Heart rate variability during 48-hour recovery from incremental cycle ergometer test in type 1 diabetes patients and healthy controls

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Heart rate variability (HRV) is a noninvasive tool for investigating cardiac autonomic nervous system, especially its parasympathetic branch. HRV is known to be reduced for example in cardiovascular autonomic neuropathy and after myocardial infarction. Several studies have reported reduced HRV in type 1 diabetes patients. Regular exercise is known to increase parasympathetic tone but the effect of training on HRV is somewhat unclear.

This study examined HRV during 48 hours after incremental cycling until volitional fatigue in type 1 diabetes patients without cardiac autonomic neuropathy and in healthy controls. HRV was analyzed both at day and night. Subjects’ aerobic capacity was measured. Part of the subjects underwent 6-12 months training intervention, after which the same measurements were repeated. Time-domain, frequency-domain and nonlinear methods were used in HRV analysis.

Aerobic capacity was significantly higher in healthy controls than in type 1 diabetes patients (p=0.036). No differences in HRV were found between type 1 diabetes patients and healthy controls. In healthy controls aerobic capacity correlated with LF/HF-ratio (r=-0.711, p=0.014). In type 1 diabetes patients glycosylated hemoglobin (HbA1c) correlated with SDNN (r=-0.645, p=0.023), absolute VLF power (r=-0.648, p=0.023) and SD2 of Poincare plot (r=-0.646, p=0.023). There was a significant increase in aerobic capacity in both groups after training intervention (p<0.05). Training intervention did not cause a significant change in HRV.

Diabetes Mellitus, Type 1; Autonomic nervous system, Heart rate variability, Exercise
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1 Introduction

1.1 Physiological basis and analyses of heart rate variability

Heart rate variability (HRV) analysis has become a popular non-invasive tool for investigating activity of cardiac autonomic nervous system. HRV analysis is more widely used in research than in medical practice. The autonomic nervous system regulates visceral functions through the sympathetic and parasympathetic branches. In the cardiovascular system this nonstationary balance results in the fluctuation between intervals of consecutive heart beats, so called heart rate variability. (1-3)

Cardiac intrinsic automaticity of various pacemaker tissues is under the control of the autonomic nervous system (ANS). The parasympathetic influence is mediated via acetylcholine by the vagus nerve. Acetylcholine activates muscarinic receptors. The sympathetic influence is mediated via noradrenalin released by sympathetic nerves, and adrenalin released by adrenal medulla. Both noradrenalin and adrenalin activate both $\alpha$- and $\beta$-adrenergic receptors. Under resting conditions vagal tone dominates. Heart rate variability reflects the function of ANS, especially parasympathetic division. Vasomotor and respiratory centers in the central nervous system and humoral factors circulating in blood affect also heart rate variability. (4)

Traditional methods in analyzing heart rate variability are time domain analysis and frequency domain analysis. In addition, nonlinear methods of analysis are used. They are more recently developed and interpretation of them is more complicated, i.e. there is not a consensus how they reflect the function of ANS. Ectopic and artefactual beat corrections of RR interval data are essential prior to HRV analysis. Heart rate variability analyzing software includes usually an automatic RR interval correction but the data should be also inspected visually to evaluate validity of the automatic correction. (3)
In time domain analysis each QRS complex is detected in a continuous ECG record, and the RR intervals or the so-called normal-to-normal (NN) intervals (that is, all intervals between adjacent QRS complexes resulting from sinus node depolarizations) or the instantaneous heart rate is determined. The simple time domain variables that can be calculated include for example the mean RR interval and the mean heart rate. Also statistical time domain measures can be calculated. Those are for instance the standard deviation of the NN intervals (SDNN), the square root of the mean squared differences of successive NN intervals (RMSSD), the number of interval differences of successive NN intervals greater than 50 ms (NN50), and the proportion derived by dividing NN50 by the total number of NN intervals (pNN50). (4)

In frequency domain analysis the power spectral density is calculated. Three main spectral components are distinguished in short-term recordings: very low frequency (VLF, 0-0.04 Hz), low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz) component. VLF, LF and HF can be measured in absolute values of power (milliseconds squared). LF and HF can also be measured in normalized units, which present the relative value of each component. In long-term recordings there is possible to analyze also ultra low frequency component in the spectrum. (4) Detrended fluctuation analysis (DFA) and Poincare plot are some of many nonlinear methods in HRV analyses (2).

The parasympathetic activity is the major contributor to the HF component. Also RMSSD, NN50 and pNN50 reflects vagal modulation. There is a disagreement if the LF component reflects sympathetic activity or both sympathetic and parasympathetic activity. (2,4) LF/HF-ratio represents sympathovagal balance and interaction (2). SD1 of Poincare plot represents parasympathetic activity (5). Alpha 1 of DFA has been reported to correlate with LF:HF ratio related to ANS function tests (cold face immersion and cold hand immersion) (6).
1.2 The effect of gender, age, physical fitness and illnesses on heart rate variability

Several factors affect heart rate variability, such as age and gender (7). Heart rate variability declines with age till 80 years (8-10) and after that HRV indices reflecting vagal tone increases again (11). The results of HRV differences between genders are somewhat conflicting (7,12,13). This difference between genders diminishes with age (12). In addition, both psychological and physical stress decreases the HRV indices reflecting parasympathetic activity (14,15). Heart rate variability has also circadian profile. Parameters reflecting vagal tone are higher during the night than during the day. (7)

The importance of diabetes in the epidemiology of cardiovascular diseases cannot be overemphasized (16). Cardiovascular autonomic neuropathy (CAN) is a frequent complication of diabetes mellitus, which is associated with increased morbidity and mortality. It involves both the parasympathetic and sympathetic nervous system. CAN is usually diagnosed with dynamic tests involving measurement of heart rate and/or blood pressure. CAN is associated with poor prognosis. It is a complication of both type 1 and type 2 diabetes. (17,18) It is also associated with silent ischemia (17). CAN is found to be associated with modifiable risk factors (central fat distribution, hypertension, dyslipidemia, worse diabetes control, and smoking, and with the other microvascular complications of diabetes) (19).

In many studies heart rate variability has been decreased in diabetics with autonomic neuropathy as well as in those without (1,20-23). Reduced HRV has been reported already in children with type 1 diabetes (24). It also seems that in insulin-dependent diabetes patients high frequency power i.e. vagal tone of cardiac ANS decreases with the increasing duration of diabetes (25). It has been reported that decrease of LF power and HF power highly correlates with CAN severity. CAN is also reported to correlate in stepwise regression with age, retinopathy, nephropathy, bladder dysfunction, erectile dysfunction, peripheral neuropathy and hypertension. (26) One study reported that in
type 1 diabetics, high serum lipoprotein levels were related to a lower HRV while in healthy controls and chronic stable angina patients serum lipoprotein levels did not associate with lower HRV (27). It has been reported that in active standing and handgrip tests, which are used for testing ANS function, the change in alpha 1 of DFA was smaller in type 1 diabetes patients than in healthy controls, while other HRV parameters did not differ significantly between groups. DFA of nonlinear methods might be sensitive tool for detecting ANS dysfunction in early phase. (28)

Many studies have related the imbalance of the autonomic nervous system as assessed by HRV to several pathophysiological conditions, particularly in the setting of cardiovascular disease. Sudden death, coronary artery disease, heart failure, or merely cardiovascular risk factors (smoking, diabetes, hyperlipidemia, and hypertension) are the best-known clinical circumstances that can affect and/or be affected by the autonomic nervous system. (2) Heart rate variability is decreased and sympathetic tone increased after myocardial infarction (3). HRV is also decreased in chronic angina patients (29). Decreased activity of parasympathetic nervous system is related to a greater risk for cardiac event (30). The lower HRV is also associated with progression of calcification of coronary arteries in adults with and without type 1 diabetes (31). Smoking decreases HRV acutely and it also has an effect on baseline HRV (32,33). Body mass index (BMI) does not correlate with HRV (34,35).

There is a connection with physical fitness and cardiac ANS function. Regular exercise increases vagal tone and decreases resting heart rate. However, it is somewhat conflicting whether regular aerobic exercise increases heart rate variability. (30) It is controversial how a single bout of exercise affects heart rate variability during the next 24 hours. In one research exercise at approximately 65% of maximal oxygen uptake increased the HRV indices reflecting parasympathetic tone (36) while in three other researches moderate and heavy aerobic exercise decreased the overall HRV and indices reflecting parasympathetic tone (9,37,38). Endurance training also seems to increase high frequency power at nighttime (37-39). Also, one study found that in overtrained athletes parasympathetic cardiac modulation was slightly diminished after awakening
compared to control athletes. However, there was no difference in HRV between overtrained and control athletes during sleep. (40)

1.3 Type 1 diabetes and aerobic capacity

In several studies type 1 diabetic subjects has been reported to have lower aerobic capacity than healthy subjects (28,41-45). However, there are also studies which have reported similar maximal oxygen uptake in similarly trained diabetic and non-diabetic subjects (46,47). One study reported that type 1 diabetic athletes with optimal glycemic control had significantly higher aerobic capacity than type 1 diabetic athletes with poor glycemic control. Glycemic control possibly can have an independent effect on aerobic capacity. (48) Regular exercise and higher aerobic capacity is associated with enhanced vagal modulation (30,49) so type 1 diabetes patients who are at risk of CAN or who have reduced HRV can benefit from regular exercise and good aerobic capacity because decreased parasympathetic activity is related to a higher risk for cardiac event (30).

2 Objectives

Heart rate variability has been studied in type 1 diabetes patients during their normal daily activities and at rest but not during long term recovery from maximal performance. HRV in type 1 diabetes patients has also been studied during ANS function tests as deep breathing, active standing and handgrip tests. The aim of this study was to examine differences in heart rate variability during 48-hour recovery from incremental cycle ergometer test between type 1 diabetes patients and healthy controls. Heart rate variability was recorded for 24-48 hours after maximal exercise. The study paid special attention to HRV during nighttime and the first hours after exercise. This study aimed to examine the associations of HRV during recovery with maximal oxygen uptake and with diabetes. We also studied HRV after 6-12 months training intervention.
Study questions:

1. Is there a difference in HRV during 48-hour recovery between type 1 diabetes patients and healthy controls?

2. Is the potential difference in heart rate variability during recovery between type 1 diabetes patients and healthy controls associated with maximal oxygen uptake and/or diabetes?

3. Does heart rate variability during recovery in type 1 diabetes patients and healthy controls increase with regular exercise?

3 Subjects and methods

3.1 Subjects

The study was performed in University of Helsinki, Department of Sports and Exercise Medicine. There were 29 subjects, all of them were men, aged 25-46 years. Subject characteristics are presented in Table 1 and Table 2. Type 1 diabetes patients (n=13) were normo- or microalbuminuric and they did not have autonomic neuropathy or, at the most, were classified to have borderline ANS dysfunction. Healthy controls (n=16) were age-, gender-, anthropometry- and self-reported physical activity matched. Smoking and snuff using subjects were excluded from the study.
### Table 1. Subject characteristics in baseline measurements.

<table>
<thead>
<tr>
<th></th>
<th>T1D patients</th>
<th>Healthy controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>13</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.3 ± 6</td>
<td>32.8 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.6 ± 8.3</td>
<td>181.3 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.8 ± 10.5</td>
<td>83.3 ± 12.1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 2.3</td>
<td>25.3 ± 3.5</td>
<td>NS</td>
</tr>
<tr>
<td>LTPA (min/week)</td>
<td>281 ± 140</td>
<td>328 ± 177</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>10.6 ± 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (%) (n=12)</td>
<td>7.2 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>36.9 ± 4.7</td>
<td>42.9 ± 9.2</td>
<td>0.036</td>
</tr>
<tr>
<td>VO₂max (l/min)</td>
<td>2.88 ± 0.58</td>
<td>3.49 ± 0.45</td>
<td>0.007</td>
</tr>
</tbody>
</table>

T1D=type 1 diabetes, BMI=body mass index, LTPA=leisure-time physical activity, HbA1c=glycosylated hemoglobin, VO₂max=maximal oxygen uptake, NS=non-significant.

### Table 2. Subject characteristics in measurements after training intervention.

<table>
<thead>
<tr>
<th></th>
<th>T1D patients</th>
<th>Healthy controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>8</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.5 ± 5.9</td>
<td>36.3 ± 7.4</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.1 ± 9.4</td>
<td>180.9 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.4 ± 9</td>
<td>84.1 ± 9.6</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 2.2</td>
<td>25.7 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>40.9 ± 3.6</td>
<td>44.5 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>VO₂max (l/min)</td>
<td>3.1 ± 0.5</td>
<td>3.7 ± 0.4</td>
<td>0.02</td>
</tr>
</tbody>
</table>

T1D=type 1 diabetes, BMI=body mass index, HbA1c=glycosylated hemoglobin, VO₂max=maximal oxygen uptake, NS=non-significant.

### 3.2 Exercise protocol and determination of aerobic capacity

Subjects performed a cycle spiroergometer test to evaluate maximal aerobic capacity (VO₂max). Before the cycle spiroergometer test physician evaluated the subject’s suitability for maximal performance. The step incremental protocol was preceded by 5 minutes rest while the subjects sat relaxed on a cycle ergometer followed by 5 minutes baseline unloaded cycling. The step incremental exercise protocol (40 W/3 min) was then initiated, and the subjects continued exercising until volitional fatigue. The test was monitored by an experienced physician. Ventilation and alveolar gas exchange were
measured breath-by-breath by a low-resistance turbine (Triple V, Jaeger Mijnhardt, Bunnik, Netherlands) and a mass spectrometer (AMIS 2000, Innovision, Odense, Denmark). To obtain VO$_{2\text{max}}$ values, gas delay determinations were performed to measure raw breath-by-breath data, and then alveolar gas exchange was calculated with the slightly modified algorithms of Beaver et al. (50). Moving averages of individual test data were calculated over 5 seconds periods to reduce breath-by-breath variability, and interpolated to obtain values second by second, after which a maximal oxygen uptake was determined as the highest value of a 60-second moving average window.

3.3 Heart rate variability analysis

Firstbeat heart rate monitor recorded subjects' RR intervals from the beginning of the cycle ergometer test for the next 24 hours (baseline measurements) or 48 hours (measurements after training intervention). The data from heart rate monitor were analyzed with both Firstbeat software and Kubios software. Before analyzing the data, it was manually checked and corrected for artefacts and ectopic beats in addition to automatic correction properties of Firstbeat Kuntovalmentaja -software. The data with more than 20% of erroneous data was excluded.

The analyzed periods were: six-hour period starting from one hour after maximal performance (day 1) and 4-hour period between 00:00 and 4:00 or starting 30 minutes after reported bedtime (night). The third period was on the next day and it was at the same time of the

![Figure 1. Analyzed periods.](image-url)
day as the first period (day 2) (Figure 1). Day 2 was analyzed only after training intervention due to shorter RRI data (24 hours) in baseline measurements.

We used time and frequency domain analyses. In addition we used Poincare plot analysis and detrended fluctuation analysis (DFA) as nonlinear methods. In time domain analyses mean RRI (RR interval), SDNN (standard deviation of RR intervals), RMSSD (root mean square of successive differences) and pNN50 (proportion of number of successive intervals differing more than 50 ms) were analyzed. In frequency domain analysis Fast Fourier transformation (FFT) was used and very low frequency (0-0.04 Hz), low frequency (0.04-0.15 Hz) and high frequency (0.15-0.4 Hz) powers were analyzed in absolute values and LF and HF also as normalized units. Also LF/HF ratio was calculated. We analyzed SD1 and SD2 by Poincare plot and alpha 1 by DFA. (4)

3.4 Training intervention

Part of the subjects underwent 6-12 months training intervention. After that, their aerobic capacity and HRV were measured again. The goals of the 6-12 months training intervention were agreed with the subjects and the results from the clinical and exercise tests were used to individualize exercise prescription. Both endurance and strength training were included in the exercise program. The training groups performed exercise sessions individually and as guided by the University Sports. The subjects also participated in other guided exercise sessions as a part of their exercise prescription, if they wished.

3.5 Statistical analysis

The R software (2.13.0) for statistical computing and PASW statistics 18 were used for statistical analysis. We excluded data with more than 20 % erroneous data thus the number of subjects varied between 11 and 13 in baseline measurements and between 4
and 7 in measurements after training intervention. Subjects varied in different analyses (day 1, night and day 2) due to more than 20% erroneous data in some subjects’ RRI-data. The T-test was used for testing differences in aerobic capacity and HRV between type 1 diabetes patients and controls. Welch’s T-test was used for testing training intervention induced changes in HRV variables and aerobic capacity. Correlations were tested by Pearson’s correlation. Significance was defined as p<0.05.

4 Results

4.1 Aerobic capacity

Type 1 diabetes patients had significantly lower VO$_{2\text{max}}$ than healthy controls (36.9 ml/kg/min vs. 42.9 ml/kg/min, p=0.036) even though they were self-reported leisure-time physical activity matched (Table 1). VO$_{2\text{max}}$ correlated significantly with leisure-time physical activity in healthy controls (r=0.609, p=0.012) but not in type 1 diabetes patients (r=0.082, p=0.791) (Figure 2).

![Figure 2. Correlations between leisure-time physical activity and aerobic capacity. For healthy controls r=0.609, p=0.012. For type 1 diabetes patients r=0.082, p=0.791.](image-url)
4.2 Heart rate variability

There was not a significant difference in any of the studied HRV parameters between type 1 diabetes patients and healthy controls at day or night (Table 3). Mean RRI i.e. resting heart rate did not differ between groups in spite of the difference in VO$_{2\text{max}}$.

Mean RRI at day 1 correlated with leisure-time physical activity in healthy controls ($r=0.637$, $p=0.035$) but not in type 1 diabetes patients ($r=0.214$, $p=0.482$) (Figure 3). Also LF/HF-ratio at day 1 correlated significantly with VO$_{2\text{max}}$ in healthy controls ($r=-0.711$, $p=0.014$) but not in type 1 diabetes patients ($r=0.272$, $p=0.369$) (Figure 4). At day 1 glycosylated hemoglobin (HbA1c) in type 1 diabetes patients correlated significantly with SDNN ($r=-0.645$, $p=0.023$) (Figure 5), absolute VLF power ($r=-0.648$, $p=0.023$) (Figure 6) and SD2 of Poincare plot ($r=-0.646$, $p=0.023$) (Figure 7). At night there were no correlations in either group between HRV and VO$_{2\text{max}}$, LTPA, HbA1c or duration of diabetes.

Figure 3. Correlations between leisure-time physical activity and mean RR interval at day 1. For healthy controls $r=0.637$, $p=0.035$. For type 1 diabetes patients $r=0.214$, $p=0.482$. 
Figure 4. Correlations between aerobic capacity and LF/HF-ratio at day 1. For healthy controls $r=-0.711$, $p=0.014$. For type 1 diabetes patients $r=0.272$, $p=0.369$.

Figure 5. Correlation between HbA1c and SDNN in type 1 diabetes patients at day 1. $r=-0.645$, $p=0.023$. 
Figure 6. Correlation between HbA1c and absolute VLF power in type 1 diabetes patients at day 1. $r=-0.648$, $p=0.023$.

Figure 7. Correlation between HbA1c and SD2 of Poincare plot in type 1 diabetes patients at day 1. $r=-0.646$, $p=0.023$. 
4.3 Effects of training intervention on aerobic capacity and heart rate variability

After training intervention there was not a significant difference in VO$_{2\text{max}}$ (ml/kg/min) or in HRV between type 1 diabetes patients and healthy controls who underwent training intervention. The difference in absolute VO$_{2\text{max}}$ persisted (3.1±0.5 l/min vs. 3.7±0.4 l/min, p<0.05) (Table 2). There was a tendency toward higher LF/HF-ratio in type 1 diabetes patients at night (4.5±2.7 vs. 2.2±0.9, p=0.06) (Table 4). The change in VO$_{2\text{max}}$ and HRV was analyzed only in those subjects who underwent the training period. Training intervention induced a significant increase in VO$_{2\text{max}}$ both in type 1 diabetes patients (+4.4 ml/kg/min, p<0.05) and in healthy controls (+3.4 ml/kg/min, p<0.05). Due to varying subjects in different analysis, statistically significant increase in VO$_{2\text{max}}$ in healthy controls was seen only in night analysis.

The change in HRV after 6-12-months training-period was not statistically significant in type 1 diabetes patients or in healthy controls (Table 5). Mean RR interval at night was significantly increased in type 1 diabetes patients after training period meaning that their resting heart rate decreased. After training intervention there were no significant correlations between VO$_{2\text{max}}$, HbA1c and HRV in type 1 diabetes patients or healthy controls who performed 6-12 months training period.
Table 3. HRV in baseline measurements. Results are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1D (n=13)</td>
<td>HC (n=11)</td>
</tr>
<tr>
<td>Mean RRI (ms)</td>
<td>752.0 ± 110.3</td>
<td>806.8 ± 110.7</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>108.8 ± 38.0</td>
<td>127.1 ± 47.2</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>26.8 ± 12.4</td>
<td>35.0 ± 22.0</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>7.2 ± 6.7</td>
<td>11.8 ± 11.0</td>
</tr>
<tr>
<td>Absolute VLF (ms²)</td>
<td>8048.5 ± 4954.0</td>
<td>11538.9 ± 9366.3</td>
</tr>
<tr>
<td>Absolute LF (ms²)</td>
<td>1220.9 ± 782.6</td>
<td>1824.8 ± 1212.6</td>
</tr>
<tr>
<td>Absolute HF (ms²)</td>
<td>272.1 ± 228.6</td>
<td>556.9 ± 650.4</td>
</tr>
<tr>
<td>LF power (n.u.)</td>
<td>82.7 ± 5.7</td>
<td>82.8 ± 9.8</td>
</tr>
<tr>
<td>HF power (n.u.)</td>
<td>17.3 ± 5.7</td>
<td>17.2 ± 9.8</td>
</tr>
<tr>
<td>LF/HF-ratio</td>
<td>5.6 ± 3.0</td>
<td>6.8 ± 4.4</td>
</tr>
<tr>
<td>SD1 (Poincare) (ms)</td>
<td>18.9 ± 8.8</td>
<td>24.7 ± 15.5</td>
</tr>
<tr>
<td>SD2 (Poincare) (ms)</td>
<td>152.6 ± 53.2</td>
<td>177.9 ± 65.3</td>
</tr>
<tr>
<td>α1 (DFA)</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

T1D=type 1 diabetes patients, HC= Healthy controls, RRI=RR interval, SDNN=standard deviation of RR intervals, RMSSD= the square root of the mean squared differences of successive RR intervals, pNN50=the percentage of number of successive intervals differing more than 50 ms, VLF=very low frequency, LF=low frequency, HF=high frequency, n.u.=normalized units, DFA=detrended fluctuation analysis.
Table 4. HRV in post-training measurements. Results are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Night</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1D (n=5)</td>
<td>HC (n=6)</td>
<td>P</td>
</tr>
<tr>
<td>Mean RRI (ms)</td>
<td>780.6 ± 79.8</td>
<td>729.2 ± 100.0</td>
<td>0.38</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>99.6 ± 26.4</td>
<td>107.9 ± 34.2</td>
<td>0.67</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>24.9 ± 8.0</td>
<td>27.9 ± 23.9</td>
<td>0.80</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>5.5 ± 5.3</td>
<td>7.7 ± 11.9</td>
<td>0.72</td>
</tr>
<tr>
<td>Absolute power</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(VLF)</td>
<td>6405.3 ± 3365.6</td>
<td>8195.9 ± 5565.3</td>
<td>0.55</td>
</tr>
<tr>
<td>Absolute power</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LF)</td>
<td>1319.1 ± 608.1</td>
<td>1560.6 ± 1605.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Absolute power</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(HF)</td>
<td>222.2 ± 195.7</td>
<td>450.5 ± 727.5</td>
<td>0.52</td>
</tr>
<tr>
<td>LF power (n.u.)</td>
<td>86.3 ± 7.2</td>
<td>85.6 ± 8.4</td>
<td>0.89</td>
</tr>
<tr>
<td>HF power (n.u.)</td>
<td>13.7 ± 7.2</td>
<td>14.4 ± 8.4</td>
<td>0.89</td>
</tr>
<tr>
<td>LF/HF-ratio</td>
<td>8.1 ± 4.4</td>
<td>7.6 ± 3.6</td>
<td>0.84</td>
</tr>
<tr>
<td>SD1 (Poincare) (ms)</td>
<td>17.6 ± 5.7</td>
<td>19.7 ± 16.9</td>
<td>0.80</td>
</tr>
<tr>
<td>SD2 (Poincare) (ms)</td>
<td>139.7 ± 37.4</td>
<td>151.0 ± 46.5</td>
<td>0.67</td>
</tr>
<tr>
<td>α1 (DFA)</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>0.98</td>
</tr>
</tbody>
</table>

T1D=type 1 diabetes patients, HC= Healthy controls, RRI=RR interval, SDNN=standard deviation of RR intervals, RMSSD= the square root of the mean squared differences of successive RR intervals, pNN50=the percentage of number of successive intervals differing more than 50 ms, VLF=very low frequency, LF=low frequency, HF=high frequency, n.u.=normalized units, DFA=detrended fluctuation analysis.
Table 5. Change in HRV in type 1 diabetes patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th></th>
<th></th>
<th>Night</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T1D (n=5)</td>
<td></td>
<td>HC (n=7)</td>
<td></td>
<td>T1D (n=5)</td>
<td></td>
</tr>
<tr>
<td>Mean RRI (ms)</td>
<td>MD</td>
<td>95 % CI</td>
<td>p</td>
<td>MD</td>
<td>95 % CI</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>15.16</td>
<td>(-32.8-63.1)</td>
<td>0.43</td>
<td>-33.5</td>
<td>(-86.4-19.4)</td>
<td>0.16</td>
</tr>
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</tr>
<tr>
<td>SDNN (ms)</td>
<td>-8.0</td>
<td>(-21.4-5.3)</td>
<td>0.17</td>
<td>-10.1</td>
<td>(-43.9-23.7)</td>
<td>0.48</td>
</tr>
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<tr>
<td>RMSSD (ms)</td>
<td>-0.9</td>
<td>(-5.1-3.3)</td>
<td>0.57</td>
<td>-2.6</td>
<td>(-7.1-1.9)</td>
<td>0.19</td>
</tr>
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</tr>
<tr>
<td>pNN50 (%)</td>
<td>-1.1</td>
<td>(-3.6-1.4)</td>
<td>0.29</td>
<td>-1.1</td>
<td>(-4.1-1.9)</td>
<td>0.39</td>
</tr>
<tr>
<td>Absolute VLF (ms²)</td>
<td>1199.2</td>
<td>(-3168.4-770.1)</td>
<td>0.17</td>
<td>-2786.5</td>
<td>(-10749.8-5176.7)</td>
<td>0.41</td>
</tr>
<tr>
<td>Absolute LF (ms²)</td>
<td>-24.5</td>
<td>(-243.7-194.7)</td>
<td>0.77</td>
<td>-130.8</td>
<td>(-754.5-492.8)</td>
<td>0.61</td>
</tr>
<tr>
<td>Absolute HF (ms²)</td>
<td>-20.1</td>
<td>(-75.5-35.3)</td>
<td>0.37</td>
<td>-71.3</td>
<td>(-179.2-36.6)</td>
<td>0.15</td>
</tr>
<tr>
<td>LF power (n.u.)</td>
<td>1.4</td>
<td>(-1.5-4.2)</td>
<td>0.26</td>
<td>0.0</td>
<td>(-3.4-3.5)</td>
<td>0.98</td>
</tr>
<tr>
<td>HF power (n.u.)</td>
<td>-1.4</td>
<td>(-4.2-1.5)</td>
<td>0.26</td>
<td>0.0</td>
<td>(-3.5-3.4)</td>
<td>0.98</td>
</tr>
<tr>
<td>LF/HF-ratio</td>
<td>1.5</td>
<td>(-0.7-3.6)</td>
<td>0.13</td>
<td>-1.3</td>
<td>(-3.0-0.3)</td>
<td>0.08</td>
</tr>
<tr>
<td>SD1 (Poincare) (ms)</td>
<td>-0.7</td>
<td>(-3.6-2.3)</td>
<td>0.57</td>
<td>-1.9</td>
<td>(-5.0-1.3)</td>
<td>0.19</td>
</tr>
<tr>
<td>SD2 (Poincare) (ms)</td>
<td>-11.4</td>
<td>(-30.3-7.7)</td>
<td>0.17</td>
<td>-14.3</td>
<td>(-62.3-33.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>α1 (DFA)</td>
<td>0.01</td>
<td>(-0.09-0.07)</td>
<td>0.83</td>
<td>-0.02</td>
<td>(-0.04-0.01)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

T1D=type 1 diabetes patients, HC= Healthy controls, MD=mean difference, CI=confidence interval, RRI=RR interval, SDNN=standard deviation of RR intervals, RMSSD= the square root of the mean squared differences of successive RR intervals, pNN50=the percentage of number of successive intervals differing more than 50 ms, VLF=very low frequency, LF=low frequency, HF=high frequency, n.u.=normalized units, DFA=detrended fluctuation analysis.
5 Discussion

The aim of this study was to examine if there is a difference in 48-hour heart rate variability (HRV) after incremental cycle ergometer test in type 1 diabetes patients and healthy controls and if the possible difference is related to diabetes and/or aerobic capacity. The main findings were as follows: Aerobic capacity (VO$_{2\text{max}}$) was significantly lower in type 1 diabetes patients than in healthy controls. There was not a significant difference in HRV between these groups. Leisure-time physical activity correlated significantly with VO$_{2\text{max}}$ and mean RR interval during daytime in healthy controls but not in type 1 diabetes patients. Also, in healthy controls there was a correlation between VO$_{2\text{max}}$ and LF/HF-ratio at daytime. Glycosylated hemoglobin (HbA1c) in type 1 diabetes patients correlated significantly with SDNN, absolute VLF power and SD2 of Poincare plot at daytime. There were no significant correlations between aerobic capacity, leisure-time physical activity, glycosylated hemoglobin, duration of diabetes and heart rate variability at night in either group.

In addition, part of the subjects performed 6-12 months training period and after that aerobic capacity and HRV were measured again. Training significantly increased VO$_{2\text{max}}$ in type 1 diabetes patients and healthy controls. The increase in aerobic capacity was higher in type 1 diabetes patients than in healthy controls. After training intervention there was not a significant difference in aerobic capacity or in HRV between type 1 diabetes patients and healthy controls who underwent this training intervention. In our study there was not a significant change in HRV in either of these training groups even though VO$_{2\text{max}}$ significantly increased.

5.1 Aerobic capacity

In our study aerobic capacity was significantly lower in type 1 diabetes patients than in healthy controls as it has been previously widely reported (41,42,44,45). Possible explanation can be independent effect of glycemic control on aerobic capacity. It has
been reported that when comparing type 1 diabetic athletes with optimal glycemic control and with poor glycemic control, VO$_{2\text{max}}$ has been significantly higher in type 1 diabetic athletes with optimal glycemic control (48). It has been suggested that in type 1 diabetes patients with poor glycemic control cardiac pump function is impaired. Impairment is resulted from inability to reach age-determined maximal heart rate and from diastolic dysfunction leading to lower stroke volume. (51) Also pulmonary diffusion in chronically hyperglycemic type 1 diabetes patients seems to be reduced (52), but according one review the reduction is too modest to explain the lower aerobic capacity (51).

In this same study population as in this study hemoglobin mass has been studied. This study suggests that difference in VO$_{2\text{max}}$ between type 1 diabetes patients can also be due to lower total hemoglobin mass and blood volume (43). Also active muscle deoxygenation has been studied in this same study population. During cycle ergometer test earlier tissue deoxygenation in leg muscles was found in type 1 diabetes patients suggesting lower cardiac output and/or impaired peripheral vascular function. (44)

VO$_{2\text{max}}$ significantly correlated with leisure-time physical activity in healthy controls but not in type 1 diabetes patients. Leisure-time physical activity had also significant correlation with mean RR interval i.e. heart rate, but only in healthy controls. Leisure-time physical activity includes all the exercise subjects usually have during the week, so it did not discriminate between light, moderate or heavy exercise and so there is possibility that type 1 diabetes patients’ training intensity could have been lower. Also, the leisure-time physical activity was self-reported so there is a slight possibility of reporting bias. In addition, in type 1 diabetes patients the variation in VO$_{2\text{max}}$ was smaller than in healthy controls and this could be a reason why there is not a significant correlation between leisure-time physical activity and aerobic capacity in type 1 diabetes patients.
5.2 Heart rate variability

To our knowledge, this was the first study researching HRV in type 1 diabetes patients during long-term recovery. There was not a significant difference in HRV between type 1 diabetes patients and healthy controls during long-term recovery in spite of diabetes and difference in VO$_{2\text{max}}$. We wanted to examine HRV in type 1 diabetes patients without diabetes-related complications so our type 1 diabetic subjects did not have autonomic neuropathy. Several previous studies which have reported a difference in HRV between type 1 diabetes patients and healthy controls have not excluded cardiac autonomic neuropathy (CAN) as we did (4,20,23,24). In aforementioned studies HRV was studied during rest, during autonomic function tests and during daily activities. One study reported type 1 diabetes patients without clinical evidence of CAN to have significantly lower HRV than healthy controls. In that study HRV was examined over 60 minutes period in resting conditions. (21) One study did not find a significant difference in HRV between type 1 diabetes patients without CAN and healthy controls. HRV was recorded for 24 hours during subjects’ daily activities. (25) Our study population did not have CAN and HRV was recorded during their daily activities so our result is consistent with last-mentioned study but our analyzed periods were four or six hours. However, it cannot be overemphasized that this was the first study to examine HRV in type 1 diabetes patients during long-term recovery so our results are not directly comparable with previous studies.

In our study population ANS function has been previously evaluated by HRV, systolic blood pressure variability and baroreflex sensitivity at rest and during handgrip and active standing tests. There were no differences at rest but during maneuvers alpha1 responses in DFA were smaller in type 1 diabetes patients. (28) In this study maximal performance did not help to show up possible autonomic dysfunction in type 1 diabetes patients studied by HRV during 48-hour recovery. A few studies suggest that single bout of exercise reduces HRV parameters reflecting parasympathetic tone for next 24 hours so maximal performance is an interesting maneuver to affect HRV (9,37,38). This also means that we cannot make conclusions of our subjects’ normal long-term HRV based on HRV after maximal performance.
One review suggests that among time domain measures of HRV, pNN50 < 3% and RMSSD < 25 milliseconds are used to assess impairment of vagal activity; SDNN < 100 milliseconds are considered as a rough index of abnormal sympathetic outflow (2). In our study, only in day 1 analysis after training intervention type 1 diabetes patients had RMSSD lower than 25 milliseconds. In type 1 diabetes patients in three of five analyses SDNN was lower than 100 milliseconds. Otherwise these parameters were normal. Based on these values our type 1 diabetes patients did not have an impairment of vagal activity. It is controversial if there is abnormal sympathetic outflow in type 1 diabetes patients because SDNN varied in different analyses being both under and over 100 milliseconds. In healthy controls there was not abnormality in vagal or sympathetic activity based on time-domain measurements. However, there is not official consensus for normal HRV values or the length of HRV data so it is hard to make conclusions based only on these time domain measures.

In type 1 diabetes patients HbA1c correlated with SDNN, absolute VLF power and SD2 of Poincare plot so that lower HbA1c correlated with higher HRV parameters. SDNN represents overall HRV, whereas the role of VLF power remains unclear. (2) SD2 represents long-term heart rate variability and it seems to correlate with LF power and LF/HF-ratio (53-55). This might indicate that in our subjects higher HbA1c reflecting chronically higher blood glucose levels could have slightly reduced their HRV and disturbed their sympathovagal balance. The correlation was not found between $VO_{2\text{max}}$ and HRV, this could mean that long-term blood glucose level has stronger effect on overall HRV than aerobic capacity has. The variation in HbA1c was not higher than in $VO_{2\text{max}}$ so this does not explain why there was a significant correlation between HbA1c and HRV and not between $VO_{2\text{max}}$ and HRV.

Type 1 diabetes usually causes very notable symptoms (polyuria, polydipsia, polyphagia and losing weight, in severe cases ketoacidosis) and because of that diagnosis is usually set at early phase of the disease whereas in type 2 diabetes the delay to diagnosis can be quite long. The long-term goal of insulin treatment is the prevention
of chronic complications by maintaining blood glucose levels as close to normal as possible. Long-term blood glucose levels can be monitored by HbA1c. HbA1c-goal is usually under 7% without severe hypoglycemias. Usually HbA1c-goal can be defined to level 7.0 – 7.5%. Our type 1 diabetes patients’ mean HbA1c was 7.2 – 7.8% so glycemic control was fairly good. Nowadays insulin therapy is based on multiple daily injections (long-lasting and short- or rapid-acting insulin) or insulin pump and this way it mimics normal insulin secretion by pancreas. (56) That was also the case in our type 1 diabetes patients. Long-term blood glucose levels as well as genetic factors affect development of autonomic neuropathy, that is, in same blood glucose levels some individuals develop autonomic neuropathy when some individuals do not (Camilla Schalin-Jäntti, personal communication, 14.1.2013).

In healthy controls there was a significant correlation between VO_{2max} and LF/HF-ratio at daytime so that higher aerobic capacity correlated with lower LF/HF-ratio. LF/HF-ratio reflects sympathovagal balance. This means that healthy subjects with higher aerobic capacity had increased parasympathetic or decreased sympathetic tone. As mentioned previously, regular aerobic exercise increases vagal tone (30) so this correlation can indicate enhanced vagal tone in healthy subjects with higher aerobic capacity. It is possible that chronically higher blood glucose levels in type 1 diabetes patients disturbs their sympathovagal balance and good aerobic capacity cannot compensate that. One study also reported that HF power significantly decreases with increasing duration of diabetes so this can increase type 1 diabetes patients’ LF/HF-ratio (25).

Probably differences in HRV during recovery could have been seen if type 1 diabetes patients had had autonomic neuropathy. We studied type 1 diabetes patients without remarkable complications and fair glycemic control. Some HRV parameters reflecting parasympathetic tone (RMSSD, pNN50) were lower in type 1 diabetes patients than in healthy controls even though the difference was not significant. It could be possible that with more subjects the result would have been statistically significant. Number of subjects was low especially in analyses after training intervention as well as in analyses comparing baseline and post-training intervention HRV and this weakens the statistical
power. HRV during recovery would be interesting to study with more subjects to figure out if there could be a statistically significant difference in HRV. Also studying HRV during long-term recovery and effect of training intervention would be interesting to study in subjects with known reduced HRV to assess the effect of exercise on reduced HRV during recovery.

5.3 Effects of training intervention on aerobic capacity and heart rate variability

Training increased aerobic capacity both in type 1 diabetes patients and healthy controls. In our study training did not induce a significant change in HRV either in type 1 diabetes patients or healthy controls who performed training intervention in spite of significant increase in aerobic capacity. One study reported that aerobic exercise increases HRV (49). There is also study in which six year training intervention had no effect on heart rate variability (57) but it must be taken into consideration that HRV declines with age (2). In one review the effect of training on HRV was found to be controversial. The suggested explanation was that subject’s baseline HRV has an effect on change in HRV i.e. if the baseline HRV is high the increase in HRV is small and vice versa. (30) According to previous studies it is controversial whether there is correlation between aerobic capacity and HRV so that higher VO\textsubscript{2max} is correlated with higher HRV (35,58). It is also reported that exercise intensity has an effect on nighttime HRV. In recreational endurance runners moderate- and high-intensity training increased nighttime vagal activity studied by HRV but low-intensity training did not. (59)

5.4 Study design

Subjects performed their cycle ergometer test in different times of day thus the analyzed RRI periods were also in different times of day. Accordingly, we could not take into account circadian variation in HRV when analyzing daytime HRV (7). Also there was a slight difference how much time has passed after maximal performance when analyzing
HRV at night. This could have affected HRV results. However, the analyzed periods were at daytime and during sleep in every subject. The conditions were not the same in different subjects while recording the RR interval data because they continued their normal activities after maximal performance. This should not have affected HRV based on the study reporting that physical activity can affect short-term HRV studied by Poincare plot but not long-term HRV (SDNN, VLF power and SD2) (60).

The manual correction i.e. removing artefacts and ectopic beats should not have had a significant effect on time-domain analysis even though it caused variation in the length of RRI data. When comparing time-domain analysis results, 20% removal of RRI data should not affect these results (Esa Hynynen, personal communication, 21.3.2012).

6 Conclusion

In this study there was a significant difference in VO$_{2\text{max}}$ even though type 1 diabetes patients and healthy controls were self-reported leisure-time physical activity matched. However, this did not reflect as differences in HRV during the next 48 hours after the incremental exercise test between type 1 diabetes patients and healthy controls. Lower aerobic capacity in type 1 diabetes patients has been previously widely reported. Six to twelve months training period increased aerobic capacity both in type 1 diabetes patients and healthy controls but it did not induce changes in HRV. Even though there was not a sign of impaired autonomic function in type 1 diabetes patients, regular exercise is recommended both type 1 diabetes patients as well as healthy controls to prevent cardiovascular diseases and to maintain normal weight. Intensive treatment of type 1 diabetes is proven to reduce complications (61) and regular exercise has a positive effect on glycemic control. Maximal performance is an interesting maneuver to affect HRV and HRV during long-term recovery in type 1 diabetes patients with known reduced HRV would be an interesting field of research.
References


(59) Vesterinen VF, Hakkinen KF, Hynynen EF, Mikkola JF, Hokka LF, Nummela A. Heart rate variability in prediction of individual adaptation to endurance training in recreational endurance runners. LID - 10.1111/j.1600-0838.2011.01365.x doi]. Scandinavian journal of medicine & science in sports JID - 9111504 EDAT- 2011/08/05 06:00 MHDA- 2011/08/05 06:00 CRDT- 2011/08/05 06:00 AID - 10.1111/j.1600-0838.2011.01365.x doi] PST - aheadofprint 0804.
