assess the linearity of the relationships between HRV and inflammatory biomarkers we evaluated SDNN, HF and LF measures across quartiles of individual inflammatory biomarkers in separate multivariable models. P values for trend were calculated using quartile-specific medians. After confirming approximately linear relationships, we entered inflammatory biomarkers as continuous parameters in the multivariable models. Due to the skewed distribution, inflammatory variables were log-transformed for all analyses. To improve comparability across biomarkers, we calculated β-coefficients (95% confidence interval (CI)) per one standard deviation (SD) increase. In order to have a better comparability of the effect sizes all HRV variables were transformed into z-scores. The multivariable models were adjusted for age, sex, BMI, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, fruit and vegetable consumption, physical activity, LDL-C, HDL-C and body fat. To assess whether HRV has an incremental effect beyond HR, we additionally adjusted all models for ambulatory HR in a separate step.

Finally, in similar models we used 24-h HR instead of HRV as the outcome variable to assess its relationship with inflammatory biomarkers. A p-value <0.05 was pre-defined to indicate statistical significance. Statistical analyses were done using SAS 9.4 (SAS Institute Inc, Cary, North Carolina, USA).

Results

Baseline characteristics stratified by sex are presented in *Table 1*. The median age of the population was 36.9 years and 46.7% were men. Compared to women, men had a significantly higher BMI (median 25.6 versus 22.5 kg/m^2, p<0.0001), were more often current smokers (25.1 versus 19.1%, p<0.0001), had higher systolic and diastolic blood pressure values and a worse cholesterol profile (all p-values <0.0001). There were no significant differences for hs-CRP (0.9 versus 0.9 mg/l, p=0.39) and total LC count (5.3 and 5.3 G/l, p=0.38). Men had a significantly higher lymphocyte (1.8 versus 1.7 G/l, p=0.004) and monocyte count (0.5 versus 0.4 G/l, p<0.0001) and women had a higher neutrophil count (2.9 versus 2.8 G/l, p=0.02). The SDNN, TP and normalized LF were significantly higher in men than in women with (156 versus 145 ms, 4096 versus 3060 ms^2 and 57 versus 51 ms^2, respectively; all p-values <0.0001). Normalized HF was significantly higher among women compared to men (16 versus 13 ms^2, p<0.0001).

Relationships between heart rate variability and inflammatory biomarkers

Results of the multivariable linear regression analyses comparing SDNN values across quartiles of inflammatory parameters are shown in *Table 2*. There were significant inverse and linear associations of SDNN with hs-CRP, LC, neutrophils, lymphocytes and monocytes after multivariable adjustment. Additional adjustment for ambulatory HR strongly attenuated these effect sizes, but all associations remained statistically significant *(Table 2)*. The association between TP and inflammatory biomarkers was similar, except the not significant relationship with lymphocytes *(Table S1)*. Normalized HF was inversely related to neutrophils and monocytes *(Table S2)*. There was no relationship with hs-CRP and lymphocytes, and the association with total LC count was of borderline significance (p=0.08). There were positive linear relationships of normalized LF with total LC count and neutrophils as well as an inverse relationship with hs-CRP *(Table S3).* The relationships of both LF and HF with inflammatory biomarkers were strongly attenuated after the additional adjustment for 24-h HR and most became non-significant *(Tables S2 and S3)*.

Results of the relationships between HRV variables and continuous inflammatory biomarkers were similar to these results and are presented in *Figures 1-3 and S1.* After adjustment for 24-h HR, the relationships with SDNN attenuated by 36.4% for hs-CRP, 53.9% for LC, 66.7% for neutrophils and 50.0% for monocytes, even though all of them remain significant. The attenuation effect of the correlation between TP and inflammatory biomarkers was even stronger, such that only the relationship with hs-CRP remained significant *(Figure S1)*. The multivariable adjusted β-coefficients for normalized HF and LF are shown in *Figures 2 and 3*.

Again, all relationships were strongly attenuated after the adjustment for 24-h HR and none of them remained statistically significant.

Relationships between ambulatory heart rate and inflammatory biomarkers

A positive and linear association was found for the association of ambulatory HR with hs-CRP, LC, neutrophils and monocytes *(Table S4)*. Per one SD increase on the log-scale the multivariable adjusted β-coefficients (95%CI) were 0.68 (0.30; 1.07), p=0.0005 for hs-CRP, 1.21 (0.83; 1.58), p<0.0001 for LC, 1.38 (1.01; 1.75), p<0.0001 for neutrophils and 0.66 (0.28; 1.04), p=0.0007 for monocytes *(Figure 4)*. Additional adjustment for SDNN lowered the effect sizes of these relationships by 74% for hs-CRP, 47% for LC, 38% for neutrophils and 4.5% for monocytes, however with the exception of hs-CRP, all of them remained statistically significant. Lymphocyte count was not significantly associated with HR after multivariable adjustment.

Discussion

In this large population-based study we found strong and independent relationships of HR and HRV as measured by SDNN with a large set of inflammatory biomarkers, including hs-CRP, total LC count and several LC subpopulations. These relationships persisted after comprehensive multivariable adjustment but were strongly attenuated after additional adjustment for either ambulatory HR or HRV. Although these results suggest that HR and HRV carry similar information with regard to their association with inflammatory biomarkers,[23] it is important to emphasize that most relationships remained statistically significant after mutual adjustment, suggesting that both parameters carry some incremental information over each other. Finally, TP and normalized HF and LF did not provide additional information over and above ambulatory HR alone.

Our findings confirm and expand prior studies showing negative associations of HRV with mainly hs-CRP.[16-19] Importantly, our results were additionally adjusted for HR, providing incremental information. The consistent results across various inflammatory biomarkers found in our study may also indicate a broad interplay between different inflammatory pathways and the ANS. These data are in line with experimental studies showing direct relationships between of the ANS and inflammatory cells.[14,15] To our knowledge this is one of the first studies from the general population showing the relationships of HRV and HR with not only hs-CRP but also total LC count and its subtypes. Thus, a direct link between HRV and inflammation may be one factor that explains the increased cardiovascular risk previously shown for both entities.[2,12]

Inflammation is based on complex processes and various pathways are involved. Our findings showed that several inflammatory pathways seem to be involved in the relationship with HRV. Potential underlying mechanisms for these relationships are multifaceted. Norepinephrine, as the main sympathetic neurotransmitter, has been negatively associated with HRV and positively related to inflammatory parameters.[17] Experimental studies have shown expression of adrenoceptors on immune cells, suggesting a direct influence of the immune system by adrenoceptor agonists.[27-29] Additionally, other studies showed a reduced inflammatory reaction as a consequence of medical sympathectomy.[30,31] Vagal activity seems to have an anti-inflammatory effect via a cholinergic pathway, which results in a down-regulation of pro-inflammatory cytokines.[14]

Some of the described mechanisms partly explain and support our findings. HF is mainly modulated by parasympathetic activity.[10,32] An inverse relationship of HF with neutrophils and monocytes was found, assuming either an anti-inflammatory effect of vagal activity for those immune cells or an increased vagal activity as a consequence of low neutrophil and

monocyte cell counts. In contrast, lymphocytes were positively related to HF. This finding could be supported by the results of a previous study showing a protective effect of lymphocytes and an adverse effect for neutrophils for the occurrence of cardiovascular events.[33,34] Others have showed that inflammation could increase lymphocyte apoptosis, which may contribute to unfavorable outcomes.[35] SDNN was negatively associated with all inflammatory biomarkers, although the relationship with the lymphocyte cell count was weakest and reached only borderline significance. The association of lymphocyte cell count with different HRV variables should further be investigated to understand the underlying mechanisms of the relationship between lymphocytes and autonomic function.

Based on previous studies, resting and 24-h HR are known to be positively associated with hs-CRP, fibrinogen and interleukin, which is in line with our findings.[17-19,21] Additionally, our data showed a positive association with total LC count, neutrophils and monocytes, but similar to HRV, no significant relationship with lymphocytes. Physical activity and other healthy lifestyle habits reduce HR[36] and improve autonomic function, which may in consequence lower inflammatory biomarker levels. Others suggest that the protective effect of physical activity on inflammation is mediated by autonomic function.[37] This cross-sectional study cannot prove the above mentioned pathways, but nevertheless highlights the need for future studies to better understand the relationship between inflammation and the ANS.

Strengths and Limitations

Major strengths of the present study are the population-based study design, the well-characterized study population, the availability of 24-h ECG data to quantify HRV and the inclusion of young and healthy participants with a relatively short exposure history to environmental factors. However there are several potential limitations, which should be taken into account for the interpretation of our results. First, this is a cross-sectional analysis such that causality of the relationship between HRV and inflammatory biomarkers cannot be addressed. Second, we enrolled mainly Caucasians in our study and the generalizability of our results to other populations is uncertain. Third, as in any observational study, residual confounding could be an issue despite our comprehensive multivariable adjustment. Fourth, it is unclear if variables, such as body fat mass or BMI are confounders or mediators of the observed relationships. If some mediators should have been included in our models, we would expect that the true relationships between inflammation, HR and HRV would be somewhat stronger.

Conclusion

In this large population of young and healthy adults from the general population, we found strong and independent relationships of HR and HRV with a broad set of inflammatory biomarkers. Relationships between SDNN and inflammatory biomarkers were weakened but remained significant after additional adjustment for ambulatory HR, suggesting incremental information of HRV over HR. Thus, our findings suggest important interrelationships between inflammatory pathways and the ANS.

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Disclosures

None

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Figure legend

Figure 1 Relationship between SDNN and inflammatory biomarkers

Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. SDNN = standard deviation of all normal RR intervals: Hs-CRP = high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, systolic blood pressure, prediabetes, physical activity, low- and high density lipoprotein cholesterol and body fat. Model 3 was additionally adjusted for 24-hour heart rate. n = 2096.

Figure 2 Relationship between normalized HF and inflammatory biomarkers

Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. HF = high frequency; Hs-CRP = high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, systolic blood pressure, prediabetes, physical activity, low- and high density lipoprotein cholesterol and body fat. Model 3 was additionally adjusted for 24-hour heart rate. n = 2096.

Figure 3 Relationship between normalized LF and inflammatory biomarkers

Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. LF = low frequency; Hs-CRP = high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, systolic blood pressure, prediabetes, physical activity, low- and high density lipoprotein cholesterol and body fat. Model 3 was additionally adjusted for 24-hour heart rate. n = 2096.

Figure 4 Relationship between ambulatory heart rate and inflammatory biomarkers

Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. Hs-CRP = high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, systolic blood pressure, prediabetes, physical activity,

low- and high density lipoprotein cholesterol and body fat. Model 3 was additionally adjusted for the standard deviation of all normal RR intervals. SDNN = standard deviation of all normal RR intervals; n = 2096.

Table 1 Baseline Characteristics stratified by sex

n=2064	Men n=979 (46.7%)	Women n=1117 (53.3%)	p-value*
Age, years	36.9 (31.6; 40.5)	36.7 (30.9; 40.1)	0.19
BMI, kg/m^2	25.6 (23.6; 27.9)	22.5 (20.6; 25.2)	<0.0001
Smoking, %			0.0003
Current	246 (25.1)	213 (19.1)	
Former	240 (24.5)	250 (22.4)	
Never	493 (50.4)	654 (58.6)	
Systolic BP, mmHg	127 (121; 135)	113 (107; 120)	<0.0001
Diastolic BP, mmHg	82.0 (77.5; 87.5)	74.5 (69.5; 79.5)	<0.0001
LDL-C, mmol/l	3.2 (2.7; 3.8)	2.6 (2.2; 3.1)	<0.0001
HDL-C, mmol/l	1.3 (1.1; 1.5)	1.7 (1.5; 1.9)	<0.0001
HbA_{1c}, %	5.4 (5.2; 5.7)	5.4 (5.2; 5.6)	0.001
Education, %			<0.0001
Basic	64 (6.5)	108 (9.7)	
Middle	489 (50.0)	651 (58.3)	
University	426 (43.5)	358 (32.0)	
Hs-CRP, mg/l	0.9 (0.5; 1.8)	0.9 (0.5; 2.1)	0.39

Leukocytes, G/l	5.3 (4.6; 6.2)	5.3 (4.5; 6.1)	0.38
Neutrophils	2.8 (2.3; 3.4)	2.9 (2.3; 3.6)	0.02
Lymphocytes	1.8 (1.5; 2.1)	1.7 (1.4; 2.1)	0.004
Monocytes	0.5 (0.4; 0.6)	0.4 (0.3; 0.5)	<0.0001
SDNN, ms	156 (132; 185)	145 (121; 168)	<0.0001
Total power, ms^2	4096 (2846; 5901)	3060 (2184; 4241)	<0.0001
LF normalized, ms^2	57 (53; 62)	51 (46; 56)	<0.0001
HF normalized, ms^2	13 (10; 17)	16 (12; 21)	<0.0001
Heart Rate 24-h, bpm	72.2 (66.6; 78.8)	76.9 (71.9; 82.2)	<0.0001

Data are median (interquartile range) or numbers (percentages). BMI = body mass index; BP = blood pressure; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; HbA_{1c} = glycated hemoglobin A_{1c}; hs-CRP = high-sensitivity c-reactive protein; SDNN = standard deviation of all normal RR intervals; LF = low frequency; HF = high frequency.

* P-values are based on Wilcoxon rank sum tests or chi-square tests, as appropriate.

Table 2 SDNN across quartiles of inflammatory biomarkers

n=2064	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value
			high-sensitivity c-reactive protein		
Hs-CRP, mg/l	<0.50	0.50-0.90	0.91-1.83	>1.83	
Age-sex adj.	Ref.	-0.12 (-0.24; -0.008)	-0.18 (-0.31; -0.06)	-0.46 (-0.58; -0.34)	<0.0001
Model 1	Ref.	-0.05 (-0.17; 0.06)	-0.05 (-0.17; 0.08)	-0.26 (-0.39; 0.08)	<0.0001
Model 2	Ref.	-0.03 (-0.13; 0.07)	-0.07 (-0.18; 0.04)	-0.17 (-0.28; -0.06)	0.0008
			Leukocytes		
LC, G/l	<4.6	4.6-5.3	5.4-6.1	>6.1	
Age-sex adj.	Ref.	-0.20 (-0.32; -0.09)	-0.29 (-0.40; -0.17)	-0.50 (-0.62; -0.38)	<0.0001
Model 1	Ref.	-0.14 (-0.25; -0.02)	-0.17 (-0.29; -0.06)	-0.35 (-0.47; -0.22)	<0.0001
Model 2	Ref.	-0.08 (-0.18; 0.02)	-0.08 (-0.19; 0.02)	-0.19 (-0.30; -0.09)	0.0004
			Neutrophils		
Neutrophils, G/l	<2.4	2.4-2.8	2.9-3.5	>3.5	
Age-sex adj.	Ref.	-0.19 (-0.31; -0.07)	-0.24 (-0.35; -0.12)	-0.47 (-0.58; -0.35)	<0.0001
Model 1	Ref.	-0.13 (-0.24; -0.02)	-0.13 (-0.24; -0.02)	-0.32 (-0.44; -0.20)	<0.0001
Model 2	Ref.	-0.07 (-0.17; 0.03)	-0.05 (-0.15; 0.05)	-0.13 (-0.23; -0.03)	0.02
			Lymphocytes		
Lymphocytes, G/l	<1.5	1.5-1.7	1.8-2.1	>2.1	
Age-sex adj.	Ref.	-0.10 (-0.22; 0.02)	-0.14 (-0.26; -0.02)	-0.24 (-0.37; -0.12)	0.0001
Model 1	Ref.	-0.07 (-0.18; 0.05)	-0.07 (-0.19; 0.04)	-0.12 (-0.25; -0.00)	0.06
Model 2	Ref.	-0.12 (-0.22; -0.02)	-0.14 (-0.24; -0.04)	-0.13 (-0.24; -0.03)	0.03
			Monocytes		
Monocytes, G/l	<0.4	0.4-0.4	0.5-0.5	>0.5	
Age-sex adj.	Ref.	-0.21 (-0.33; -0.09)	-0.20 (-0.33; -0.08)	-0.33 (-0.46; -0.21)	<0.0001
Model 1	Ref.	-0.15 (-0.26; -0.03)	-0.13 (-0.25; -0.01)	-0.21 (-0.34; -0.09)	0.003
Model 2	Ref.	-0.07 (-0.17; 0.03)	-0.07 (-0.02; 0.03)	-0.11 (-0.22; -0.006)	0.05

Data are β-coefficients (95% confidence interval). Model 1 adjusted for age, sex, body mass index, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, diet, physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body fat; Model 2 additionally adjusted for ambulatory heart rate. LC = Leukocytes, Hs-CRP = high-sensitivity C-reactive protein.

Figure 1 Relationship between SDNN and inflammatory biomarkers

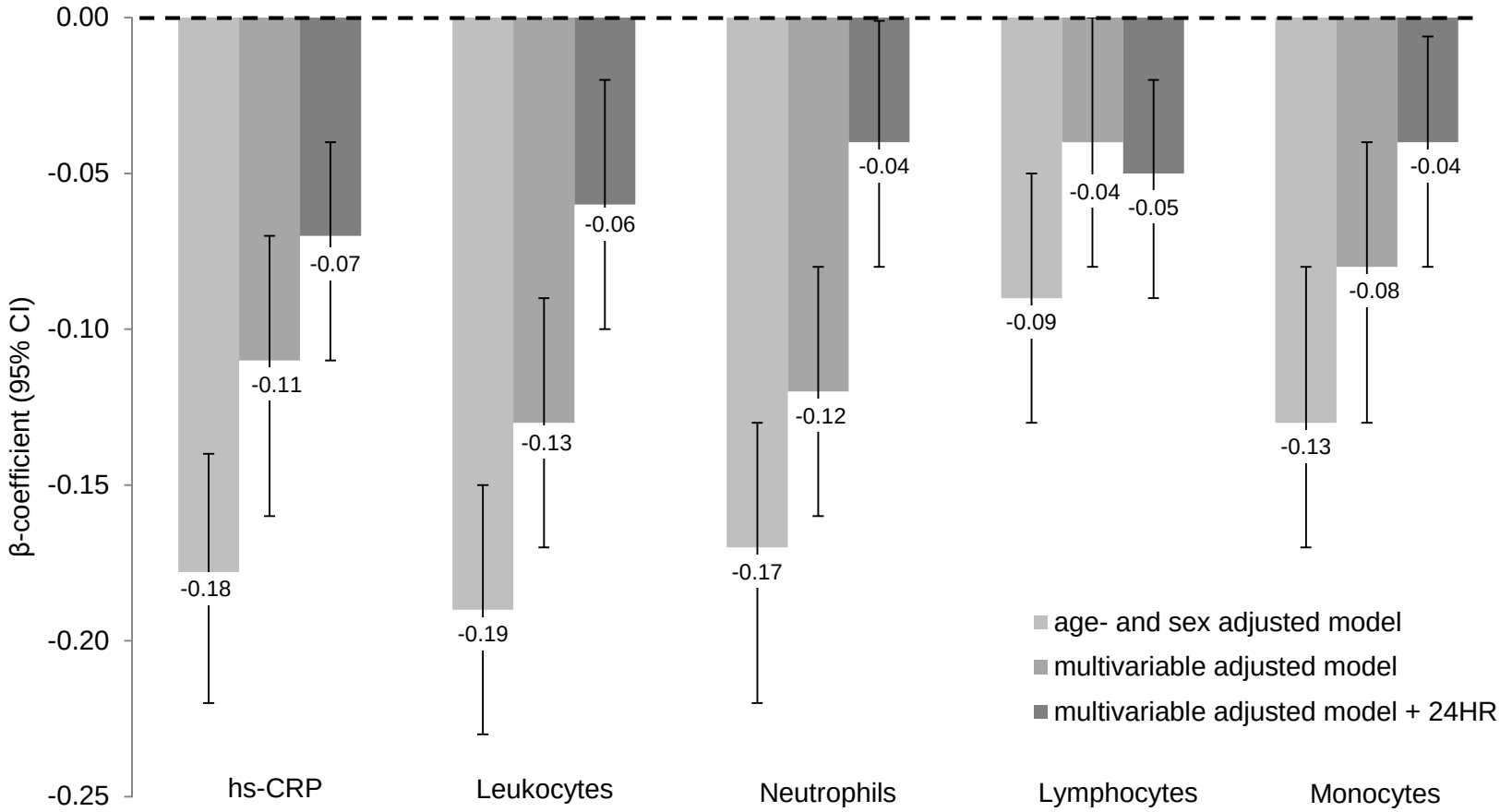


Figure 2 Relationship between normalized HF and inflammatory biomarkers

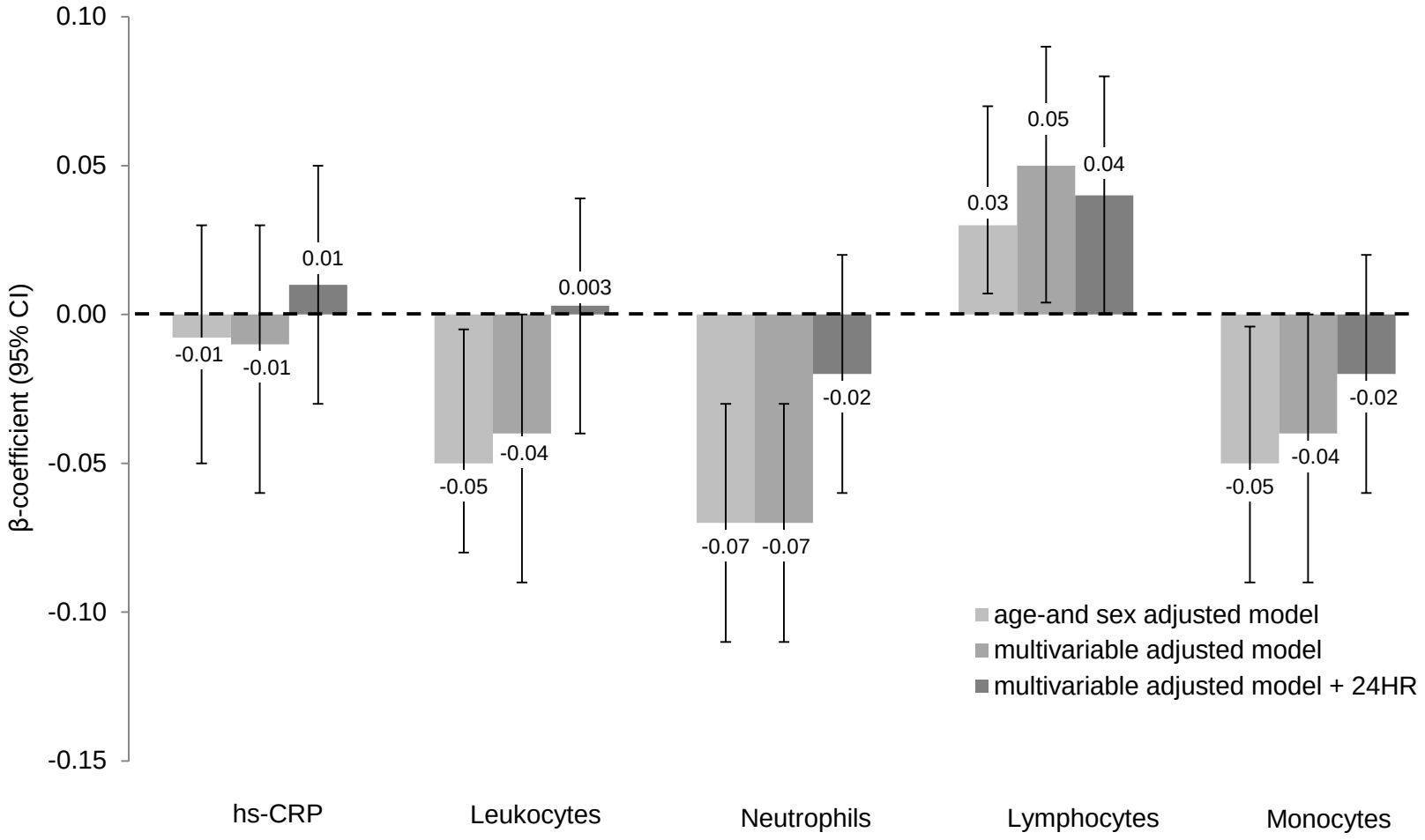


Figure 3 Relationship between normalized LF and inflammatory biomarkers

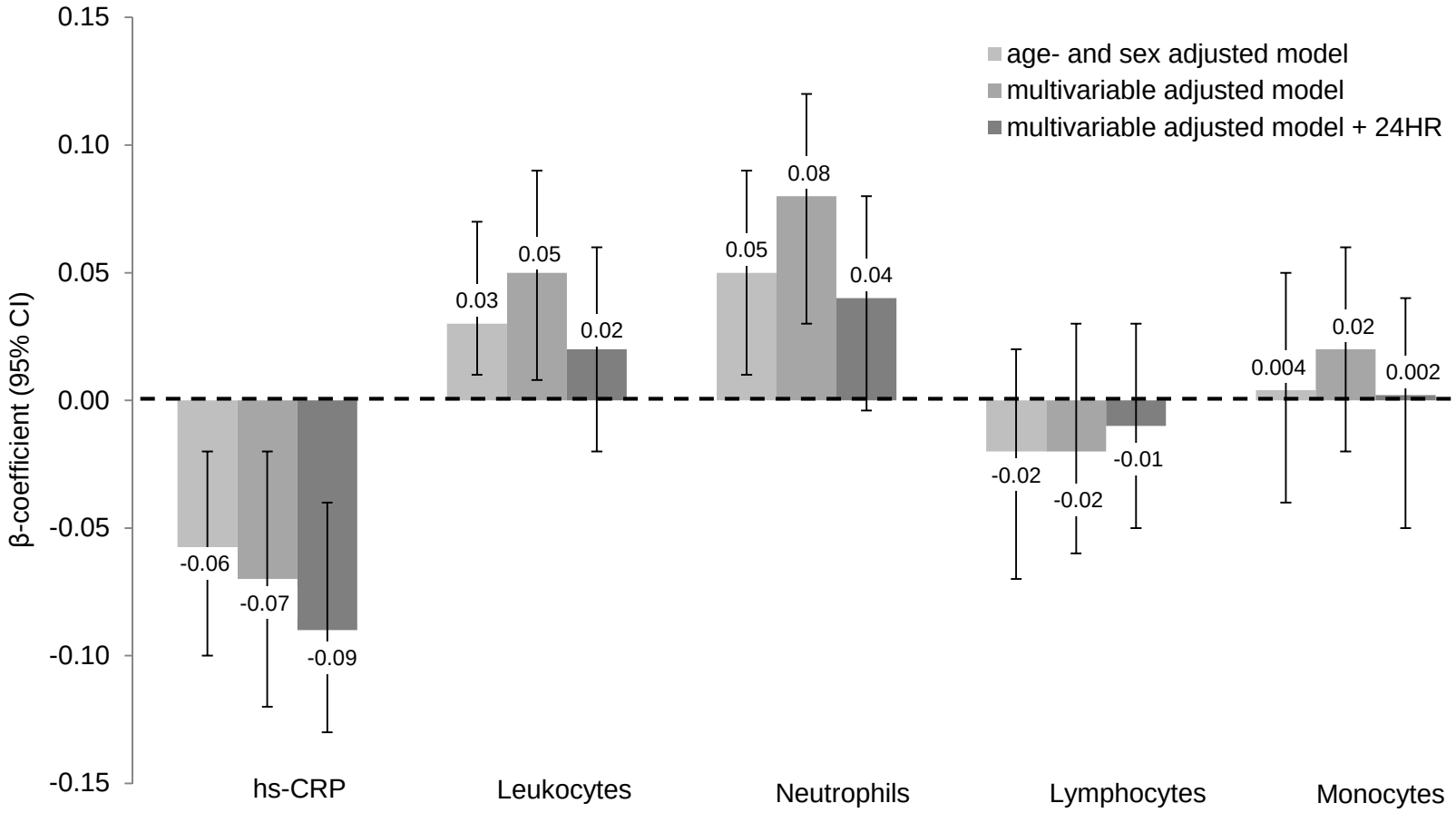
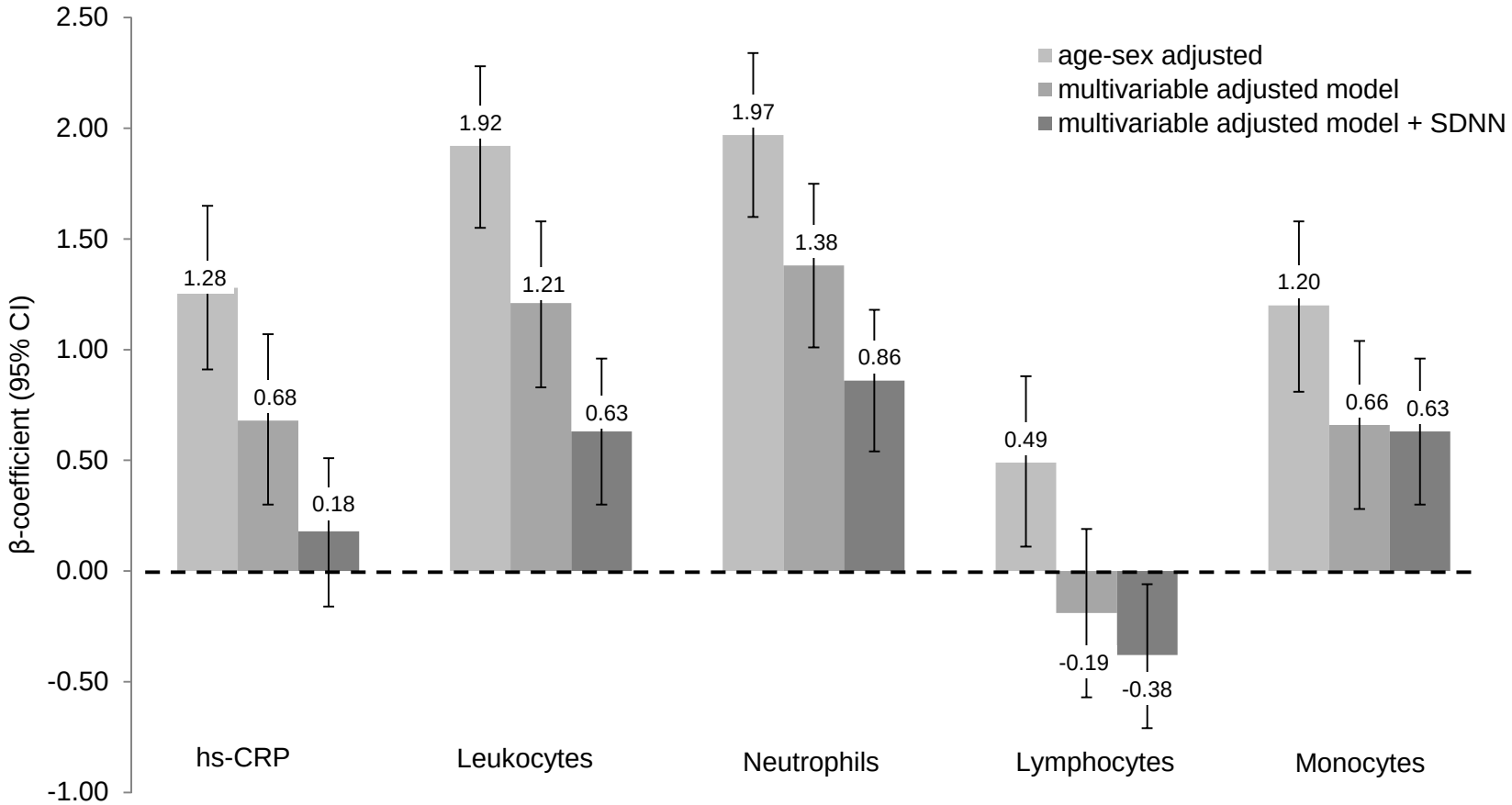


Figure 4 Relationship between ambulatory heart rate and inflammatory biomarkers



Supplement

Heart rate, heart rate variability and inflammatory biomarkers among young and healthy adults

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Figure legend

Figure S1 Relationship between total power and inflammatory biomarkers

Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. Hs-CRP = high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, systolic blood pressure, prediabetes, physical activity, low- and high density lipoprotein cholesterol and body fat. Model 3 was additionally adjusted for 24-hour heart rate. n = 2096.

Table S1 Total power across quartiles of inflammatory markers

n=2064	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value
			high-sensitivity c-reactive protein		
Hs-CRP, mg/l	<0.50	0.50-0.90	0.91-1.83	>1.83	
Age-sex adj.	Ref.	-0.14 (-0.25; -0.02)	-0.12 (-0.24; 0.00)	-0.44 (-0.55; -0.32)	<0.0001
Model 1	Ref.	-0.09 (-0.20; 0.02)	-0.01 (-0.14; 0.11)	-0.27 (-0.39; -0.15)	<0.0001
Model 2	Ref.	-0.06 (-0.14; 0.03)	-0.04 (-0.13; 0.06)	-0.15 (-0.25; -0.06)	0.001
			Leukocytes		
LC, G/l	<4.6	4.6-5.3	5.4-6.1	>6.1	
Age-sex adj.	Ref.	-0.12 (-0.23;-0.01)	-0.24 (-0.36; -0.12)	-0.48 (-0.59; -0.36)	<0.0001
Model 1	Ref.	-0.05 (-0.16; 0.06)	-0.11 (-0.22; 0.008)	-0.26 (-0.37; -0.14)	<0.0001
Model 2	Ref.	0.02 (-0.06; 0.11)	0.003 (-0.09; 0.09)	-0.07 (-0.16; 0.02)	0.07
			Neutrophils		
Neutrophils, G/l	<2.4	2.4-2.8	2.9-3.5	>3.5	
Age-sex adj.	Ref.	-0.11 (-0.22; 0.007)	-0.18 (-0.30; -0.007)	-0.49 (-0.60; -0.38)	<0.0001
Model 1	Ref.	-0.05 (-0.16; 0.06)	-0.08 (-0.19; 0.03)	-0.31 (-0.42; -0.19)	<0.0001
Model 2	Ref.	0.02 (-0.06; 0.11)	0.02 (-0.07; 0.10)	-0.08 (-0.17; 0.01)	0.05
			Lymphocytes		
Lymphocytes, G/l	<1.5	1.5-1.7	1.8-2.1	>2.1	
Age-sex adj.	Ref.	0.02 (-0.10; 0.14)	-0.07 (-0.18; 0.05)	-0.14 (-0.26; -0.02)	0.009
Model 1	Ref.	0.07 (-0.04; 0.18)	0.04 (-0.08; 0.15)	0.06 (-0.06; 0.18)	0.50
Model 2	Ref.	0.002 (-0.09; 0.09)	-0.04 (-0.13; 0.04)	0.05 (-0.05; 0.14)	0.41
			Monocytes		
Monocytes, G/l	<0.4	0.4-0.4	0.5-0.5	>0.5	
Age-sex adj.	Ref.	-0.14 (-0.26; -0.02)	-0.16 (-0.28; -0.04)	-0.32 (-0.44; -0.20)	<0.0001
Model 1	Ref.	-0.08 (-0.19; 0.03)	-0.07 (-0.18; 0.05)	-0.14 (-0.26; -0.01)	0.05
Model 2	Ref.	0.02 (-0.07; 0.10)	-0.00 (-0.09; 0.09)	-0.02 (-0.11; 0.08)	0.61

Data are β-coefficients (95% confidence interval). Model 1 adjusted for age, sex, body mass index, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, diet, physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body fat; Model 2 additionally adjusted for ambulatory heart rate. LC = Leukocytes, Hs-CRP = high-sensitivity C-reactive protein

Table S2 Normalized high frequency across quartiles of inflammatory markers

n=2064	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value
			high-sensitivity c-reactive protein		
Hs-CRP, mg/l	<0.50	0.50-0.90	0.91-1.83	>1.83	
Age-sex adj.	Ref.	0.01 (-0.10; 0.12)	0.09 (-0.03; 0.20)	-0.00 (-0.12; 0.11)	0.84
Model 1	Ref.	0.01 (-0.10; 0.12)	0.09 (-0.03; 0.21)	0.05 (-0.12; 0.13)	0.90
Model 2	Ref.	0.03 (-0.08; 0.13)	0.08 (-0.04; 0.19)	0.07 (-0.05; 0.18)	0.31
			Leukocytes		
LC, G/l	<4.6	4.6-5.3	5.4-6.1	>6.1	
Age-sex adj.	Ref.	-0.06 (-0.17; 0.05)	-0.07 (-0.18; 0.05)	-0.12 (-0.23; -0.07)	0.05
Model 1	Ref.	-0.07 (-0.18; 0.05)	-0.07 (-0.18; 0.05)	-0.12 (-0.23; 0.003)	0.08
Model 2	Ref.	-0.03 (-0.13; 0.08)	-0.008 (-0.12; 0.10)	-0.01 (-0.13; 0.10)	0.94
			Neutrophils		
Neutrophils, G/l	<2.4	2.4-2.8	2.9-3.5	>3.5	
Age-sex adj.	Ref.	-0.06 (-0.17; 0.05)	-0.08 (-0.19; 0.03)	-0.15 (-0.26; -0.04)	0.007
Model 1	Ref.	-0.06 (-0.17; 0.05)	-0.08 (-0.20; 0.03)	-0.15 (-0.27; -0.04)	0.01
Model 2	Ref.	-0.02 (-0.13; 0.08)	-0.03 (-0.14; 0.07)	-0.03 (-0.06; 0.08)	0.64
			Lymphocytes		
Lymphocytes, G/l	<1.5	1.5-1.7	1.8-2.1	>2.1	
Age-sex adj.	Ref.	0.04 (-0.07; 0.16)	0.04 (-0.07; 0.15)	0.04 (-0.08; 0.16)	0.55
Model 1	Ref.	0.06 (-0.05; 0.17)	0.06 (-0.05; 0.18)	0.07 (-0.05; 0.19))	0.29
Model 2	Ref.	0.02 (-0.08; 0.13)	0.02 (-0.08; 0.13)	0.07 (-0.05; 0.18)	0.27
			Monocytes		
Monocytes, G/l	<0.4	0.4-0.4	0.5-0.5	>0.5	
Age-sex adj.	Ref.	-0.12 (-0.23; -0.005)	-0.09 (-0.21; 0.02)	-0.15 (-0.27; -0.03)	0.03
Model 1	Ref.	-0.12 (-0.23; -0.009)	-0.09 (-0.21; 0.03)	-0.15 (-0.27; -0.03)	0.04
Model 2	Ref.	-0.07 (-0.18; 0.04)	-0.05 (-0.16; 0.06)	-0.09 (-0.20; 0.03)	0.21

Data are β-coefficients (95% confidence interval). Model 1 adjusted for age, sex, body mass index, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, diet, physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body fat; Model 2 additionally adjusted for ambulatory heart rate. LC = Leukocytes, Hs-CRP = high-sensitivity C-reactive protein.

Table S3 Normalized low frequency across quartiles of inflammatory markers

n=2064	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value
			high-sensitivity c-reactive protein		
Hs-CRP, mg/l	<0.50	0.50-0.90	0.91-1.83	>1.83	
Age-sex adj.	Ref.	0.009 (-0.11; 0.12)	-0.09 (-0.21; 0.04)	-0.13 (-0.25; -0.01)	0.008
Model 1	Ref.	-0.004 (-0.12; 0.11)	-0.10 (-0.23; 0.03)	-0.14 (-0.27; -0.01)	0.01
Model 2	Ref.	-0.02 (-0.13; 0.10)	-0.09 (-0.21; 0.03)	-0.19 (-0.31; -0.06)	0.0008
			Leukocytes		
LC, G/l	<4.6	4.6-5.3	5.4-6.1	>6.1	
Age-sex adj.	Ref.	0.11 (-0.002; 0.23)	0.07 (-0.05; 0.18)	0.11 (-0.003; 0.23)	0.14
Model 1	Ref.	0.12 (0.01; 0.24)	0.09 (-0.03; 0.20)	0.18 (0.06; 0.30)	0.01
Model 2	Ref.	0.10 (-0.01; 0.21)	0.04 (-0.07; 0.16)	0.10 (-0.01; 0.22)	0.18
			Neutrophils		
Neutrophils, G/l	<2.4	2.4-2.8	2.9-3.5	>3.5	
Age-sex adj.	Ref.	0.11 (-0.01; 0.22)	0.12 (0.006; 0.23)	0.12 (0.008; 0.24)	0.06
Model 1	Ref.	0.11 (-0.001; 0.23)	0.13 (0.01; 0.24)	0.17 (0.05; 0.29)	0.008
Model 2	Ref.	0.09 (-0.03; 0.20)	0.09 (-0.02; 0.20)	0.08 (-0.04; 0.20)	0.24
			Lymphocytes		
Lymphocytes, G/l	<1.5	1.5-1.7	1.8-2.1	>2.1	
Age-sex adj.	Ref.	-0.02 (-0.13; 0.10)	-0.02 (-0.13; 0.10)	-0.05 (-0.17; 0.07)	0.41
Model 1	Ref.	0.00 (-0.12; 0.12)	-0.01 (-0.13; 0.10)	-0.30 (-0.10; 0.13)	0.61
Model 2	Ref.	0.03 (-0.09; 0.14)	0.02 (-0.09; 0.13)	-0.02 (-0.15; 0.10)	0.61
			Monocytes		
Monocytes, G/l	<0.4	0.4-0.4	0.5-0.5	>0.5	
Age-sex adj.	Ref.	-0.01 (-0.13 ; 0.11)	0.01 (-0.11; 0.13)	0.02 (-0.10; 0.14)	0.66
Model 1	Ref.	0.002 (-0.11; 0.12)	0.04 (-0.09; 0.16)	0.07 (-0.06; 0.16)	0.23
Model 2	Ref.	-0.04 (-0.15; 0.08)	0.01 (-0.11; 0.13)	0.02 (-0.10; 0.14)	0.55

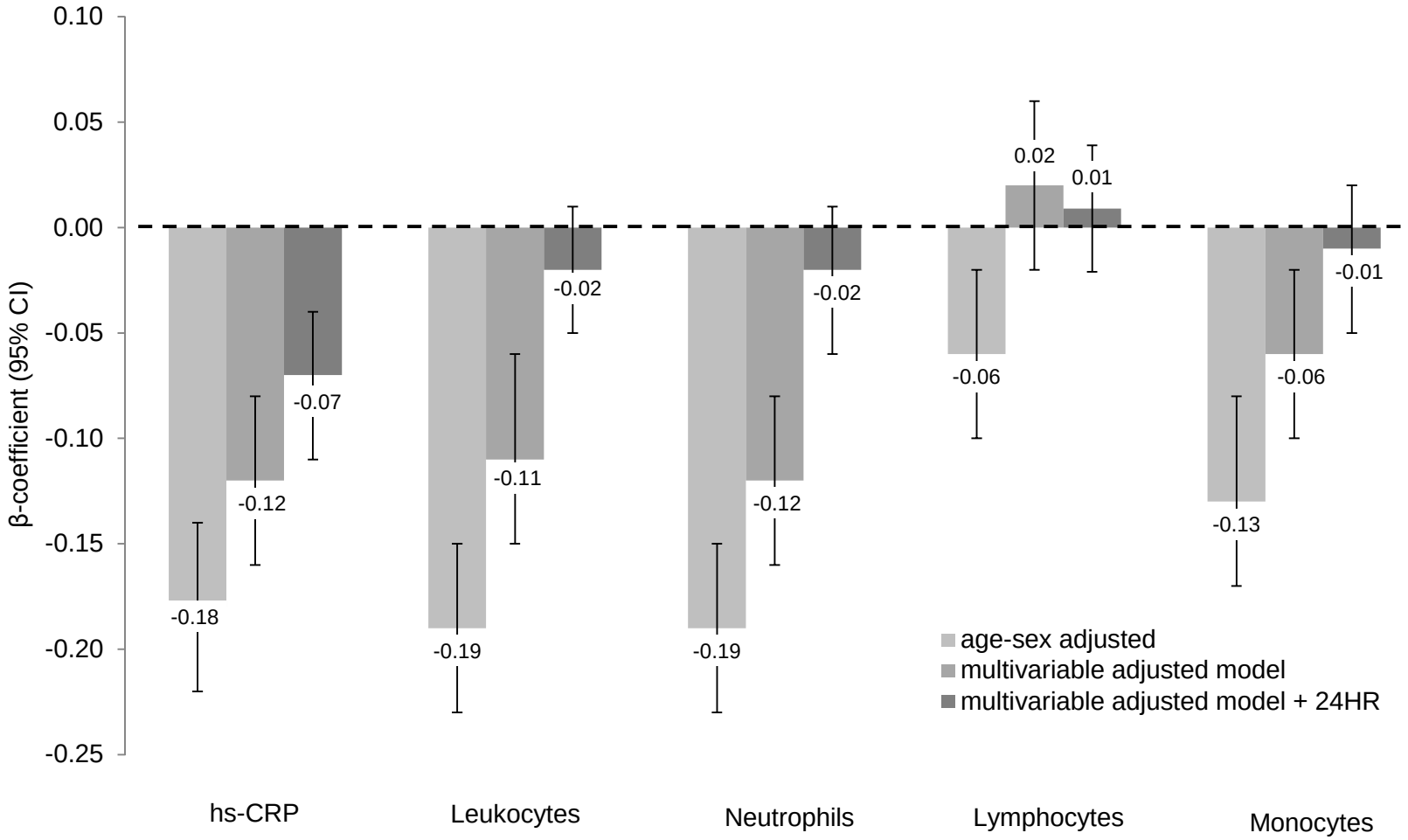
Data are β-coefficients (95% confidence interval). Model 1 adjusted for age, sex, body mass index, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, diet, physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body fat; Model 2 additionally adjusted for ambulatory heart rate. LC = Leukocytes, Hs-CRP = high-sensitivity C-reactive protein.

Table S4 Ambulatory heart rate across quartiles of inflammatory markers

n=2064	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value
			high-sensitivity c-reactive protein		
Hs-CRP, mg/l	<0.50	0.50-0.90	0.91-1.83	>1.83	
Age-sex adj.	Ref.	0.71 (-0.36; 1.78)	0.51 (-0.62; 1.65)	3.44 (2.34; 4.55)	<0.0001
Model 1	Ref.	0.45 (-0.55; 1.45)	-0.36 (-1.46; 0.74)	1.62 (0.81; 2.72)	0.002
Model 2	Ref.	0.21 (-0.66; 1.07)	-0.57 (-1.52; 0.37)	0.44 (-0.52; 1.40)	0.31
			Leukocytes		
LC, G/l	<4.6	4.6-5.3	5.4-6.1	>6.1	
Age-sex adj.	Ref.	1.53 (0.48; 2.59)	2.58 (1.49; 3.67)	4.33 (3.26; 5.40)	<0.0001
Model 1	Ref.	0.98 (-0.01; 1.97)	1.56 (0.52; 2.59)	2.69 (1.64; 3.74)	<0.0001
Model 2	Ref.	0.38 (-0.47; 1.23)	0.78 (-0.11; 1.68)	1.15 (0.23; 2.06)	0.01
			Neutrophils		
Neutrophils, G/l	<2.4	2.4-2.8	2.9-3.5	>3.5	
Age-sex adj.	Ref.	1.40 (0.34; 2.46)	2.41 (1.36; 3.46)	5.09 (4.04; 6.14)	<0.0001
Model 1	Ref.	0.99 (-0.00; 1.98)	1.33 (0.34; 2.32)	3.26 (2.25; 4.28)	<0.0001
Model 2	Ref.	0.42 (-0.44; 1.27)	0.76 (-0.10; 1.61)	1.86 (0.97; 2.74)	<0.0001
			Lymphocytes		
Lymphocytes, G/l	<1.5	1.5-1.7	1.8-2.1	>2.1	
Age-sex adj.	Ref.	-0.91 (-2.01; 0.19)	-0.88 (-1.95; 0.18)	1.07 (-0.04; 2.18)	0.003
Model 1	Ref.	-0.95 (-1.97; 0.06)	-1.13 (-2.14; -0.12)	-0.16 (-1.24; 0.92)	0.95
Model 2	Ref.	-1.25 (-2.13; -0.38)	-1.46 (-2.33; -0.59)	-0.72 (-1.65; 0.21)	0.23
			Monocytes		
Monocytes, G/l	<0.4	0.4-0.4	0.5-0.5	>0.5	
Age-sex adj.	Ref.	1.23 (0.04; 2.21)	0.42 (-0.71; 1.54)	1.49 (0.37; 2.62)	<0.0001
Model 1	Ref.	1.39 (0.39; 2.40)	0.93 (-0.13; 1.94)	1.72 (0.63; 2.81)	0.01
Model 2	Ref.	0.73 (-0.13; 1.60)	0.36 (-0.55; 1.27)	0.77 (-0.17; 1.71)	0.24

Data are β-coefficients (95% confidence interval). Model 1 adjusted age, sex, body mass index, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, diet, physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body fat; Model 2 additionally adjusted for the standard deviation of all normal RR intervals. LC = Leukocytes, Hs-CRP = high-sensitivity C-reactive protein.

Figure S1 Relationship between total power and inflammatory biomarkers



7.SUMMARY OF THE MAIN FINDINGS

The results of this project will be summarized in this chapter and illustrated in *Figure 6.*

7.1 Healthy lifestyle and heart rate variability

The validated lifestyle-score is based on seven cardiovascular health metrics and lifestyle factors, such as smoking, BMI, physical activity, diet, blood pressure, cholesterol and HbA_{1c}. The score is scaled from 0-7, where 0=very unhealthy and 7=very healthy. Around 5% of 2070 study participants had a very unhealthy score of 0 or 1 and 11% had a very healthy score of 6 or 7. Overall, a healthy lifestyle was more often adopted by women compared to men with a prevalence of 18% and 3%, respectively. There was a strong positive association of the lifestyle-score with HRV and an inverse relationship with ambulatory and resting HR. The relationship between HRV and the lifestyle-score was strongly attenuated after additional adjustment for either resting or ambulatory HR, however most relationships remained statistically significant. Never smoking cigarettes, regular physical activity and having a normal BMI were the strongest individual and independent predictors of SDNN.

7.2 Heart rate variability and sleep-related breathing disorders

Overall, 1255 participants without known OSA were included in this analysis. Among this young and relatively healthy population, 9.6% had an AHI defined sleep-related breathing disorder, where men were significantly more often affected compared to women (18% versus 3%). SDNN and TP were linear inversely associated with AHI and ODI categories. Moreover, normalized LF was positively associated across ODI categories. SDNN and TP were significantly associated with sleep-related breathing disorder, using either the AHI or ODI based definition. Additional adjustment for resting HR has weakened these relationships in order that only associations with ODI remained significant. The adjustment for ambulatory HR has even stronger attenuated these relationships and virtually all of them lost level of significance. Resting and ambulatory HR by itself was positively associated with AHI and ODI categories and sleep-related breathing disorders. However, only the relationships of ambulatory HR with AHI and ODI were associated independently of HRV.

7.3 Heart rate variability, heart rate and inflammation

We found a strong, linear and inverse relationship of SDNN with hs-CRP, LC, neutrophils, lymphocytes and monocytes, even after comprehensive multivariable adjustment. These results were strongly attenuated after additional adjustment for ambulatory HR, suggesting only little incremental information of HRV over and above HR. Normalized HF, mainly modulated by parasympathetic activity was inversely associated with neutrophils and

monocytes, however positively related to lymphocytes. In contrast, normalized LF, modulated by sympathetic and parasympathetic activity, was positively associated with neutrophils. There was a strong positive association between ambulatory HR and all available inflammatory biomarkers with the exception of lymphocytes.

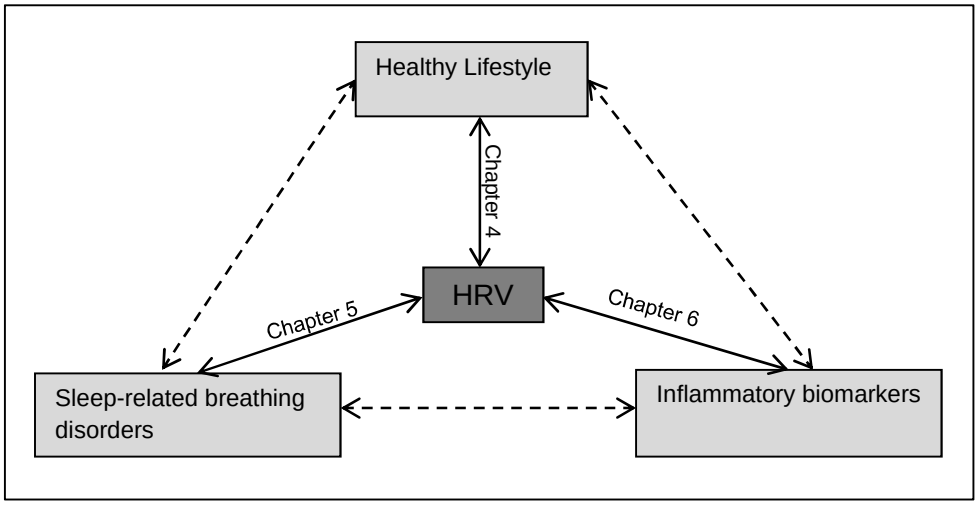


Figure 6 Simplified diagram of the study results.

Solid lines are showing new relationships; dashed lines are corresponding to associations based on current literature.

8.DISCUSSION

In the following paragraph our findings will be compared with the existing literature and potential underlying mechanisms will be described. Additionally, methodological and technical aspects will be critically elucidated, to avoid misunderstandings when interpreting the results. Finally, the relevance of this project and potential implications for further research will be outlined to even improve and deepen the knowledge in this field of research.

8.1 Subject-specific discussion

8.1.1 Heart rate variability and its meaning

It is well known that the ANS plays an important role in several processes of the cardiovascular system.[41] To find a way to measure the function of the ANS was therefore important to further investigate the role of the ANS in the development diseases. By performing intraneural recordings, activity of the ANS can be measured. Due to the complex intervention to obtain intraneural recordings, this approach is not useful in cohort studies. The HRV, as a relative easily measurable variable, is a suitable tool to estimate the function of the ANS. As elucidated in the background (chapter 1), HRV is based on the variation of the HR, which is determined by autonomic inputs to the sinus node. The HRV is a measure of the modulation of the HR and does not reflect autonomic tone.[43-45] Therefore, it is very important to distinguish between the terms “autonomic modulation” and “autonomic tone” (activity), which are often used interchangeably in the existing literature.[44]

An experimental study 30 years ago showed that the HF component of the HRV is mainly modulated by the parasympathetic activity. The parasympathetic induced modulation causes short cycle lengths (0.15-0.40 Hz) of the normal sinus rhythm. However, to interpret HF as a general measure of the parasympathetic tone is not fully correct and one has to be cautious when interpreting such results. Increased HF levels rather indicates an increased modulation of parasympathetic activity.[44] The LF component of the HRV is modulated by sympathetic and parasympathetic activity and the interpretation of this parameter is controversially discussed. Absolute LF and HF values are based on the total variance of HRV. Since total variance is depending on different factors (e.g. recording length of the ECG), changes in total variance influence both components (LF and HF) in the same direction.[43,84] Based on this phenomenon, it is crucial to normalize LF and HF to assess these components independently of the total variance.

In contrast to the frequency-domain variables, SDNN is a measure of the total variance and the circadian rhythm and cannot be assigned to a physiologic correlate, such as sympathetic or parasympathetic activity.[45] SDNN is very simple to calculate and commonly used in HRV analysis, even though the interpretation of the SDNN is limited. Moreover, SDNN has showed to be a strong predictor for cardiovascular outcomes and mortality over the last 30 years, which increases the importance of this variable.[49,85]

It should be highlighted that further research is needed in this area to better understand the physiologic correlate of the single HRV variables. This would allow a more accurate interpretation of the results.

8.1.2 Associations of HRV with lifestyle factors, sleep-related breathing disorders and inflammation

It is known that lifestyle-factors, cardiovascular health metrics, sleep-related breathing disorders and inflammation are associated with an increased risk of cardiovascular outcomes and mortality. However, the role of the ANS in these associations is unclear. Based on this evidence it was aimed to determine the association of lifestyle and lifestyle-related factors with the autonomic function, measured using HRV. As summarized in chapter 7, HRV was associated with lifestyle and cardiovascular health metrics, sleep-related breathing disorders and inflammatory biomarkers. Due to several possible influencing factors, the context of these associations is complex. To thoroughly think about possible confounders, mediators or potential modifying factors of each association is an enormously important process. The risk of residual confounding or over-adjustment exists and should to be minimized as best as possible. To investigate our research questions in a young and healthy population without established cardiovascular disease, reduces the risk of confounding factors.

<u>Heart rate variability, lifestyle factors, cardiovascular health metrics and potential underlying mechanisms</u>

HRV was found to be linearly associated with increasing lifestyle-score, suggesting an incremental effect of combined lifestyle factors on the autonomic function. We are not aware of other large population-based studies showing this association. We have checked the relationship for different potential effect modifier, such as sex or educational status. The results were consistent across all strata. The underlying mechanisms that connect an unhealthy lifestyle with the ANS are multifaceted and not completely understood. According

to our study, overweight, physical inactivity and smoking were the main drivers of this association.

Recent studies showed that smoking cigarettes can influence the ANS.[86-88] Fine particulate matter and nicotine may stimulate different cascades, leading to sympathetic overactivity. Impaired baroreceptor sensitivity with a deficient negative feedback-loop plays an important role in this context.[87,88] Moreover, a direct effect of nicotine on the central nervous system via nicotinic receptors is discussed.[86] Additionally, lung oxidative stress and inflammation, mainly triggered by fine particulate matter, might increase sympathetic nerve activity due to stimulation of receptors on lung C-fibers.[86] These triggered pathways may lead to the strong association between smoking cigarettes and the autonomic function.

Regarding the association of physical activity and HRV, our findings are in line with prior published studies, showing positive associations of HRV with physical activity.[58,60-62] Among middle-aged women randomized to different physical activity intervention programs over 6 months, time- and frequency domain HRV variables were significantly higher compared to controls without intervention. Even moderate activity was sufficient to significantly improve HRV.[62] One reason for the favorable effect of regular physical activity on the autonomic function may include beneficial functional and structural modifications of the cardiovascular system.[89] Moreover, regular physical activity has shown to have the ability to lower inflammation.[37,38] Since there is an interrelationship between inflammation and the ANS, the anti-inflammatory effect of physical activity might also contribute to a more balanced ANS in physically active individuals.

Further, BMI previously showed to be strongly associated with HRV. Based on data of the SAPALDIA study, the authors presented markedly lower HRV levels among overweight and obese participants compared to normal weight individuals.[60] However, obese but physically active participants had HRV values comparable to normal weight individuals. Losing weight based on a 12-week dietary program resulted in significantly higher HRV levels.[90] Thus, body fat mass, mainly visceral fat, could be involved in the relationship between BMI and HRV. In recent studies, inflammation has shown to be associated with BMI and WHR[36] as well as the ANS,[91,92] which supports the assumption that inflammation might play an important role in the association of BMI and HRV. Since inflammation is highly influenced by lifestyle factors and cardiovascular health metrics, it may play an important role in the relationship with the autonomic function.

Based on the linear relationship between the health metric score and the HRV, the different underlying pathways seem to add up their negative influence on the ANS.

Heart rate variability, sleep-related breathing disorders and potential underlying mechanisms

Among our young population without known OSA, AHI and ODI were significantly associated with HRV, mainly SDNN and TP. Additionally, ODI was positively associated with normalized LF, which is modulated by sympathetic and parasympathetic nerve activity. Normalized HF values were significantly higher among individuals with an AHI<5 compared to others. However, normalized HF was not associated with AHI and ODI after a comprehensive adjustment. Based on these findings, increased sympathetic activity, but not decreased parasympathetic activity might be assumed in this young and mainly asymptomatic population.

Population-based studies investigating the association of sleep-related breathing disorders with HRV are scarce. Few small studies have shown a markedly worse HRV profile among OSA patients compared to healthy controls.[67-69] This was confirmed in our study with participants without clinically overt OSA. The positive association of AHI and ODI with HRV is supported by another study that assessed autonomic activity using direct intraneural recordings. Compared to healthy controls, patients with overt OSA had markedly higher sympathetic nerve activity during the entire day.[93] Thus, repeated apneas among individuals with overt OSA followed by arousals are strongly associated with an increased sympathetic activity, potentially explaining the strong relationship between OSA and the occurrence of cardiovascular events. In contrast to our study, the current evidence in this field of research is mainly based on patients with a severe OSA, which could affect the comparability of the studies.

Patients with a manifest OSA have repetitive interruptions of the ventilation due to collapsing airways over at least 10 seconds.[16] Such interruptions might provoke stress reactions and activate arterial chemoreceptors, which in turn increase sympathetic activity via the brainstem (medulla oblongata).[94] These changes in the ANS activity might be reflected by the autonomic modulation and therefore the HRV. According to our data, differences in HRV profile are an early phenomenon in the pathophysiology of sleep-related breathing disorders and do exist already among healthy participants with early, subclinical stages of sleep-related breathing disorders. Whether there are long-term effects in individuals with early stages of breathing disorders during sleep is currently not known. However, it is plausible that modifications in direction of an autonomic dysbalance among individuals with early stages of sleep-related breathing disorders could have serious consequences later on. Future studies should explore ways on how to best approach individuals with subclinical forms of sleep-related breathing disorders.

Heart rate variability, inflammatory biomarkers and potential underlying mechanisms

Inflammatory biomarkers were independently associated with HRV even after comprehensive adjustment for lifestyle factors and other cardiovascular health metrics. This multivariable adjustment is very important, given the known relationships of lifestyle factors and cardiovascular health metrics with inflammation and HRV. Consistent with other studies, we could show an inverse association between SDNN and hs-CRP.[71,74,75] Our studies expand these prior studies by showing inverse associations with other inflammatory markers such as LC, neutrophils, lymphocytes and monocytes. Hs-CRP and LC are representing different inflammatory pathways. Therefore we hypothesize that different inflammatory pathways are involved in the relationship with the ANS.

Experimental studies have shown the expression of adrenoceptors on immune cells, assuming a direct influence of adrenoceptor agonists on the immune system.[95,96] As a consequence of sympathectomy, other studies could demonstrate reduced inflammatory reactions.[97] Moreover, vagal activity seems to have an anti-inflammatory effect, resulting in a down-regulation of pro-inflammatory cytokines.[91] In addition, immune cells may directly influence the ANS via the cytokine interleukin-1.[92]

Normalized HF, mainly modulated by parasympathetic activity, showed negative associations with neutrophils and monocytes. These inverse relationships may show an anti-inflammatory effect of vagal modulation or increased vagal modulation based on low immune cell count. In contrast, a positive association was found between normalized HF and lymphocytes. A previously published study among over 3300 patients scheduled for coronary angiography, could show an adverse effect for neutrophils and a protective effect of lymphocytes regarding the risk for cardiovascular events.[98,99] The strong association of hs-CRP with normalized LF and SDNN, but not with normalized HF is supported by another small study among middle-aged men,[74] suggesting an association with mainly sympathetic modulation of HR. Based on our data, it is not possible to fully explain the accurate underpinnings of these findings, which highlights the importance to perform further basic and clinical research to completely understand the interrelationship of individual inflammatory biomarkers and the ANS.

8.1.3 Heart rate variability, heart rate and its relationship

Similar to HRV, resting and ambulatory HR are associated with an increased risk of cardiovascular events in general populations.[76,77] Moreover, HR has shown to be an important predictor of mortality in diseased populations.[100,101] Among our young population,

resting and ambulatory HR were strong linearly associated with the lifestyle-score, sleep-related breathing disorders and inflammatory biomarkers.

As the term HRV already implies, HRV is based on two quantities: the HR and its variability. A certain dependence of both entities is therefore assumable. However, it is highly discussed to what extent HRV is depending on HR and whether HR is playing the principal role in the prognostic value of HRV. About 20 years ago, Tsuji et al. showed a tight inverse relationship between HR and time- and frequency domain HRV.[81] This strong association was resumed in recently published studies, explaining the physiological and mathematical interaction of the relationship between HR and HRV.[55,102] Monfredi et al. conducted an experimental study using different cardiac cells of healthy and diseased humans and animals.[55] Plotting HR and time-domain HRV of these preparations, they could graphically show an inverse, non-linear relationship *(Figure 4, page 8)*. The authors conclude to always consider changes of HR when investigating relationships with HRV.[55] Tsuij et al. could show similar relationships not only with time- but also with frequency-domain variables (HF and LF).[81] Simultaneously, they found that this relationship markedly differs across age decades with a strong relationship in young individuals and a weak inverse relationship among older individuals.[81] In other words, the dependence of HRV (mainly LF and HF) and HR is weaker in the elderly compared to the young.[81] This finding could be explained by the impaired autonomic modulation of the sinus node in the elderly. The age-dependent relationship between HR and HRV could be one reason for the strong attenuation of our relationships when adjusting for HR. Sacha et al. demonstrated that the prognostic value of HRV regarding cardiovascular outcomes is mainly based on HR.[102,103] However this assumption is based on complex mathematical models. The mentioned studies highlight the complexity of the relationship between HRV and HR. It is necessary to further investigate the additional benefit and the prognostic value of HR and HRV, independent of each other.

On the basis of the above mentioned studies, we have decided to additionally adjust all our analyses for resting and ambulatory HR. All relationships were strongly attenuated by the additional adjustment for HR. The attenuation of these results was stronger for 24-h HR compared to resting HR, in order that only few relationships remained significant. In contrast, adjusting the relationships between HR and lifestyle, inflammatory biomarkers or sleep-related breathing disorders with HRV, most relationships were attenuated but remained significant. We therefore suggest that HRV and mainly 24-h HR carry a high amount of common information. Whether HR is on the path of the relationship between the variables of interest and HRV remains unclear.

8.1.4 Prevalence of a healthy lifestyle and sleep-related breathing disorders

Adopting a healthy lifestyle is highly promoted by different institutions to reduce risk factors for morbidity and mortality. To estimate the current prevalence of people adopting a healthy lifestyle is of high interest. To be able to intervene and to launch new prevention programs, the current situation has to be known and analyzed. Among our young western European study population, only a minority of the population, namely 11%, has adopted a comprehensive healthy lifestyle (score of 6 or 7), which is based on the recommendations of the American Heart Association (AHA). Women had a higher lifestyle-score compared to men. Our results are in line with other studies among US populations, using the identical score to estimate lifestyle. According to data of the ARIC study, only 3% of the study population adopted a healthy lifestyle. Women and Caucasians were more likely to adopt a healthy lifestyle compared to men and African Americans.[8] Another US study, using data of the National Health and Nutrition Examination Survey (NHANES), showed an increasing prevalence of individuals with an unhealthy lifestyle over the last 20 years.[9] Consumption of a healthy diet had a very low prevalence in our study population. This result is supported by another study, where they also showed a low prevalence of people consuming a healthy diet.[9,104] In our cohort, the prevalence of men having an ideal BP was impressively low with 18% compared to 66% among women. Thinking about the high influence of high-normal BP and manifest hypertension on the risk for cardiovascular events,[105,106] it is crucial to intervene and to educate and sensitize the population.

As elucidated in the background, there is a close link between the occurrence of sleep-related breathing disorders, lifestyle factors and cardiovascular risk factors, especially BMI. According to data of the Wisconsin Sleep Cohort Study, the prevalence of sleep-related breathing disorders has markedly increased over the last 20 years among men and women.[107] This increase parallels the increasing prevalence of obesity.[9] Overall, 9.6% of our study participants had sleep-related breathing disorders, of whom 83% were male. The Epworth Sleepiness Scale, as an indicator for daytime sleepiness, was similar among participants with an AHI≥5 or an AHI<5, assuming that affected participants may be at an early, asymptomatic stage of the disorder. Based on data of the population-based CoLaus/PsyCoLaus study, Heinzer et al. found a prevalence of mild sleep-related breathing disorders (AHI≥5) of 83.8 and 60.8% among men and women, respectively.[15] In contrast to our study, the median age of the population was 57 years, individuals had more co-morbidities and the diagnosis was obtained by polysomnography. In accordance with other studies, we could show a distinct difference in the prevalence of sleep-related breathing disorders among both sexes. In the population investigated by Heinzer et al., this difference seems to diminish with the age of 60 years, assuming an influence of the hormonal status in

women.[15] This finding is supported by another study, showing that the prevalence of sleep-related breathing disorders among postmenopausal women with hormone replacement therapy was markedly lower compared to women without replacement therapy.[108] Due to the collapsing airways and the interruption of ventilation in patients with OSA, hypertension often co-exists.[16,18] We also made this observation in our young and relatively healthy population with subclinical and early forms of sleep-related breathing disorders. Based on the tight link between sleep-related breathing disorders, lifestyle and cardiovascular risk factors, it is very likely to lower the prevalence of both obesity and sleep-related breathing disorders when adopting a healthy lifestyle. Our results therefore implicate that promoting a healthy lifestyle should remain a key issue on the agenda of professional societies and governments.

As an example of a prevention program, the American Heart Association formulated public health goals, which target cardiovascular health metrics and lifestyle factors. The American Heart Association's 2020 impact goals have the aim to improve cardiovascular health by 20% and reduce deaths from CVD and stroke by 20% while targeting seven lifestyle factors and cardiovascular health metrics, which are known as Life's simple 7 behaviors.[109] Such nation-wide prevention programs, but also a lot of smaller interventions programs will be necessary in order to reduce cardiovascular risk and adverse cardiovascular outcomes in the general population.

8.2 Methodological aspects – Strengths, limitations and challenges

Conducting observational research is challenging and needs a lot of financial, technical and personal resources. Study investigators have to perform a balancing act between the feasibility of a study and the requirements and own ideas to investigate a certain research question. The feasibility of a study is of high importance and does often co-determine the choice of measurement instruments.[110] Repeatedly, important decisions have to be made to find a compromise between the ideal assessment of an item and the practicability of the measurement in the planned context. In case of the GAPP-study we have tried to assess items best possible without ignoring the feasibility and the time expenditure for the study participants. As the GAPP-study is a prospective study, it is of high important that participants remain motivated to participate in the study. In general, it is also crucial to use validated measurement instruments to be sure that the instrument measures what it is supposed to measure. All measurement procedures in our study were highly standardized using written standard operating procedures (SOP) to minimize the risk of systematic errors.

Moreover, all employees of the GAPP-study are familiar with Good Clinical Practice (GCP) and are working according the GCP guidelines.

Even though we gave our best to perform reliable research, several aspects have to be discussed regarding the methods of the study and the interpretation of our study results. In the following paragraphs, the assessment of main study variables will be discussed, as well as the main study limitations, the relevance of the study and finally further implications for research.

8.2.1 Measurement instruments and assessment of study variables

Assessment of heart rate variability

In this study, HRV was assessed over 24 hours, while participants performed their daily activities. Compared to short-term HRV recordings, the long-term HRV assessment is commonly known as the gold-standard. However there are plenty of studies, where HRV is assessed using short-term ECG recordings of 2, 5, 10 minutes or even 2 hours. A common convention about the duration of short-term recordings does not exist. However, at least 2 minutes of recording are necessary to assess the HF and LF components of HRV.[43] Since total variance of HRV is changing with the duration of ECG recording, the absence of a standardized short-term recording duration might be a disadvantage. To compare absolute HRV values across different individuals is therefore difficult.[43] Based on the change in total variance, the distribution of frequency-domain variables is markedly different in short-term recordings compared to long-term HRV recordings. In any case, one should be careful when comparing results of HRV analysis with different ECG recording durations and should be aware of this problem. However, independent of the recording length, short-and long-term HRV has shown to be associated with cardiovascular risk and events.[47,48,58]

Another important point to consider is to use of a well-functioning software to edit ECG recordings. Artefacts may have a crucial influence on HRV values, which makes the editing process enormously important. This process includes the removal of artefacts but also the redefinition of premature ventricular and atrial beats. The quality of the Holter ECG recordings in our study was very high, which resulted in an editing time of 10.2 minutes per Holter-ECG. Two researchers were trained by a cardiologist to perform the editing process. Trainings were regularly repeated in order to maintain high quality standards. Difficult cases were discussed together with an experienced electrophysiologist.

Due to feasibility reasons, study participants carried the 24-h ECG device simultaneously with a 24-h BP monitoring device. To wear both devices simultaneously could be impairing for some participants. Therefore, it is possible that the behavior of some individuals was different compared to normal days. Unfortunately, there are no possibilities of controlling for this behavior. Nevertheless, we are confident that this phenomenon is attenuated with increasing sample size.

The task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommend a recording duration of at least 18 hours for long-term ECG recordings.[43] In our study we have defined a cut-off of at least 80% of the maximal duration of 24 hours, which results in a cut-off of 19.2 hours. Participants with recording durations below this cut-off were asked and motivated to repeat the recording. Even though overall quality of the ECG recordings was very high, we had to exclude ECG recordings of 22 participants. Moreover, 20 participants have denied to perform a 24-h ECG or had technical device issue during 24-h ECG recording.

Assessment of physical activity and other lifestyle factors

Lifestyle factors, such as physical activity, diet and smoking status were self-assessed using standardized questionnaires. Questionnaires are commonly used in epidemiological studies with a large sample size and are a helpful tool to assess different items. Using questionnaires has several advantages and disadvantages, which should be taken into account before deciding for or against their use. Important advantages are the low costs, the easy way of distribution as well as the potential of saving time. Moreover, the questionnaires used in the GAPP-study were scanned and data were automatically entered in the dataset. One main disadvantage of using questionnaires is that the assessment is subjective and there remain a potential uncertainty. Recall and response bias, which also includes social desirability bias, may occur and are difficult to control.[111,112] Social desirability bias is based on the phenomenon that some individuals tend to answer what they think is more esteemed in the society. As an example, individuals might overestimate their physical activity because they know that being physically active is healthy. Even though these sources of uncertainty when using questionnaires, we expected that these potential biases can be minimized when increasing the number of study participants.

Diet was assessed using the official questionnaire of the Federal Office of Public Health (Swiss health survey 2007), where information about the frequency of fruit, vegetable, meat, fish or dairy consumption was assessed. While diet and smoking status are often ascertain

using questionnaires there are several options to assess physical activity.[113] The choice of one specific method to assess physical activity is depending on the research question and the resources and possibilities of the study. The short IPAQ is an international and commonly used questionnaire assessing habitual moderate and vigorous physical activity and time spent for walking and sedentary activities. The validity and reliability of the short IPAQ was evaluated in an international population aged 18 to 65 years and achieved acceptable measurement properties.[83] As always with questionnaires, one should be aware that collected data remain an approximation of the true result. The IPAQ is assessing information about the frequency and duration of physical activity, which could be misjudged by study participants. Moreover, seasonal changes might play an important role and could be included in the estimation of the participants.[114] Underreporting may occur if individuals do not remark physical activity (mainly moderate physical activity) in their daily routine. In contrast, individuals may include socializing, theoretical instructions or refreshments in the physical activity duration.

Lifestyle and cardiovascular health metrics score

Adopting a healthy lifestyle is highly promoted by professional societies and governments.[6,7] To investigate associations of a healthy lifestyle as a whole construct with different outcomes is therefore essential. As a consequence, different scores, based on lifestyle factors and/or cardiovascular health metrics, were constructed. Up to now, there is no consensus for the use of one specific score.

For our project, we have chosen a score, which is based on the American Heart Association (AHA) definition of ideal cardiovascular health metrics. As reported in the manuscript in chapter 4, this score consists of a broad set of cardiovascular health metrics and lifestyle factors, such as smoking, physical activity, diet, BMI, blood pressure, total cholesterol and HbA_{1c} levels. Until now, this score was used in different US cohort studies with middle-aged adults from the general population, where they showed a strong association with cardiovascular outcomes and mortality.[8,9] However, there remain several issues concerning the score that should be discussed in the following section.

BMI is a widely used variable to assess body weight in relation to height with a universal classification independent of sex. The most important disadvantage of the BMI is that body composition is not taken into account. Based on the higher average muscle mass of men compared to women, BMI in men is often overestimated. Using the BMI as a risk predictor is therefore not always reliable. Sex-specific calculations or cut-offs might be one solution.

WHR for example, an easily measurable marker, takes into account the distribution of body fat. Universal and sex-specific cut-offs for WHR exists. Several studies showed that WHR is a stronger predictor for future cardiovascular events compared to BMI.[115-117] Therefore, including WHR instead of BMI into the score should be considered as a meaningful alternative.

Total cholesterol is one of seven components of the lifestyle-score. Taking into account the strong association of LDL with cardiovascular outcomes,[118] the inclusion of LDL instead of total cholesterol could be a good option. For a sensitivity analysis, we have modified this score accordingly, using a LDL cut-off of 160 mg/dl. As presented in chapter 4, this modification had no consequences on the relationship between HRV and the lifestyle-score.

High BP is one of the most important cardiovascular risk factors. To include this strong risk factor in the score is therefore justified. However, it could be discussed whether conventional BP levels, measured in the doctor's office, or ambulatory 24-h BP levels are more useful and reliable. Ambulatory 24-h BP levels have shown to be a stronger predictor for cardiovascular outcomes compared to conventional BP.[119,120] Another study showed a high proportion of young individuals with a masked hypertension (27% among men), meaning that they have normal conventional BP, but an elevated ambulatory BP.[121] With regard of our young study population, a remarkable proportion of our study participants could be misclassified. Nevertheless, compared to conventional BP measurement, ambulatory 24-h BP monitoring needs markedly more time and financial resources and it seems therefore not realistic to commonly include 24-h ambulatory BP levels into the score.

In the original AHA based score, diet relies on five dietary items, including salt consumption, fish intake, fruit- and vegetable intake, consumption of sweet beverages and whole grain consumption. The questionnaire we used for our study does not assess information about the consumption of sweet beverages and whole grains. This forced us to modify the definition of an ideal diet. We are aware of this problem and cannot rule out that this modification might have an influence on our study results.

Assessment of sleep-related breathing disorders

Polysomnography is the current standard diagnostic tool for the diagnosis of sleep-related breathing disorders. This procedure is rather complex and expensive and is done stationary in a sleep laboratory. Polysomnography is a multichannel recording of electromyographic, electroencephalographic and respiratory activity together with an ECG to detect any breathing disorders during sleep.[16] Because of the financial resources, the sample size and

feasibility of the study, we have decided to instead perform a nighttime pulse oximetry including nasal airflow measurement, which can be handled by the study participants independently.

This portable device (ApneaLink, Resmed, USA) was validated in a validation study and data were compared to simultaneously recorded polysomnographic data.[122] AHI and ODI assessed using the ApneaLink device were compared to the AHI based on the polysomnography. The sensitivity for AHI was ≥ 90% with a very high specificity. The ODI had a good overall sensitivity (≥80%). However, there was a lower specificity, especially at lower polysomnographic AHI levels. This may lead to a higher number of false positive results.[122] In our analysis (chapter 5), 120 individuals had an AHI≥5 while 141 participants had an ODI ≥5. This difference might partly be reasoned by the lower specificity of the ODI. Despite we have not used the gold-standard method for the assessment of sleep-related breathing disorders, we are confident that the usage of the ApneaLink device including pulse oximetry and nasal airflow was the best possible solution to investigate our study research questions.

Nocturnal pulse oximetry and nasal air flow measurement was routinely implemented about one year after the official start of the baseline examination. Therefore, 610 participants were included in the study before having initiated this measurement. In addition, 127 participants have declined to perform this analysis or had artificial fingernails, which did not allow the nocturnal pulse oximetry. Unfortunately, among several participants the recording duration of either nocturnal pulse oximetry or nasal airflow measurement was too low, leading to the exclusion of these recordings (n=114). One reason for a shorter recording duration was a shift of the nasal cannula in order that nasal airflow could not be measured. To avoid this problem, we have instructed participants to fix the nasal cannula with tape on both cheeks. A few participants reported that they could only breathe through the mouth, which of course resulted in no recording time of the air flow measurement. Other participants reported of having lost the pulse oximetry device during night. Overall, the device was well tolerated of the participants and easy to handle.

8.2.2 Study limitations – Consequences for the interpretation of the results

The GAPP-study is a population-based cohort study in the Principality of Liechtenstein, which was designed to investigate the development of cardiovascular risk factors in an initially young and healthy population. The study population is large and well-characterized and we are not aware of another study with similar population characteristics. Based on our inclusion and exclusion criteria, we are confident that our study sample is representing a western European population in a given age range. Despite various strengths, several limitations have to be taken into account when interpreting the study results in order to avoid wrong conclusions and misunderstandings.

Based on the nature of cross-sectional data it is not possible to draw any causal assumptions. Prospective, longitudinal data are needed to make statements about the directionality of relationships. This is a very important issue to consider in order to avoid misunderstandings when interpreting the results. Causality is a complex term and different definitions exists.[123] In brief, causality means that an exposure is fully or partly causing a certain outcome. Causality is based on several criteria. Bradford Hill has established 'Hill's criteria for causation', which are consisting of strength (effect size), specificity, plausibility, consistency, biological gradient, temporality, coherence, experiment and analogy. These criteria should be met to have enough evidence for causality.[124] Thus, lot of research is needed to detect causal relationships.

When interpreting the study results, it is important to consider that the generalizability of our results is not certain. Even though we are confident that the GAPP study population is representing a general western European population in the given age range, it is uncertain if similar results could be expected in other populations. Generally, the generalizability of study results depends on several aspects, such as internal and external validity, the representativeness of the sample, potential bias and the statistical power.[125]

Study errors may happen. It is crucial to minimize the occurrence of random errors and to avoid systematic errors as efficiently as possible. The research process consists of different steps[126] where both types of errors may originate. To reduce the risk of errors, the individual research steps, such as selecting a sample, collecting or processing data, should be thoroughly studied when planning the study.[126] Since random and systematic errors have different consequences regarding the results, the discrepancies are further elucidated in this paragraph. Random errors are also known as 'noise' and have no directionality. Therefore it is expected that random errors will not have an effect on the results, especially with increasing sample size. In contrast, systematic errors or so called bias might have a high impact on the results and have to be avoided as best as possible.[127] There exist no valid

methods to correct for systematic errors. Bias may occur at different levels of the study, e.g. due to a poor recruitment strategy, unfavorable measurement procedures, uncalibrated devices, deficient data entry or data analysis. Selection bias is one of the most important biases that may occur in cohort and randomized studies. Based on our recruitment strategy and given circumstances in the Principality of Liechtenstein we are confident that we could minimize the risk of selection bias. First, we had the opportunity to invite all inhabitants of the Principality of Liechtenstein in the given age range due to a collaboration with local authorities. Second, since Liechtenstein is a rather small country and the study center is well located, it is assumable that all inhabitants are able to reach the study center in less than 20 minutes by car. Third, inhabitants of the Principality of Liechtenstein get a biannual health check by their general practitioner, which is paid by the government. Therefore, we expect no selection of individuals with less financial resources, who might have a high interest of getting a health check for free. In contrast, there was a certain selection regarding the age. It has to be noted that markedly more individuals around 40 years were participating in the study than younger individuals around 25 years. This might be explained by a higher health interest of older participants. A further potential bias might be the information bias, including observer and recall bias. Applying standardized procedures, we tried to minimize the risk of an observer bias. Moreover, it is an advantage to have an experienced study team in our study center.

As in any observational study, confounding is one of the most important problems and may lead to false associations. By definition, a confounder has an association with the independent and dependent variable but is not on the casual pathway of the relationship of interest.[128] Potential confounders have to be taken into account and need to be included into statistical models to adjust for them. The GAPP study population is well-characterized, which allows the adjustment for many various potential confounders. However, to be able to adjust for a certain confounder, researchers have to be aware of these possible confounders. Of course these variables also need to be assessed and available for calculations. Therefore, despite a well-performed characterization, residual confounding is nearly unavoidable. To minimize the risk and influence of residual confounding, investigators have to think about potential confounders when planning and setting up a study and of course before starting a statistical analysis.

8.2.3 Relevance, implication and perspective

Relevance and implications of the study

In our study population, the prevalence of a healthy lifestyle was relatively low, especially among men. These findings highlight the importance that promoting a healthy lifestyle in the general population should remain a key priority of governments and public health associations. Based on our data, public health associations may detect and analyze certain problems, which allow them to develop specific public health interventions. Educational programs, including presentations or workshops, could help to further educate the population regarding their lifestyle, the most important cardiovascular risk factors and the resulting consequences. It can be assumed that individuals of a western European population are well educated and are theoretically aware of the adverse consequences of an unhealthy lifestyle and cardiovascular risk factors. However, to increase the awareness regarding unhealthy habits and the willingness to modify habitual behaviors of young adults should further be promoted. On the other hand, the personal environment of every individual should be formed in order to keep the needed effort to be physically active or to consume a healthy diet as low as possible. Employers or health insurances may have great potential to increase the motivation or willingness of individuals to adopt a healthy lifestyle. Employers and insurances should both be interested in keeping their employees or clients as healthy as possible, which clearly results in a win-win situation for both sites.

The investigated relationships showed a strong link between the autonomic function and the investigated entities. This is even more impressive when thinking about the young and relatively healthy study population and shows that these links are an early and linear phenomenon. Early abnormalities in the function of the ANS might partly be responsible for the occurrence of cardiovascular events later on and are therefore important to further investigate. This current project was necessary to further develop new research questions and to analyze the association in a longitudinal setting in order to assess the directionality.

Outlook and recommendations for future studies

New research is based on current evidence and existing gaps in knowledge. Based on the results of our study, several new research questions can be develop with the aim to better understand the influence of possible predictors on changes in HRV over time, the meaning of the HRV and its relationship with HR, and finally to elaborate the potential role in clinical routine.

The current project is based on cross-sectional analyses and no assumptions about the direction of the association are allowed. However, since the GAPP-study is a prospective cohort study and the first follow-up is currently ongoing with 800 participants completed, it is foreseeable to perform longitudinal analysis to be able to investigate associations of the ANS and assessed variables in a longitudinal setting. First of all, it would be of great interest to observe the progression of individual HRV parameters and to investigate potential predictors for the change in HRV values over time. Longitudinal population based studies of an initially healthy population are rare and could therefore shed new light on HRV and the mechanisms of the ANS. Independent of HRV, observing the change in prevalence of the lifestyle-score and sleep-related breathing disorders over the years could be of high interest not only from a scientific point of view, but also from a public health point of view.

24-h HRV was associated with sleep-related breathing disorders. To check, whether this there is the same association during day- and nighttime would be interesting. Therefore, separate day- and nighttime analyses are desirable and should be performed in the future. Moreover, we have the opportunity to prospectively analyze this association. Population-based studies in this area are very rare, so that we could highly contribute to improve knowledge in this area.

Two new measurement instruments were integrated into our study procedure 1) to measure physical activity over several days using an activity tracker and 2) to estimate psychological well-being using a validated questionnaire. Participants are wearing the activity tracker on their non-dominant wrist in form of a bracelet, which is mainly counting the steps performed. Using this device, we hope to better evaluate physical activity as we have now the reported habitual physical activity as well as the measured activity. First of all, it would be interesting to compare reported activity versus measured activity. In a second step the role of measured physical activity on the change of HRV should be investigated. Besides, to investigate psychological well-being as a potential predictor of the change in HRV would be interesting, in order to check the influence of the psychological status on physiological processes.

With the recent publication of Monfredi et al.,[55] where they showed a strong inverse relationship between HR and HRV, this topic became of high interest, even though Tsuij et al.[81] described similar associations 20 years ago. Considering their thoughts as well as our study results, HR and HRV seem to have a lot of common information. In contrast to HR, individual HRV variables have a physiological correlation and are therefore directly linked to the ANS.[43] However, it is important to point out that mainly the HF component of the HRV could be experimentally assigned to parasympathetic modulation.[129] The evidence for the physiological correlation of other HRV variables is rather poor. Thus, the question rises why

all these HRV variables are absolutely needed. In order to take this question up, it is necessary and would be of high interest to better evaluate the benefit and the additional information of HRV variables over HR. Mainly the complex frequency domain variables have some potential and should further be researched. Since there exists several HRV variables, it would additionally be relevant to investigate whether all HRV variables are necessary or if some variables have the same content or meaning.

Further studies are needed to investigate the utility of the HRV in the clinical routine. HRV values are depending on various physiological factors as well as the recording time. Comparisons of absolute HRV levels between individuals are therefore difficult, even though some reference levels exist.[130] However, HRV might have prognostic impact when observing it over time in the same patient. The observation of the progression of HRV might allow statements about the course of the patient's health. To clarify this assumption and the potential in the clinic, clinical studies should be planned to assess the relationship between the relative change in HRV and clinical outcomes. Moreover short-term HRV might become an important screening tool for paroxysmal atrial fibrillation, which could help to decide whether further accurate evaluation is needed. Though, it is essential to point out that a lot of research and resources are needed to integrate HRV one day into clinical routine.

Finally, basic research should be supported, in order to better understand the influence of autonomic dysbalances on the development of CVD. Moreover, the development of new therapeutic approaches that directly intervene and modify the ANS in direction of a more balanced system are needed and may probably prevent future cardiovascular events.

8.3 Conclusion

In order to evaluate the multifaceted association between lifestyle and lifestyle-related factors with the autonomic function, we have assessed the relationship of HRV with a healthy lifestyle, sleep-related breathing disorders and inflammatory biomarkers in a young and relatively healthy population. Based on the strong associations with HRV, we suggest interrelationships between the ANS and these entities. Not only HRV, but also resting and 24-h HR was strongly associated with these independent factors. Adjusting the associations of HRV and lifestyle, sleep-related breathing disorders and inflammatory biomarkers with HR, most relationships lost level of significance. Therefore it is assumable that most of the information of HRV seems to be contained in mainly 24-h HR and the incremental information of HRV parameters was modest. Even though the directionality of our association cannot be proven, these data may allow some insights in the pathophysiology of CVD

occurrence. Additionally, the adoption of a healthy lifestyle was rather low in this young population, which underscores the importance of healthy lifestyle promotion in the society. Based on this research project new research questions can be developed to further understand the role of the ANS in the relationship between lifestyle and lifestyle-related factors and the increased risk for cardiovascular events.

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CURRICULUM VITAE

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Educational Qualifications

2012-2016	PhD Candidate in Sport Sciences, University of Basel, Switzerland Thesis “Lifestyle and heart rate variability in general population” Supervisor: Prof. Dr. med. D. Conen. MPH
2013-2016	PhD program in Public Health at the Swiss School of Public Health (SSPH+), Basel, Switzerland
2009-2011	Master in Exercise and Health Sciences, University of Basel, Switzerland Thesis “Einfluss eines 12-wöchigen Rehabilitationsprogramms (KARAMBA) auf die HRV und HR-Recovery bei Patienten mit koronarer Herzkrankheit“ Supervisor: Dr. rer. nat. Melissa Jehn, Prof. Dr. med. Arno Schmidt-Trucksäss
2006-2009	Bachelor in Sport Sciences (Major) and Biology (Minor)

Professional Experience

2011-	Research Fellow, Cardiovascular Research Institute Basel, University Hospital Basel, Switzerland
2011	Exercise therapist KARAMBA, cardiac rehabilitation program, University Hospital Basel
06.2011-09.2011	Internship biomechanics, Rennbahnklinik, Muttenz

07.2010-08.2010	Internship exercise therapist, cardiac rehabilitation, Seewis
11.2008-01.2009	Internship sport physiology, Federal office for sport (BASPO), Magglingen

Awards

2016	Pfizer Forschungspreis (CHF 15'000)
2015	Young Investigator Award, Congress of the European Congress of Cardiology, London; Runner-up (CHF 1'000)
2012	Poster Prize, Swiss Med Lab Congress, Bern (CHF 500)

Editorial Activity – Review of manuscripts

Journal of the American Heart Association

Teaching

Supervision and support of medical master students

Additional Qualifications

Languages:

German	native language
English	good (TOEFL 2012)
French	fair
Italian	fair

Software Skills:

Operating Systems: MS Windows

Data analysis: SAS, R, STATA

Good Clinical Practice (GCP): 2014

Publications (peer-reviewed)

1. Krisai P., Leib S., **Aeschbacher S.**, Kofler T., Assadian M., Maseli A., Todd, J., Estis J., Risch M., Risch L., Conen D. Relationships of iron metabolism with insulin resistance and glucose levels in young and healthy adults. European Journal of Internal Medicine. 2016, accepted.
2. Kofler T, Bossard M, **Aeschbacher S**, Tabord A, Ruperti Repilado FJ, van der Lely S, Berger S, Risch M, Risch L, Conen D. The interrelationship of birth weight, inflammation and body composition in healthy adults. European Journal of Clinical Investigation. 2016.
3. Ruperti Repilade JF, **Aeschbacher S**, Bossard M, Schoen T, Gugganig R, van der Stouwe JG, Krisai P, Kofler T, Buser A, Risch M, Risch L, Müller C, Conen D. Relationship of N-Terminal fragment of Pro-B-Type Natriuretic Peptide and copeptin with erythrocytes-related parameters: A population-based study. Clinical Biochemistry. 2016.
4. **Aeschbacher S**, Bossard M, Ruperti Repilado FJ, Good N, Schoen T, Zimny M, Probst-Hensch NM, Schmidt-Trucksäss A, Risch M, Risch L, Conen D. Healthy lifestyle and heart rate variability in young adults. Eur J Prev Cardiol, 2015.
5. **Aeschbacher S***, Metin F*, Bossard M, Schoen T, von Rotz M, Mettler H, Abächerli R, Risch M, Risch L, Conen D. Relationships of electrocardiographic parameters with ambulatory hypertension in young and healthy adults. International Journal of Cardiology. 2015;202:300-4.

   *equal contribution
6. Van der Stouwe JG., **Aeschbacher S.**, Krisai P., Schoen T., Meyre P., Todd J., Estis, J., Risch M., Risch L., Conen D. Plasma Levels of Glucagon-Like Peptide 1 and markers of obesity among young and healthy adults. Clinical Endocrinology. 2015;83(5):636-42.
7. Schoen T., Hohmann E.M., van der Lely S., **Aeschbacher S**., Reusser A., Risch L., Risch M., Conen D. Plasma copeptin levels and ambulatory blood pressure characteristics in healthy adults. Journal of Hypertension. 2015;33(8):1571-9.
8. Bossard M, Pumpol K, van der Lely S, **Aeschbacher S,** Schoen T, Krisai P, Lam T, Todd J, Estis J, Risch M, Risch L, Conen D. Plasma endothelin-1 and cardiovascular risk among young and healthy adults. Atherosclerosis. 2015;239(1):186-91.

9. Zimmermann AJ, Bossard M, **Aeschbacher S**, Schoen T, Voellmin G, Suter Y, Lehmann A, Hochgruber T, Pumpol K, Sticherling C, Kuhne M, Conen D, Kaufmann BA. Effects of sinus rhythm maintenance on left heart function after electrical cardioversion of atrial fibrillation: implications for tachycardia-induced cardiomyopathy. The Canadian journal of cardiology. 2015;31(1):36-43.

10. Krisai P, **Aeschbacher S**, Schoen T, Bossard M, van der Stouwe JG, Dorig L, Todd J, Estis J, Risch M, Risch L, Conen D. Glucagon-like peptide-1 and blood pressure in young and healthy adults from the general population. Hypertension. 2015;65(2):306-12.

11. **Aeschbacher S**, Schoen T, Bossard M, van der Lely S, Glattli K, Todd J, Estis J, Risch M, Mueller C, Risch L, Conen D. Relationship between high-sensitivity cardiac troponin I and blood pressure among young and healthy adults. American journal of hypertension. 2015;28(6):789-96.

12. Blum J, **Aeschbacher S,** Schoen T, Bossard M, Pumpol K, Brasier N, Risch M, Risch L, Conen D. Prevalence of prediabetes according to hemoglobin A1c versus fasting plasma glucose criteria in healthy adults. Acta diabetologica. 2015;52(3):631-2.

13. **Aeschbacher S**, Schoen T, Clair C, Schillinger P, Schonenberger S, Risch M, Risch L, Conen D. Association of smoking and nicotine dependence with pre-diabetes in young and healthy adults. Swiss medical weekly. 2014;144:w14019.

14. Conen D, **Aeschbacher S**, Thijs L, Li Y, Boggia J, Asayama K, Hansen TW, Kikuya M, Bjorklund-Bodegard K, Ohkubo T, Jeppesen J, Gu YM, Torp-Pedersen C, Dolan E, Kuznetsova T, Stolarz-Skrzypek K, Tikhonoff V, Schoen T, Malyutina S, Casiglia E, Nikitin Y, Lind L, Sandoya E, Kawecka-Jaszcz K, Mena L, Maestre GE, Filipovsky J, Imai Y, O'Brien E, Wang JG, Risch L, Staessen JA. Age-specific differences between conventional and ambulatory daytime blood pressure values. *Hypertension*. 2014

15. Marti-Soler H, Gubelmann C, **Aeschbacher S**, Alves L, Bobak M, Bongard V, Clays E, de Gaetano G, Di Castelnuovo A, Elosua R, Ferrieres J, Guessous I, Igland J, Jorgensen T, Nikitin Y, O'Doherty MG, Palmieri L, Ramos R, Simons J, Sulo G, Vanuzzo D, Vila J, Barros H, Borglykke A, Conen D, De Bacquer D, Donfrancesco C, Gaspoz JM, Giampaoli S, Giles GG, Iacoviello L, Kee F, Kubinova R, Malyutina S, Marrugat J, Prescott E, Ruidavets JB, Scragg R, Simons LA, Tamosiunas A, Tell GS, Vollenweider P, Marques-Vidal P. Seasonality of cardiovascular risk factors: An analysis including over 230 000 participants in 15 countries. *Heart (British Cardiac Society)*. 2014;100:1517-1523.

16. Bossard M*, **Aeschbacher S*,** Schoen T, Hochgruber T, von Rotz M, Blum J, Risch M, Risch L, Conen D. Serum bilirubin levels and risk of prediabetes in young and healthy adults. *International journal of cardiology*. 2014;171:e24-25.

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17. Conen D, Schon T, **Aeschbacher S**, Pare G, Frehner W, Risch M, Risch L. Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors (gapp). *Swiss Med Wkly*. 2013;143:w13728.

Active Conference Participation

2015	Poster presentation: 'Sleep-related breathing disorders and heart rate variability in young and healthy adults from the general population'. Congress of the American Heart Association (AHA), 2015, Orlando
2015	Abstract Presentation Young Investigator Award Session (Population Sciences): 'A healthy lifestyle is strongly related to an increased heart rate variability in healthy adults.' Congress of the European Society of Cardiology (ESC), 2015, London
2014	Moderated poster: 'Healthy Lifestyle and heart rate variability in general population'. Congress of the American Heart Association (AHA) 2014, Chicago.
2014	Poster presentation: 'Thyroid-stimulating hormone and premature atrial contractions in a young population'. Congress of the European Society of Cardiology (ESC), 2014, Barcelona
2013	Moderated poster: 'Relationship between copeptin and nocturnal blood pressure in young and healthy adults'. Congress of the European Society of Cardiology (ESC) 2013, Amsterdam
2012	Poster presentation: 'Current smoking and prediabetes in young and healthy adults'. Congress of the European Society of Cardiology (ESC) 2012, Munich

2012 Oral presentation: Body mass index, muscle mass and NT-proBNP levels in healthy adults. Swiss MedLab 2012, Berne