# Human longevity : crosstalk between the brain and periphery

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# CHAPTER 11

# CHARACTERISATION OF HEART RATE RHYTHMS AND THEIR VARIABILITY IN AGEING AND FAMILIAL LONGEVITY

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# ABSTRACT

Age related cardiac changes occur in everyone, but not at the same rate. Here, we compared heart rate rhythms and their variability in offspring from long-lived siblings enriched for familial longevity (offspring) versus age-matched controls, and in young versus older adults. Heart rate (HR) and heart rate variability (HRV) were continuously measured over three consecutive days in a total of 28 offspring, 23 age-matched aged controls and 19 young controls (mean age 65, 66 and 23 years respectively). To take the differences in the awake/ sleep rhythms into account, HR and HRV were calculated during 24h, and also during awake and self- reported sleep periods separately.

Heart rate rhythms and HRV were not different in offspring compared to middle- aged controls. While the difference between HR during awake and sleep periods ($HR_{awake}$ – $HR_{sleep}$), was not different between offspring and controls, the young participants had a higher difference compared to middle- aged controls (P<0.001). The mean (standard error (SE)) standard deviation of NN intervals (SDNN) over 24- hours was higher in young (108.5 (6.3)) compared to the older adults (83.7 (6.3)) (P=0.01). Similar results were obtained for NN50 and pNN50 over 24h and also during awake and sleep periods.

Thus, we observed beat-to-beat heart rate variability were increased with age but not with familial longevity.

# INTRODUCTION

Cardiovascular health, like many other biologic processes, is modified by time. Age-associated changes in cardiac autonomic function (autonomic dysfunction) [1] have been linked with increased risk for a number of cardiac outcomes, including left ventricular hypertrophy, hypertension, heart failure, ventricular arrhythmias and sudden cardiac death [2-6]. Autonomic dysfunction has been suggested as a common pathway leading to increased morbidity and mortality from cardiovascular diseases [7]. Changes in cardiac autonomic function can be non- invasively estimated by quantifying sympathetic and parasympathetic inputs by measuring beat-to-beat changes in heart rate (heart rate variability (HRV)).

Existing literature show that HRV is believed to decline with age [8, 9]. Although age related changes occur in everyone, these do not occur at the same rate [10]. Since cardiovascular ageing is influenced by genetic, lifestyle and environmental factors, it thus would be imperative to investigate heart rate rhythms in relation to ageing and longevity, since longevity has a genetic component. Epidemiological studies in both model organisms and humans have focused on identifying mechanisms of healthy longevity, and the autonomic nervous system has been identified as playing a role, since it has been implicated in the onset of many adverse cardiovascular events.

The Leiden Longevity Study (LLS) was designed to investigate factors associated with human longevity [11]. Offspring of long- lived nonagenarian siblings, who are predisposed to become long-lived were included. Their partners with whom most have had a relationship and shared environment for decades, were included as age- and, environment- matched controls. These offspring carry 50% of the genetic advantage of their long- lived parent and were shown to have a 35% lower mortality rate compared to their birth cohort [11], and thus are genetically enriched for exceptional longevity. Offspring were observed to have a lower prevalence of cardiovascular diseases, myocardial infarctions, hypertension, type 2 diabetes, as well as preserved insulin sensitivity and resistance to cellular stress, compared to controls of similar chronological age [11-13]. Most studies of the association between heart rate variability and age have included subjects based on calendar age. We aimed to decipher whether heart rate and its variability is also modified by familial longevity as well as calendar age.

Here for the first time, we investigated associations of continuously measured 72- hour heart rate rhythms and HRV with propensity for longevity, and (chronological) age.

# METHODS

## Ethics statement

The present study was approved by the Medical Ethical Committee of Leiden University Medical Center under protocol P11.116. All investigations were conducted according to the principles of the Declaration of Helsinki. All study participants gave written informed consent.

## Study participants

Participants for the current study were derived from the Switchbox study, which is a satellite study of the Leiden Longevity Study (LLS). The LLS is a family based study consisting of 421 families with at least two long-lived siblings (men ≥ 89 years, women ≥ 91 years) of Dutch descent, without any selection on demographics or health [14].

For the present study, 28 offspring from long-lived siblings and 23 middle- aged controls (partners from offspring) from the LLS were included. Inclusion criteria for Switchbox study included being aged 55–77 years, having a stable body mass index (BMI) between 19 and 33 kg/m2 and having no significant chronic disease or medication use known to influence any hormonal axis. To enhance the contrast in familial longevity between groups, controls with a nonagenarian parent who had one or more nonagenarian siblings were excluded (based on telephone questioning). Exclusion criteria have been described in details elsewhere [15]. Additional inclusion criteria specific for this study were being free- living during the 5- day study period, and having good quality ECG recordings. Good quality ECQ recordings was defined as having ≤ 20% average artefacts on 5-day ambulatory ECG recording.

Additionally, we further recruited 19 young healthy volunteers from the general population via advertisement, using the same exclusion criteria that were used for the middle- aged participants, except for age, which was set at 18 – 30 years.

## Experimental protocol

At the study center, height, weight, body composition, and waist and hip circumference were measured, and body mass index (BMI) was calculated. Body composition was measured using a bioelectrical impedance analysis (BIA) meter at a fixed frequency of 50kHz (Bodystat® 1500 Ltd, Isle of Man, British Isles). Timing and duration of sleep was assessed using detailed diaries that were filled in by the participants, as well via the Pittsburg sleep quality index (PSQI) questionnaire [16] that was filled in by the participants during the study.

Fasted blood samples were collected for baseline measurement of glucose, HbA1c, cholesterol and HDL cholesterol. After blood withdrawal, the serum tubes were kept at room temperature to clot and immediately centrifuged when the samples were clotted. Serum was aliquoted into 500µl tubes and promptly stored at -80°C. HbA1c was measured on a Primus Ultra 2 HPLC analyzer (Trinity Biotech, Bray, Ireland), using Boronate affinity separation. Glucose levels were measured using fully automated equipment with the Hitachi Modular P800 from Roche Diagnostics (Almere, the Netherlands). Coefficient of variation for measurements ranged between 0.9–3.0%.

### ECG monitoring

Each participant was assigned to an Equivital EQ02 lifemonitor, which was worn over five consecutive days. The EQ02 (Equivital EQ02, Hidalgo, UK) continuously measures ECG, as well as breathing rate, skin and core body temperature, and tri- axial accelerometry. ECG was measured on two channels via three electrodes. The EQ02 monitoring system consisted of an LM 1000 Life monitor sensor electronic module ((SEM), Life monitor belts of varying sizes, a SEM lead and a charging dock, and an Equivital Manager to configure SEMs and to download and export data. For this study, SEMs were configured in clinical mode, and data reported retrospectively at local time. A lifemonitor belt, whose size was chosen based on the participant's body size and fit held the SEM onto the participant's body. It's fabric (lycra) -based electrodes were moistened with water before making contact with the participant's skin [14]. SEMs were disconnected from the belt after 12 hours of recording, for charging for approximately one hour, after which SEMs were reconnected. Upon study completion, SEM data was uploaded onto the Equivital manager; from where date- and time- stamped ECG data (raw and in mV), inter-beat interval (in sample period and in milliseconds), and summary data of all vital signs were extracted and exported. Circadian HR and HRV were calculated from the ECG.

### Data and artefact management

Raw data from EQ02, which were automatically time- and date stamped (date, time in hr., min., sec. & ms.), was extracted from the EQ02 using the Equivital manager and uploaded to the Vivosense modular physiological monitoring and analysis platform (Vivonoetics, San Diego, USA). Via the cardiac layout, EQ02 data were visualized for inspection of each ECG channel and derived R- wave markings. This layout also contained accelerometer data channels for contextual interpretation and artefact identification, raw SEM waveforms and quality control.

After removal of charging times, the ECG data was cleaned using automatic cleaning methods in Vivosense, by selecting the timeframe to be cleaned, and the automatic cleaning module and setting the lower HR limit to 30 and the upper limit to 220. For the analysis contained in this study, only files containing less than 20% artefacts were selected for HR analysis and files with less than 2% artefacts were used for HRV analysis. Thus, data from days 2-5 was used for the HR analysis, whereas the 24- hour data with than 2% artefacts were used for HRV analysis.

Five-minute trends (averages) of HR, RR and HRV parameters in time domain were extracted for analysis. Five- minute HR averages of less than 30 beats per minutes were considered non- physiological (probably caused by undetected artefacts) and excluded from analyses. Furthermore, eight HRV indices, namely, average of NN intervals (ANN), standard deviation of NN intervals (SDNN), standard deviation of 5 minute averages of NN intervals (SDANN), standard deviation of successive differences of NN intervals (SDSD), square root of the mean squared differences of successive intervals (RMSSD), mean of the standard deviation of 5 minute NN intervals (SDNNi), number of adjacent NN intervals with a difference less than 50ms (NN50) and ratio of a NN50 to total number of NN intervals (pNN50) were extracted from Vivosense for analysis .

## Statistical analysis

All data are presented as mean with standard errors (SE). Groups were compared using linear mixed effects models, with adjustment for sex. To take the differences in awake/ sleep rhythms into account, we studied first the continuous rhythm over three days and then the rhythms during awake periods and sleep periods separately, based on the self-reported sleep data provided by the participants. Differences between HR on awake and sleep periods were compared between offspring, partner and young using unpaired

T-tests with Welch's correction. All statistical analyses were performed using SPSS v.20 for Windows (SPPS Inc., Chicago, IL, U.S.A.). Graphs of 24h HR and HRV trajectories were drawn using GraphPad Prism version 5 (GraphPad, San Diego, CA). Two-sided p-values below 0.05 were considered statistically significant.

# RESULTS

Characteristics of the young individuals from the general population and middle- aged individuals with propensity for longevity and their age-matched controls from the Leiden Longevity Study are summarized in table 1. To study the relation between heart rate rhythms and familial longevity, participants were selected on the basis of the age of their parents. Thus the parents of the offspring were significantly older than those of the middle- aged controls (P<0.001) and those of the young controls of whom most parents are still alive (P<0.001). The mean age and cardiovascular risk factors were not significantly different between the middle aged controls and the middle aged participants with propensity for longevity.

The number of females was lower in the middle aged with propensity for longevity (46%) compared to middle aged controls (56.5%) and to the young group (63.2%). All subsequent analyses were therefore adjusted for sex. In all groups, BMI and serum parameters were all within the normal range.

## Heart rate rhythms

The heart rate rhythms were measured continuously over three consecutive days. Figure 1 shows the 3-day heart rate rhythms for one representative offspring enriched for familial longevity (Figure 1A), one representative middle-aged control (Figure 1B), and one representative young participant (Figure 1C).

**TABLE 11.1 |** Description of study subjects

| | Leiden Longevity Study | | General population |
|---|---|---|---|
| | **Middle- aged Offspring N=28** | **Middle- aged Controls N=23** | **Young Controls N=19** |
| **Age of parents*** | | | |
| Age of father | 90.0 (9.2) | 72.8 (12.6) | 57.1 (6.4) |
| Age of mother | 88.25 (10.0) | 76.7(17.6) | 54.7 (3.4) |
| **Anthrometrics** | | | |
| Age, years | 65.38 (6.7) | 65.7 (6.9) | 22.9 (2.1) |
| BMI kg/m$^2$ | 25.65 (3.9) | 26.4 (4.4) | 23.2 (2.4) |
| Lean mass | 52.9 (10.9) | 51.1 (9.4) | 58.1 (11.0) |
| Fat, kg | 23.3 (7.0) | 26.6 (10.1) | 14.6 (4.9) |
| Female (%) | 13 (46.4) | 13 (56.5) | 12 (63.2) |
| **Serum parameters** | | | |
| Glucose, mmol/l | 5.5 (0.4) | 5.5 (0.5) | 4.9 (0.4) |
| HbA1c, mmol/mol | 34.8 (2.0) | 35.6 (39) | |
| Cholesterol, mmol/l | 5.9 (0.9) | 5.7 (0.9) | 4.7 (0.9) |
| HDL cholesterol | 1.8 (0.6) | 1.7 (0.6) | 1.7 (0.5) |
| **Cardiovascular risk** | | | |
| Alcohol n(%) | 1 (3.6) | 1 (4.3) | 2 (10.5) |
| Systolic BP | 151.3 (18.5) | 155.7 (19.9) | 119.2 (13.0) |
| Diastolic BP | 91.3 (10.7) | 91.0 (10.8) | 75.5 (8.73) |
| Anti-hypertensives | 4 (14.3) | 5 (22.7) | 0 (0) |
| CVD n(%) | 1 (3.6) | 2 (8.7) | 0 (0) |
| **Activity** | | | |
| Working full time n(%) | 16 (57.1) | 16 (69.6) | 19 (100) |
| Hours worked per day | 5.7 (8.2) | 4.4 (2.1) | 8 (1.3) |
| Sleep duration(hours) | 7.58 (0.93) | 7.28 (0.96) | 7.82 (1.19) |
| Motion** | 36.7 (66.8) | 36.7 (52.7) | 37.6 (58.9) |

*Data presented as mean (standard deviation) unless otherwise stated. BMI: Body mass index. CVD: cardiovascular disease.*

** Age of parents indicate age at death or age at moment the questionnaire was taken.*

***Motion indicate mean tri-axial body movement per five minutes as measured by the equivital monitor.*

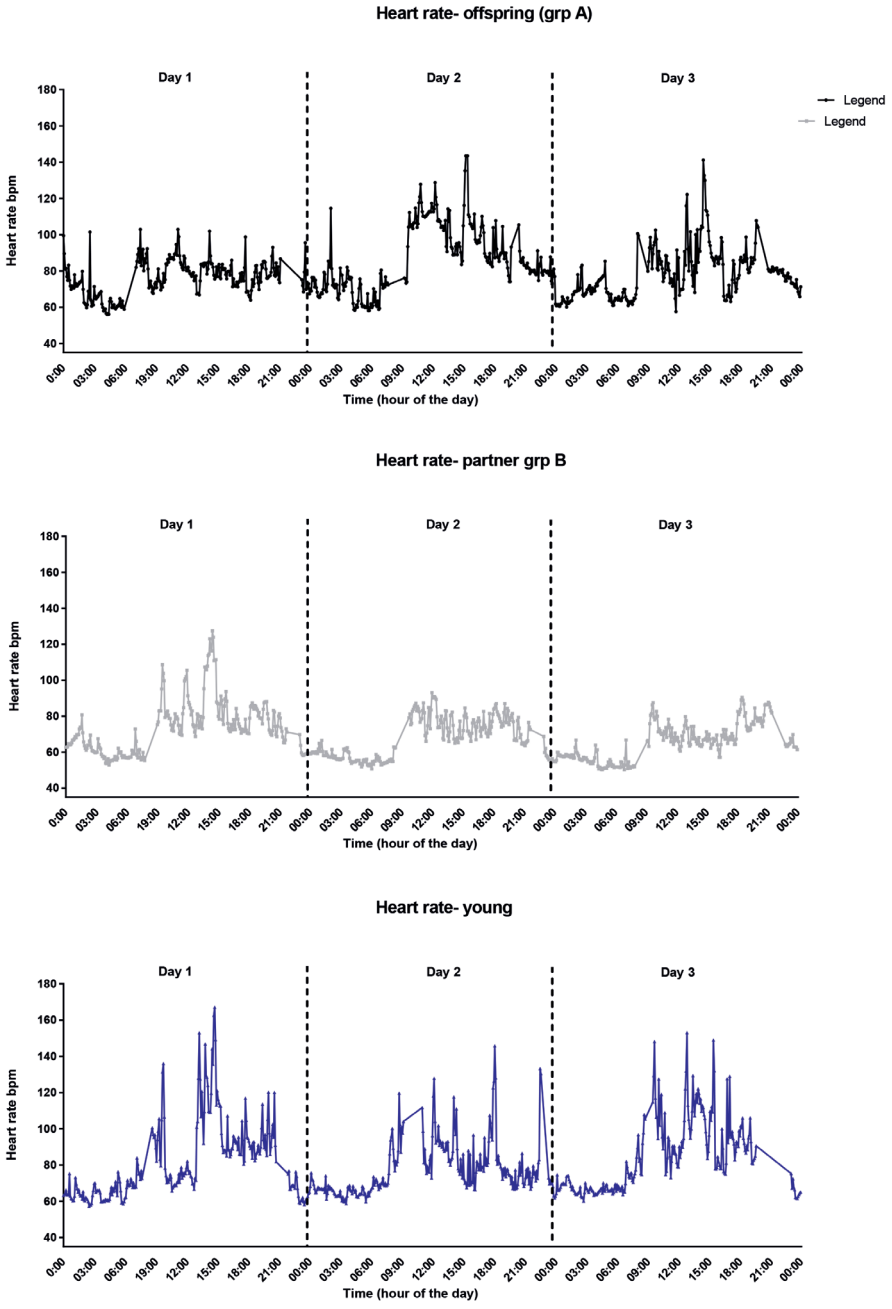


**FIGURE 11.1 |** 3-day heart rate rhythm of one representative participant each from the offspring, middle- aged controls, and the young.

## Heart rate: familial longevity

To test if heart rate rhythm is modified by familial longevity, we compared the continuously-measured 5- minute HR averages in offspring enriched for longevity to those of age-matched controls. As shown in table 2, the mean HR for the three- day period, and during awake and sleep periods was not significantly different in offspring compared to age-matched controls. The mean HR over the entire three days and when stratified to awake and sleep periods was 70.8, 76.1 and 62.6 beats per minute (bpm) respectively for the offspring and 71.4, 75.2 and 62.6 bpm respectively for the middle- aged controls. We further measured the difference between HR during awake and sleep periods, as a measure of fitness and adaptability. The mean ($HR_{awake}$ – $HR_{sleep}$) difference in HR did not significantly differ in offspring compared to controls (P=0.150).

**TABLE 11.2 |** Mean heart rate in groups that differ in familial longevity or calendar age.

| | Leiden Longevity Study | | General population | *P*-value* | *P*-value** |
|---|---|---|---|---|---|
| | Middle- aged Offspring N=28 | Middle aged controls N=23 | Young controls N=19 | | |
| Heart rate (HR) | | | | | |
| Over 3- days | 70.80 (1.4) | 71.43 (1.8) | 70.15 (2.0) | 0.760 | 0.633 |
| Awake | 76.13 (1.7) | 75.22 (1.9) | 78.03 (2.2) | 0.803 | 0.335 |
| Sleep | 62.56 (1.2) | 62.61 (1.6) | 59.72 (1.8) | 0.798 | 0.229 |
| $HR_{awake}$ – $HR_{sleep}$ | 13.57 (2.1) | 12.61 (2.5) | 18.31 (2.7) | 0.150 | <0.001 |

*Data presented as mean with standard error.*
** P-value for difference between offspring with propensity for longevity and middle aged controls.*
***P-value for difference between young and middle aged controls.*
*All analyses were adjustment for gender.*

## Heart rate: Chronological age

Table 2 also shows the result of the comparison of continuously- measured 5- minute HR averages in young participants and middle aged control participants, to assess if heart rate rhythm is modified by chronological age. The mean HR for the three- day period, during awake and during self- reported sleep periods was not significantly different in young

compared to middle aged controls. The mean HR over the entire three days and when stratified to awake and sleep periods was 71.4, 75.2 and 62.6 bpm respectively for the middle- aged controls and 70.2, 78.0 and 59.7 bpm respectively for the young controls. However, when the difference between HR during awake and sleep periods was compared in young versus middle aged controls, the mean (HRawake – HRsleep) difference in young was significantly higher in young compared to middle aged controls (P<0.001).

## Heart rate variability

The differences in variability in 24h heart rate between offspring compared to middle- aged controls and middle- aged controls compared to young was assessed using five parameters of heart rate variability, namely SDNN, RMSSD, SDNNi, NN50 and pNN50. The results are presented in table 3.

### Heart rate variability: familial longevity

To assess which parameters of heart rate variability associated with familial longevity, we compared HRV parameters between offspring and middle- aged controls, as shown in table 3. For the whole 24- hour period, the mean SDNN, RMSSD SDNNi and SDANN were consistently but not statistically significantly lower in the offspring compared to middle- aged controls. Conversely, NN50 and pNN50 seemed to be higher in the offspring, although not statistically significant. SDNN, RMSSD, NN50 and pNN50 showed a trend of increased variability during self- reported sleep- time compared to awake periods, in both offspring and middle- aged controls.

### Heart rate variability: Chronological age

We also compared parameters of heart rate variability in middle- aged controls to young controls in order to test which parameters of 24- hour HRV associated with chronological age. As depicted in table 3, there were no significant differences in RMSSD, SDNNi and SDANN in middle- aged controls compared to young controls, neither during the full 24- hour period, nor during awake or sleep- time. On the other hand, after adjustment for sex, younger participants had higher mean (standard error (SE)) SDNN compared to the older adults (108.5 (6.3) versus 83.7 (6.3), P=0.011. Similar difference was seen in SDNN during awake periods but not during sleep.

**TABLE 11.3 |** Parameters of 24- hour heart rate variability in groups of different calendar age and familial longevity

| | Leiden Longevity Study | | General population | *P*-value* | *P*-value** |
|---|---|---|---|---|---|
| | Middle- aged Offspring N=10 | Middle aged Controls N=12 | Young Controls N=12 | | |
| Measure of total HRV | | | | | |
| SDNN | | | | | |
| 24hr | 76.8 (8.1) | 83.7 (6.3) | 108.5 (6.3) | 0.634 | **0.011** |
| Awake | 72.7 (7.6) | 78.2 (6.6) | 104.3 (6.7) | 0.685 | **0.011** |
| Sleep | 81.1 (12.5) | 95.5 (1.5) | 112.0 (11.3) | 0.511 | 0.307 |
| Measures of short- term HRV | | | | | |
| SDNNi | | | | | |
| 24hr | 276.6 (99.5) | 403.5 (213) | 575.9 (238) | 0.438 | 0.598 |
| Awake | 207.0 (133) | 486.9 (131) | 196.5 (160) | 0.137 | 0.157 |
| Sleep | 403.8 (146) | 248.0 (516) | 1054.2 (513) | 0.336 | 0.265 |
| SDANN | | | | | |
| 24hr | 124.5 (65.2) | 235.5 (66.2) | 171.9 (71.5) | 0.329 | 0.542 |
| Awake | 72.5 (77.0) | 279.2 (76.0) | 122.2 (86.5) | 0.120 | 0.103 |
| Sleep | 220.6 (106) | 93.6 (117.3) | 270.2 (116) | 0.399 | 0.291 |
| Measures of vagal activity | | | | | |
| RMSSD | | | | | |
| 24hr | 68.5 (11.6) | 83.6 (9.2) | 95.4 (9.2) | 0.427 | 0.368 |
| Awake | 61.7 (11.3) | 78.0 (10.0) | 90.0 (10.1) | 0.367 | 0.406 |
| Sleep | 76.8 (17.2) | 95.9 (15.4) | 98.5 (15.1) | 0.482 | 0.905 |
| NN50 | | | | | |
| 24hr | 96.1 (25.7) | 79.4 (21.7) | 192.8 (21.9) | 0.652 | **0.002** |
| Awake | 84.2 (25.7) | 78.9 (27.7) | 181.3 (24.7) | 0.887 | **0.009** |
| Sleep | 110.0 (32.2) | 82.3 (27.2) | 200.8 (27.4) | 0.553 | **0.007** |
| pNN50 | | | | | |
| 24hr | 0.160 (0.042) | 0.112 (0.036) | 0.297 (0.037) | 0.422 | **0.002** |
| Awake | 0.122 (0.039) | 0.109 (0.039) | 0.255 (0.040) | 0.808 | **0.018** |
| Sleep | 0.207 (0.054) | 0.121 (0.045) | 0.340 (0.045) | 0.276 | **0.003** |

*Data presented as mean with standard error. *P-value for difference between young and middle aged from the general population, with adjustment for gender. ** P-value for difference between middle aged from the general population and middle- aged with propensity for longevity. All analyses were adjustment for gender.*

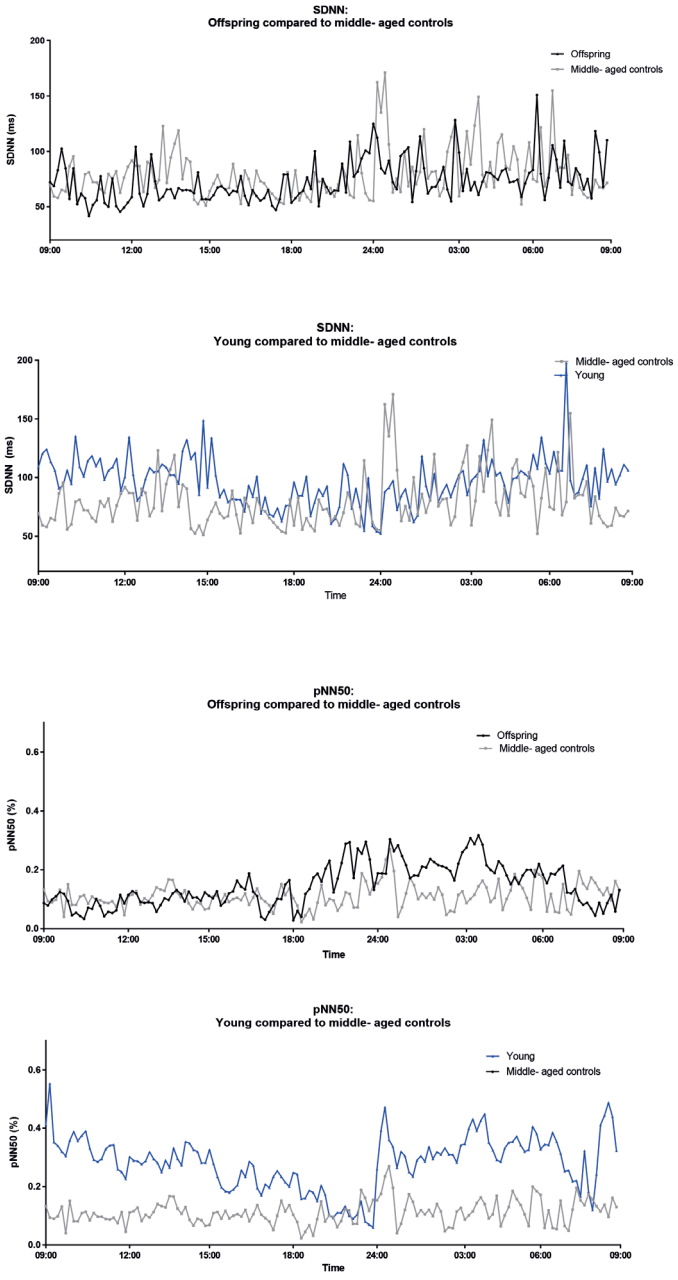


**FIGURE 11.2 |** 24h HRV parameters (SDNN and pNN50) in offspring, middle aged controls and the young.

Also, younger participants showed higher NN50 and pNN50 values, which was found from the 24hr analysis and also when stratified for awake and sleep period. The mean (SE) pNN50 in young compared to older adults was 0.297 (0.037) versus 0.112 (0.036), P= 0.002 during the 24h period, 0.255 (0.040) versus 0.109 (0.039), P=0.018 during awake periods, and 0.340 (0.045) versus 0.121 (0.045), P= 0.003 during self- reported sleep time. The differences in SDNN and pNN50 in young compared to older adults are visualized in figure 2.

# DISCUSSION

We report two main findings. First, mean 3- day heart rate was not different in offspring compared to middle- aged controls, or in middle- aged controls compared to younger participants. However, difference between HR during awake and sleep periods ($HR_{awake}$ – $HR_{sleep}$), as a measure of fitness and adaptability, was higher in young compared to middle- aged controls. Secondly, we observed significantly higher values of SDNN, NN50 and pNN50 in young compared to older adults, and the difference in the latter two HRV parameters were consistent both during awake and sleep periods.

Cardiovascular events are known to increase with age. It is well established that the autonomic nervous system, which acts as control system for the body's visceral system, associates with cardiovascular mortality, including sudden cardiac death [3, 8, 9, 17]. Heart rate is controlled on a beat- to- beat basis by the combined effects of the sympathetic and parasympathetic system. Continuous measurement of HR and its variability provides a non- invasive way to measure autonomic activity. In our study, we measured HRV in the time domain. In young compared to older adults, we found that increased variability in SDNN, NN50 and pNN50. The SDNN is considered as the most global measure of HRV because it captures total HRV while being insensitive to small measurement errors [17, 18]. Since the SDNN is a measure of total HRV, it is not surprising that we found higher SDNN in younger compared to older participants. This is in line with previous studies that show that HRV decreases with increasing age [3, 8, 17]. The differences have been ascribed to a decline in parasympathetic activity and possible sympathetic nervous system [8]. In line, we found differences also on NN50 and pNN50, which are measures of vagal activity.

Concerning heart rate, we did not find significant differences in heart rate in the main study sample, neither in offspring compared to controls, or in the controls compared to young. While the difference between HR during awake and sleep periods ($HR_{awake}$ – $HR_{sleep}$), was not different between offspring and controls, the young participants had a higher difference compared to middle- aged controls. This could be a reflection of the difference in behaviour and activity patterns, since young participants worked longer hours per day, and moved more, in comparison with the offspring and middle aged controls. The ability to maintain comparatively higher heart rate during the day and lower heart rate during sleep periods could thus suggest that the young had comparatively more adaptable day- night heart rate rhythms, or more preserved global autonomic nervous system [1].

To our knowledge, this is the first study where continuously measured heart rate and its variability were compared in offspring enriched for familial longevity and age- matched controls. Previously, offspring have been shown to differ from partners in many cardio-metabolic parameters compared to controls of similar calendar age [12, 13, 19] However, in both the main and full study samples, we found no difference in HRV parameters in offspring enriched for familial longevity and controls. In a previous study in which healthy participants aged 10 – 99 years were included, HRV parameters were found to decrease with age, and it was concluded that persistently high HRV could be predictive of longevity [20]. In line, we also found that HRV was lower in older compared younger participants. However, this was not the case in offspring enriched for longevity and age- matched controls. Thus, our findings that heart rate and its variability did not differ would suggest that familial longevity may not be characterized by changes in the autonomic nervous system. This may be in line with previous arguments that HRV studies in older adults could be confounded by increasing erratic rhythms, and so, difference found in previous studies may not be due to longevity [21,22].

A strength of this study is that the heart rate and its variability was continuously recorded over 5 consecutive days, during the home setting of the participants. A limitation of this study is that we only examined cross-sectional associations and so, our findings are only descriptive. Another limitation is the small sample size. Using continuous ECG measurement, we observed beat-to-beat heart rate variability were increased with age but not with familial longevity. These findings possibly offer more insight into the association between different parameters of heart rate variability and aging. Sufficiently powered follow-up studies are needed to evaluate cause or consequence in the relation between parameters of heart rate variability and aging.

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