Cardiovascular autonomic imbalance in patients with both T2DM and CAD

Association to glucometabolic control

Susanne Kristine Aune
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Department of Cardiology, Oslo University Hospital Ullevål;
Center for Clinical Heart Research
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Abstract

Background: Cardiac autonomic dysfunction is a common complication to type 2 diabetes mellitus (T2DM), and an association to glucometabolic control has been suggested. Autonomic dysfunction is associated with increased mortality in patients with coronary artery disease (CAD). We have investigated the association between glucometabolic control and autonomic function in patients with both T2DM and CAD.

Methods: ECG was recorded in 137 patients and a cardiopulmonary exercise test was performed to assess resting heart rate (HR), heart rate reserve (HRR) and heart rate variability (HRV) as a measure of autonomic activity. Fasting blood samples were drawn for assessment of blood glucose, HbA1c and insulin. Insulin resistance (IR) was assessed by the HOMA2-IR computer model.

Results: HOMA2-IR was inversely correlated to HRR (p=0.044) and positively correlated to HR rest (p=0.030). Fasting blood glucose correlated inversely to HRR (p=0.022). No correlation was found between HRV and glucometabolic variables, but a strong inverse correlation was present between HRV and duration of diabetes. Also HRR correlated inversely to the duration of diabetes.

Summary: In the present study we found glucometabolic factors to be associated with autonomic dysfunction, indicating that glycemic control is important in patients with both T2DM and CAD. The association to duration of diabetes confirms the importance of prevention and early detection of T2DM in patients with CAN.

Acknowledgements

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Introduction

The sinoatrial node (SA node) located in the right atrium is the heart's intrinsic pacemaker, and thus the generator of sinus rhythm. The SA node cells are called pacemaker cells. They depolarize spontaneously, creating rhythmical impulses that travel through the heart to produce simultaneous contraction of the cardiomyocytes; a heartbeat. Denervated, the SA node holds a pace at around 90-110 bpm in healthy subjects and is age and gender dependent (1, 2). However, it is under constant influence by the Autonomic Nervous System (ANS).

The ANS consist of the sympathetic and the parasympathetic nervous system, their actions on the SA node being opposite each other (3). The heart is innervated by sympathetic fibers from the T1-T4 spinal nerves. The sympathetic neurons release noradrenaline that acts through B1-receptors on SA node cells to increase heart rate and on myocytes in the atria and ventricles to increase contractility (4), the result being increased cardiac output (CO) and a rise in arterial blood pressure (BP). The parasympathetic nervous system, mediated mainly by the vagus nerve (CN X), release acetylcholine that acts through muscarinic M2 receptors on SA node cells to decrease heart rate, and thereby reducing CO and arterial BP (5). At rest, parasympathetic activity dominates, keeping the resting heart rate far below the heart's intrinsic activity (60-90 bpm) (2, 6).

The autonomic innervation of the heart is controlled by the cardiovascular center located mostly in the medulla oblongata of the central nervous system (the CNS) which in turn sends inhibitory or excitatory interneurons to the cardio inhibitory area and the cardio accelerator area in the medulla. These centers furthermore regulate parasympathetic and sympathetic activity respectively. The cardiovascular center is a complex autonomic network of interconnected brain structures (7), receiving information from the cortex, limbic structures and the respiratory center of the CNS, as well as from baroreceptors and peripheral chemo receptors in the body (8). The integrated response enhances either parasympathetic or sympathetic tone, whilst inhibiting the other one (9), keeping the autonomic output to the heart in balance.

In the day-to-day activities of healthy individuals many factors influence the heart rate, including the baroreflex, thermoregulation, hormones, sleep-wake cycle, meals, physical activity and stress. Imbalance of this fine regulation, often with a shift towards increased sympathetic and decreased vagal activity, is called cardiac autonomic dysfunction.
Cardiac autonomic neuropathy

Patients with type 2 Diabetes Mellitus (T2DM) have a high risk of developing neuropathies, and polyneuropathies affect ~50% of all diabetics (10). Diabetic polyneuropathies can be divided into at least two subgroups. The typical symmetrical, length dependent group of polyneuropathies affecting sensorimotor function, in which total hyperglycemic exposure seems to be the most important risk factor (11), and the atypical group in which we find the autonomic polyneuropathies. The cardiac autonomic neuropathy (CAN) is the most common autonomic polyneuropathy with a prevalence rate of up to 90% dependent of different autonomic testing (12). CAN results in cardiac autonomic dysfunction. It is caused by damage to the autonomic nerves, and the correlation between symptoms and severity is often weak (13). The most important factors found to influence the prevalence of CAN are patient age and duration of diabetes, and some studies have also found body mass index (BMI) to be correlated with CAN (14). Poor glycemic control is a risk factor for developing autonomic neuropathy in type 1 diabetes mellitus (15, 16), and there is broad consensus that tight glycemic control can prevent and slow the progression of CAN (17). However, the role of glycemic control in T2DM is not yet clear as there are conflicting results. Some studies have found that there is no correlation between HbA1c and autonomic activity (18), while other studies have suggested that mean HbA1c over time (19) and parameters of HbA1c variability are related to the progression of CAN in T2DM (20). Some have also suggested that blood lipids and other cardiovascular risk factors may play a role in the development of CAN in T2DM (21), while others have proposed a link between insulin resistance, hyperinsulinemia and CAN (22) although the cause-effect relationship needs further clarification.

The earliest manifestations of CAN tend to be associated with parasympathetic denervation and a relative augmentation of sympathetic tone (23). Patients with T2DM without manifest CAN or other neuropathies even show an impairment of vagal function, indicating that parasympathetic denervation is more widespread and happens earlier in the natural course of CAN (24-26).

CAN with its associated impaired vagal activity and the relative increase of sympathetic tone leads to a rise in resting heart rate (HR) (26). Resting tachycardia is a non-specific sign of CAN, and must be interpreted with care. However, increased resting HR has been shown to be an independent risk factor for cardiovascular mortality (27-32). Ewing et. al. showed as early as in 1980 that patients with symptoms of cardiovascular neuropathy and abnormal autonomic test results had a 56% higher mortality rate (24).
Heart rate reserve (HRR) is the difference between a person’s resting HR and maximum heart rate, and reflects the heart’s ability to increase its rate in response to increased activity or demands (33).

Dominating parasympathetic activity at rest and sufficient sympathetic activity in response to exercise will result in a high HRR. On the other hand, an increase in resting heart rate and a reduction in maximum heart rate due to diminished parasympathetic activity at rest and inadequate sympathetic output in response to exercise will result in a reduction in HRR. Thus, an autonomic dysfunction will result in a reduced HRR, and an impaired physical capacity (34, 35).

Normally, there is a variation in time between consecutive normal-to-normal heart beats called heart rate variability (HRV). Parasympathetic dysfunction leads to a decrease in the normal fluctuation in heart rate, thus a reduced HRV (36).

Figure 1. Figure 1 demonstrates the variation between normal R-R intervals in an ECG recording.

The predictive value of HRV, as an assessment of autonomic function, has been investigated for risk stratification in many patient populations over the years. Decreased HRV has been reported to be an independent risk factor for all-cause mortality (37), adverse cardiac outcomes in T2DM (38), arrhythmic death and cardiac death after acute MI (39). Apart from resting tachycardia, exercise intolerance and decreased HRV, clinical manifestations of the cardiovascular abnormalities associated with CAN are postural hypotension, perioperative lability and possibly silent myocardial infarctions (23, 40, 41).

Patients with T2DM or other disorders of glucose metabolism have increased risk for cardiovascular disease (CVD) and mortality (42-45). T2DM contributes to the development and progression of CVD in multiple ways. High plasma glucose, longstanding insulin resistance and increased concentration of free fatty acids leads to the production of reactive oxygen species, inflammation, endothelial and platelet dysfunction, which further stimulates the formation of fatty streaks, atherosclerotic plaques and thrombus formations (46). T2DM is also associated with obesity, physical inactivity and hypertension (47-49), all of which are risk factors for CVD (50).
Patients suffering from coronary artery disease (CAD) alone also show diminished cardiovagal activity (51) and the severity of CAD is highly correlated with impaired autonomic function (52).

The number of patients with T2DM has been increasing over the last decades, and considering their elevated risk for CAD, there is a growing population of patients suffering both diseases (46, 53). As both patients with T2DM and patients with CAD independent of each other have a high risk of developing cardiac autonomic neuropathy, it is close to think that patients suffering both diseases have an accumulated risk for autonomic impairment. The metabolic changes that come with T2DM leads to a more rapid progression of the atherosclerotic process (46) in an already diseased heart, which in combination with autonomic dysfunction makes this group of patients highly susceptible to cardiac arrhythmias and coronary death.

Tight glycemic control is a widely accepted therapeutic option in diabetics, but the evidence of its benefits on autonomic function in patients with T2DM and CAD is unclear. Current guidelines propose a multifactorial therapeutic approach, including lifestyle changes and a wide variety of medication (54).

**Assessment of cardiac autonomic function**

Cardiac autonomic function can be assessed by several simple, non-invasive bedside tests introduced in the late 70s as the Ewing battery of tests, including heart rate variability (HRV), the response to Valsalva manoeuvre, the heart rate response to standing and the postural fall in blood pressure (55, 56). The first three are primarily an assessment of parasympathetic activity, and thus vagal function.

HRV reflects beat-to-beat changes in consecutive normal RR-intervals on ECG-recordings. The beat-to-beat variation with respiration was the first clinical measure of HRV. Later, power-spectral analysis of HRV was introduced by Akselrod et.al, implementing the use of advanced computer-based algorithms and long term ECG-recordings (57). Additional measures of HRV occurred, including standard deviation of RR-interval, the mean square successive difference, the mean successive difference and the expiratory-inspiratory ratio (E:I ratio). This led to the creation of guidelines for comparing different assessment models of HRV (58), which also postulates the two clinical settings in which the practical use of HRV has actually been reached; as a predictor of mortality after acute MI and as an early warning sign of diabetic CAN.

In this study we have chosen to assess cardiac autonomic function by HRV in response to deep breathing (HRVdb), heart rate reserve (HRR) and resting heart rate (HR rest). Several studies suggest that the HRV is maximal at respiratory rates between 5 to 10 per min (59), and typically the test is
performed with a respiratory rate of six per minute (60). HRVdb is easy to perform, non-invasive, affordable and quick, and has proven to be the most sensitive bedside test for early detection of cardiovagal dysfunction (61, 62). It is also reproducible, even under non-standardized conditions (63). However, it requires active patient participation which is difficult to standardize, and it is insensitive to changes in sympathetic nervous system changes. It is also vulnerable to arrhythmic heart rates.

**Aim of study**

The main aim of this study was to investigate the association between cardiovascular autonomic imbalance and glucometabolic control, including HOMA2-IR as a measure of insulin resistance, in patients with both CAD and T2DM. Our hypothesis was that there would be an association between poor glycemic control and the severity of autonomic impairment in these patients. We also wanted to investigate the relation between other factors and autonomic function, such as BMI, smoking and use of beta-blocker medication, and to investigate known risk factors association to autonomic imbalance such as age, sex and duration of diabetes.

**Methods**

**Study population**

The study is part of a randomized, controlled, clinical trial investigating the effects of exercise training on exercise capacity, glycemic control and progression of atherosclerosis in patients with both CAD and T2DM (64). Patients (n=137) with T2DM and CAD were included in the trial between August 2010 and March 2012 at the Department of Cardiology, Oslo University Hospital, Ullevål, Oslo, Norway. All patients had known T2DM and CAD verified by coronary angiography. Exclusion criteria were end stage renal disease, proliferative retinopathy, cancer, stroke or myocardial infarction last three months, serious arrhythmia, valvular disease, unstable angina, uncompensated heart failure, COPD GOLD stadium IV, serious rheumatologic disease, thromboembolic disease, musculoskeletal disorders or ongoing infections, and disorders or disabilities seriously interfering with exercise ability. All patients received thorough information about the study and gave written informed consent to participate. The study was approved by the Regional Ethics Committee and was conducted according to the Declaration of Helsinki. It is registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov), NCT01232608. Only baseline assessments have been used in the present work.
Laboratory measures

The blood samples were drawn between 0800 and 1000AM by standard venipuncture to measure HbA1c, blood glucose and insulin, determined by routine analyses. Patients on insulin treatment were also included as insulin and glucose were thought to be in steady state at the time of sampling (65). Fasting blood glucose and insulin were used to calculate insulin resistance with the updated homeostastic model assessment (HOMA2-IR computer model).

Deep breathing ECG (ECGdb) and calculation of heart rate variability (HRV)

A 12-lead ECG was taken of all study subjects before a cardiopulmonary exercise test. The participants sat down and relaxed while the ECG-equipment was mounted. All patients were taught to breathe in a special manner by an instructor prior to the ECG registration to ensure that breathing was optimal. The deep breathing test consisted of deep, even and calm breaths of 6 respiration cycles for 60 seconds: 5 sec for each inhalation and 5 sec for each exhalation. The ECG was then recorded at a speed of 25 mm/s with the patients sitting in an upright position breathing the way they were taught under guidance of the instructor.

All ECGs were manually overlooked, and R-R intervals surrounding premature beats (SVES/VES) were excluded. All ECGs that did not show sinus rhythm were also excluded, most importantly atrial fibrillation. If in doubt about an ECG an experienced cardiologist would evaluate it.

For every patients ECG(db), the three breathing cycles with the largest RR differences between inspiration and expiration were chosen, and from these a mean RR difference (“RR-diff”) for the test was calculated. The mean shortest RR interval (“RR-min”) was then calculated from the shortest RR intervals in the three chosen breathing cycles. Finally, a highest and lowest heart rate (“HR high” and “HR low”) for the test was calculated from “RR-min” and ”RR-diff”, see formulas below. The heart rate variability (HRV) was then calculated as the difference between “HR high” and “HR low”.

**Formulas:**

\[
\text{HR high} = \frac{60s}{(\text{RR-min}/25\text{mm} \cdot \text{s}^{-1})}
\]

\[
\text{HR low} = \frac{60s}{[(\text{RR-min} + \text{RR-diff})/25\text{mm} \cdot \text{s}^{-1})}
\]

\[
\text{HRV} = \text{HR high} - \text{HR low}
\]
HR-rest and HRR

Resting heart rate (HR rest) was determined during an ultrasonogram of the carotid artery (66). The patient was lying in the supine position, in a quiet room with reduced lighting for several minutes before the test and unaware that the heart rate was being registered.

The maximal heart rate was measured during the cardiopulmonary test on a treadmill using a modified Balke protocol (67). A 12-lead ECG was continuously recorded during the test. Gas exchange and ventilatory variables were continuously measured. HR max was the highest heart rate registered during the cardiopulmonary test (64). The patients were encouraged to continue the test until exhaustion, evaluated by the BORG scale of perceived exertion (68).

Heart rate reserve (HRR) is the difference between maximal heart rate and resting heart rate (HR max – HR rest). It was assessed by subtracting the resting heart rate (HR) described above from the maximal heart rate recorded during the cardiopulmonary exercise test on a treadmill (HR max).

Statistical analysis

To evaluate the distribution of the measured variables, visual inspection of a histogram with a superimposed normal curve was used. Descriptive statistics was also analyzed to assess the value of skewness and kurtosis; the asymmetry and the peakedness of the distribution, respectively. The histogram provided us with the opportunity to judge whether the distribution was bell shaped or skewed and to identify gaps in the data and outliers. Figure 1 presents HR reserve data plotted in a histogram showing a negatively skewed distribution with positive kurtosis.

![Figure 1 - HR reserve frequency distribution](image)
The same was performed for the two other outcome variables HR rest and HRV, which both had a positively skewed distribution and positive kurtosis. As our data distribution was skewed, non-parametric analyses were used throughout.

Data are mainly presented as medians (25, 75 percentiles). For normally distributed data (Table 1) the mean ± standard deviation (SD) are given. For categorical data numbers are given.

Correlation analyses were performed using Spearman’s rho. The Mann-Whitney U-test was used for comparison of groups.

Statistical calculations were performed using SPSS version 22.0 for windows. A p-value of < 0.05 was considered statistically significant.
Results

Baseline characteristics of the study population are presented in Table 1. The study included 115 males and 22 females, aged between 41 and 81 years. All patients were medically treated according to guidelines (Table 1), one hundred and six (77%) were beta-blocker users. There were 22 (16%) current smokers.

Of the 137 patients 120 (88%) had a valid ECG and thus HRV measurements. Of the remaining 17 EKGs some were missing, while most were excluded due to atrial fibrillation. HR rest and HRR were registered in 137 (100 %) and 133 (97 %) patients respectively.

Table 1 Baseline characteristics of the total study population (n=137)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age *</td>
<td>63.1 ± 7.9</td>
</tr>
<tr>
<td>Male</td>
<td>115 (84%)</td>
</tr>
<tr>
<td>BMI (kg/m^2)*</td>
<td>29.2 ±5</td>
</tr>
<tr>
<td>Current smoker</td>
<td>22 (16.1%)</td>
</tr>
<tr>
<td>Years of diabetes</td>
<td>9 (5, 15)</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>7.4 (6.8, 8.3)</td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td>8.1 (6.9, 9.8)</td>
</tr>
<tr>
<td>Insulin pmol/L</td>
<td>57 (33, 102)</td>
</tr>
<tr>
<td>HOMA2- IR</td>
<td>1.3 (0.7, 2.1)</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>128 (93.4%)</td>
</tr>
<tr>
<td>ASA</td>
<td>124 (90.5%)</td>
</tr>
<tr>
<td>Betablockers</td>
<td>106 (77%)</td>
</tr>
<tr>
<td>Metformin</td>
<td>101 (73.7%)</td>
</tr>
<tr>
<td>ACE-inhibitors or ARB</td>
<td>98 (31.4%)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>30 (21.9%)</td>
</tr>
<tr>
<td>Insulin</td>
<td>26 (19%)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>6 (4.4%)</td>
</tr>
</tbody>
</table>

Data presented as numbers, proportions or medians (25, 75 percentiles) if not stated otherwise.

*Mean value +-SD. Abbreviations: BMI=body mass index, ACE-inhibitors= angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blockers, ASA= acetylsalicylic acid

Absolute values of HRR, HR rest and HRV

As mentioned, the distributions of HRR, HR rest and HRV were skewed. Median HHR was 77 bpm (62 , 93), median HR rest was 62 bpm (56 , 71) and median HRV 7,3 bpm (5.0 , 12.2).
Correlation between glucometabolic control and autonomic activity

Fasting blood glucose correlated inversely with HRR (p = 0.022), but not with HR rest or HRV. HOMA2-IR was inversely correlated to HRR (p = 0.044) and positively correlated to HR rest (p = 0.030). HbA1c did not correlate significantly to neither HRV, HRR nor HR rest, although a borderline significant inverse correlation between HbA1c and HRR (p = 0.057) was observed.

Table 2 Correlations between measurements of autonomic activity and glucometabolic variables.

<table>
<thead>
<tr>
<th></th>
<th>HbA1c (%)</th>
<th>Glucose (mmol/L)</th>
<th>HOMA2-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HRR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.165</td>
<td>-0.201</td>
<td>-0.183</td>
</tr>
<tr>
<td>p</td>
<td>0.057</td>
<td>0.022</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>HR rest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.149</td>
<td>0.103</td>
<td>0.194</td>
</tr>
<tr>
<td>p</td>
<td>0.082</td>
<td>0.237</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>HRV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.017</td>
<td>0.036</td>
<td>-0.059</td>
</tr>
<tr>
<td>p</td>
<td>0.853</td>
<td>0.700</td>
<td>0.543</td>
</tr>
</tbody>
</table>

Correlations between autonomic control and other variables

HRV was inversely correlated with age (p < 0.001) and years of diabetes (p = 0.021). No significant association was found between HRV and BMI.

HRR did not correlate to age or BMI. There was, however, a significant inverse correlation between HRR and duration of diabetes (p = 0.007). HR rest was significantly correlated to BMI (p = 0.024), but showed no significant correlation to age or duration of diabetes.

Taken these data together, both HRV and HRR, but not HR rest, correlated inversely to the duration of diabetes.

Table 3 Correlations between measurements of autonomic activity and other variables.

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Years of T2DM</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HRR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.067</td>
<td>-0.237</td>
<td>-0.140</td>
</tr>
<tr>
<td>p</td>
<td>0.444</td>
<td>0.007</td>
<td>0.110</td>
</tr>
<tr>
<td><strong>HR rest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.141</td>
<td>-0.130</td>
<td>0.193</td>
</tr>
<tr>
<td>p</td>
<td>0.099</td>
<td>0.133</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>HRV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.390</td>
<td>-0.213</td>
<td>-0.149</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001</td>
<td>0.021</td>
<td>0.107</td>
</tr>
</tbody>
</table>
Autonomic activity in subgroups

In the non beta-blocker users (n= 31, median = 89 bpm) compared to the beta-blocker users (n=106, median = 73 bpm), HRR was significantly higher (p = 0.001), whereas HRV and HR rest did not differ between the groups. The significant correlations between HRR and glucose, HOMA2-IR and years of diabetes shown for the total population (Tables 2 and 3) could not be demonstrated when analyzing beta-blocker users and non-users separately.

HRV was significantly lower in men than in women (p = 0.015), whereas HRR and HR rest did not differ between the categories of sex. We could not demonstrate any statistically significant correlation between HRV and glucometabolic control in neither men nor women (data not shown), but we found a significant inverse correlation between HRV and age in men (p < 0.001), and a borderline significant inverse correlation between HRV and duration of diabetes in men (p = 0.051).

Both HR rest (r = 0.731, p < 0.001) and HRR (r = -0.451, p = 0.035) were strongly correlated to HOMA2-IR in women. We could not demonstrate the same in men. There was no correlation between HRR and HR rest and other measures of glucometabolic control in either category of sex.

Table 4 Correlation analyses in subgroups

<table>
<thead>
<tr>
<th></th>
<th>HRR (bpm)</th>
<th>HR rest (bpm)</th>
<th>HRV (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta blocker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>73 (62, 88)</td>
<td>61.5 (56 , 71)</td>
<td>7.07 (4.77 , 11.80)</td>
</tr>
<tr>
<td>No</td>
<td>89 (77 , 100)</td>
<td>65 (58.5 , 72.5)</td>
<td>9.22 (5.20 , 15.86)</td>
</tr>
<tr>
<td>p = 0.001</td>
<td></td>
<td>p = 0.191</td>
<td>p = 0.104</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>76 (62 , 92)</td>
<td>62 (56 , 69)</td>
<td>7.17 (4.70 , 11.80)</td>
</tr>
<tr>
<td>F</td>
<td>81 (63.75 , 93.50)</td>
<td>65 (58 , 76.50)</td>
<td>9.40 (7.06 , 19.95)</td>
</tr>
<tr>
<td>p = 0.460</td>
<td></td>
<td>p = 0.217</td>
<td>p = 0.015</td>
</tr>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>70 (41 , 75)</td>
<td>67 (59 , 73)</td>
<td>9.03 (3.70 , 18.68)</td>
</tr>
<tr>
<td>No</td>
<td>79.50 (63 , 94)</td>
<td>61 (56 , 68.50)</td>
<td>7.29 (5.05 , 11.0)</td>
</tr>
<tr>
<td>p = 0.001</td>
<td></td>
<td>p = 0.053</td>
<td>p = 0.812</td>
</tr>
</tbody>
</table>

M= male, F= female.

HRR was significantly higher in nonsmokers than in smokers (p = 0.001). There was neither any significant difference in HR rest and HRV (Table 4), nor in measures of glucometabolic control or BMI in either subgroup of smoking (data not shown).
Discussion

Our main finding in this study was an association between measurements of glucometabolic control and cardiovascular autonomic function in patients with both CAD and T2DM. The inverse association was most pronounced between HRR and both fasting blood glucose and HOMA2-IR. There was also a significant association between resting HR and HOMA2-IR and a borderline significant inverse association between HRR and HbA1c, but HRV did not associate with any of the glucometabolic variables.

Autonomic function and glucometabolic variables

The increase in resting HR and the reduction in HRR associated with increased HOMA2-IR and fasting blood glucose might reflect both parasympathetic and sympathetic dysfunction, indicating that high levels of blood glucose and insulin resistance are related to imbalance of the autonomic nervous system also in patients with T2DM and CAD. In T2DM several mechanisms have been proposed by which high levels of blood glucose leads to neuropathy, including accumulation of advanced glycosylated end products, increased oxidative stress and ischemia (69). Our main results are in agreement with previous studies, which have shown that high levels of blood glucose (19) or wide fluctuations of blood glucose levels may cause neuropathy (20). However, the associations in the present study were not very strong; implicating that only a minor part of the change in one variable can be explained by variance in the other. We also realize that our study is not suitable for implying any causal relationships. An alternative explanation is that autonomic dysfunction causes insulin resistance, which in turn results in hyperglycemia (70). Fasting blood glucose might be difficult to use for estimating glycemic control over time, as it reflects the level of blood glucose only at that time. However, one could also argue that high fasting blood glucose found in one random sample, often, but not always, could identify the patients with poorly controlled blood sugar or high fluctuations.

The association between insulin resistance and autonomic dysfunction found is in accordance with previous studies (22). In some studies it has been suggested that enhanced activity of the sympathetic nervous system could be a mediator of insulin resistance (70, 71), whereas others have proposed that hyperinsulinemia following insulin resistance might activate the sympathetic nervous system, leading to an imbalance of the autonomic system (72). Beck-Nielsen et. al. postulated that insulin resistance is the first detectable change in T2DM, observed several decades before the onset of hyperglycemia (73), which would advocate for the latter explanation. Again, we cannot suggest causal relationships based on our results.
Lastly, there might be common factors influencing both sympathetic function and insulin sensitivity such as genetic predisposition and a sedentary lifestyle, thereby implying no causal relationship between the two, but rather a co-existence.

Surprisingly, the association between measures of autonomic function and HbA1c was less pronounced in our study, and only a borderline significant inverse correlation between HRR and HbA1c was found. When interpreting our result, the rather low median HbA1c levels in our population have to be considered (Table 1: 7.4%), indicating that our patients have a fairly well controlled blood sugar. Previous studies have demonstrated continuous, but nonlinear relationship between HbA1c and the risk for autonomic neuropathy, and also significantly lower risk for all microvascular complications with HbA1c values ≤ 8.0% (74). In addition, as HbA1c is an indirect measure of the mean glucose level in the blood over the last three months, and neuropathy develops over a much longer period of time, a one-time measurement of HbA1c might not reflect the longstanding level of glycemic control in these patients. However, no risk for progression and development of microvascular complications has been claimed only in normoglycemic patients (74), thus, although not statistically significant, there might be an actual association in our population.

In the present study we could not demonstrate any association between HRV and the glucometabolic variables measured, in contrast to published literature (18). Our patients were treated according to current guidelines for T2DM and CAD, including use of beta-blockers, statins and different blood pressure lowering agents. Use of some of these medications has been shown to improve HRV (75). One could therefore argue that our population was too well treated to show significant reduction in HRV associated with poor glycemic control. This is in agreement with studies of HRV in patients on beta-blockade (76). In addition, our method used for calculating HRV was not quite according to established guidelines for HRV (58). Our results may therefore not be directly comparable to those of previous studies, even though HRV in response to deep breathing has proven to be the most sensitive bedside test for detection of cardiovagal dysfunction (61, 62). However, well conducted clinical trials show convincing evidence that there is an association between glycemic control and HRV (19), although we could not demonstrate it in our population of both T2DM and CAD.

**Autonomic function in beta-blocker users**

Resting HR did not differ significantly between beta-blocker users and non-users in this study. This was somewhat surprising, and is in contrast to current knowledge (77). This may simply be due to the low number in the group of non-users, but it might also reflect that the non-users were in better cardiovascular condition than the patients in need of beta-blocker medication, and therefore had a
lower intrinsic resting HR. Although not statistically significant, the resting HR was numerically lower in users.

HRR was significantly lower in the beta-blocker users compared to the non-users, and no correlation between HRR and HOMA2-IR and blood glucose could be demonstrated in either sub-group separately. The lower HRR in the users might be explained by a reduced maximal HR, due to blocking of the sympathetic innervation.

The reduction in HRR in the beta-blocker users could also reflect that the patients did not reach their true maximal HR during the exercise test. Patients with high insulin resistance have shown impaired exercise capacity independent of BMI (78, 79), and they could have bigger problems reaching their maximal HR during the test than less insulin resistant patients. Other factors, such as ischemia, functional disabilities, or even psychological factors such as the patients’ ability to push themselves, might also influence the patients’ capability to reach true maximal HR.

**Autonomic function as related to other factors**

Both HRV and HRR were inversely associated with years of diabetes, supporting the previously reported contribution of duration of diabetes to the risk of neuropathy also in patients with both T2DM and CAD (80, 81). This suggests that patients who suffer from diabetes over a long period of time have a higher risk of developing autonomic neuropathy, and points to the importance of prevention and early detection of T2DM, especially in patients with CAD (11). HRV was negatively associated with patient age, and was lower in men than in women. This is consistent with current knowledge; it is well known that even in healthy subjects HRV decreases with age and differs between sexes (82).

HRR was significantly lower and resting HR borderline significantly higher in smokers, indicating that smoking is related to autonomic dysfunction. Previous studies have proposed smoking as a risk factor for cardiac autonomic neuropathy (83), and it is already established as one of the greatest risk factors for CAD.

**Limitations**

Our population consisted of patients with CAD and T2DM, in which patients with additional severe diseases and physical limitations that would influence the ability to participate in the exercise program were excluded. The autonomic dysfunction in the excluded patients might have been more severe.
In addition, our population was well treated. Several studies have shown that intensive treatment of diabetes and its complications can improve autonomic function (75), and the median of HbA1c in the present group is evidence of an overall well treated T2DM.

As mentioned, our choice of method for HRV was not quite according to the established guidelines, and might therefore not be comparable to other studies. In the assessment of insulin resistance, the HOMA2-IR was used (78), whereas the hyperinsulinemic euglycemic glucose clamp technique is the preferred method (84).

Summary

In the present study we found glucometabolic factors to be associated with autonomic dysfunction to some extent. This confirms that tight glucose control and prevention of insulin resistance is of importance in the treatment of patients with T2DM and CAD, to prevent cardiovascular autonomic imbalance and its complications. However, other factors also seem to be of importance, including cessation of smoking and medication according to guidelines. The association to duration of diabetes highlights the importance of prevention and early detection of T2DM in patients with CAD, and our results are in accordance with current recommendations for patients with T2DM and CAD.

As this investigation is part of an interventional study, any impact of exercise training on the autonomic function of these patients would be of great interest.
References


