Lower Heart Rate Variability Is Associated With the Development of Coronary Heart Disease in Individuals With Diabetes

The Atherosclerosis Risk in Communities (ARIC) Study

Duanping Liao,[1] Mercedes Carnethon,[2] Gregory W. Evans,[4] Wayne E. Cascio,[3] and Gerardo Heiss[2]

The objective of this study was to test prospectively in a population sample whether individuals with impaired heart rate variability (HRV) are at increased risk of developing coronary heart disease (CHD) and of non-CHD mortality and to test whether this relationship is more pronounced among individuals with diabetes. We examined the association between HRV and incident CHD and non-CHD mortality in a cohort of 11,654 men and women aged 45–64 years at intake, from the biracial, population-based Atherosclerosis Risk in Communities Study. Supine, resting, 2-min beat-to-beat heart rate data were collected at the baseline examination. High frequency (HF; 0.15–0.40 Hz) and low frequency (LF; 0.04–0.15 Hz) spectral powers, LF/HF ratio, normalized HF and LF, the standard deviation of all normal R-R intervals (SDNN), and the mean of the sum of the squared differences between adjacent normal R-R intervals (MSSD) were used as the conventional indexes of HRV to measure cardiac autonomic control. From this cohort, 635 cases of incident CHD (including 346 cases of incident myocardial infarction [MI] and 82 cases of fatal CHD), and 623 non-CHD deaths were identified and validated after an average of 8 years of follow-up. Among individuals with diabetes, the multivariable adjusted proportional hazards ratios (95% CI) were 2.03 (1.28–3.23), 1.60 (1.12–2.27), 1.50 (0.65–3.42), and 1.27 (0.84–1.91) for incident MI, incident CHD, fatal CHD, and non-CHD deaths, respectively, comparing the lowest quartile to the upper most three quartiles of HF. A similar pattern of associations was found for LF, SDNN, and MSSD. By contrast, there was no consistent pattern of associations among individuals without diabetes. At the population level, a lower HRV (reflective of impaired cardiac autonomic control) is statistically significantly related to the development of CHD among individuals with diabetes, independent of markers of the duration/severity of the glucose metabolism impairment. These data suggest a contribution of an impaired cardiac autonomic control to the risk of CHD among individuals with diabetes.
***Diabetes* 51:3524–3531, 2002**

Analysis of beat-to-beat heart rate variability (HRV) has emerged as one of the noninvasive methods to quantitatively assess cardiac autonomic activity. Previous work has shown that heart rate oscillations at low frequencies (0.04–0.15 Hz) are under the influence of both the sympathetic and parasympathetic nervous systems, whereas heart rate oscillations at high frequencies (0.15–0.40 Hz) are predominantly under the influence of the parasympathetic system and serve as a marker of cardiac parasympathetic activity (1–9). Also used extensively are techniques to estimate HRV in the time domain, e.g., the standard deviation of R-R intervals. (8).

Several studies on patient populations have found that lower HRV is associated with a higher risk of all-cause mortality in survivors of an acute myocardial infarction (MI) (10–13). Lower HRV has also been found to be related to sudden cardiac death (14). Results from population-based follow-up studies also suggest that lower HRV is associated with the risk of developing coronary heart disease (CHD) (15–17). It has since been proposed that HRV be used as a prognostic factor for MI risk stratification and management (18).

People with type 2 diabetes have a two- to fourfold higher risk of cardiovascular disease (19–22). Identifying factors associated with the poor cardiovascular prognosis in individuals with diabetes may lead to more effective screening and earlier institution of primary prevention programs to slow the development and progression of cardiovascular disease among people with type 2 diabetes. We have previously reported statistically significant associations among diabetes, elevated fasting insulin, and impaired cardiac autonomic control (23,24). We have also reported a significant association between reduced heart rate variability and incident cardiovascular disease and all-cause mortality using case-cohort methods in subsamples from a large population-based sample (16,17). The

From the [1]Department of Health Evaluation Sciences, Pennsylvania State University Medical College, Hershey, Pennsylvania; the [2]Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; the [3]Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; and the [4]Department of Public Health Sciences, Wake Forest University, Winston-Salem, North Carolina.

Address correspondence and reprint requests to Dr. Duanping Liao, Department of Health Evaluation Sciences, Pennsylvania State University College of Medicine, A210, 600 Centerview Dr., Hershey, PA 17033. E-mail: dliao@psu.edu.

Received for publication 11 June 2001 and accepted in revised form 28 August 2002.

ARIC, Atherosclerosis Risk in Communities; CHD, coronary heart disease; ECG, electrocardiogram; HF, high frequency; HFnu, normalized HF; HRV, heart rate variability; LF, low frequency; LFnu, normalized LF; MI, myocardial infarction; MSSD, mean of the sum of the squares of differences; PSD, power spectral density; SDNN, standard deviation of all normal R-R intervals; TP, total HRV power; VLF, very low frequency.

present study was designed to perform more detailed analysis on the previously reported relationship between lower HRV and risk of incident CHD and non-CHD mortality (16,17) in the full cohort of a population sample followed for an average of 8 years and to examine the degree to which people with type 2 diabetes are susceptible to the risk conveyed by a lower HRV.

RESEARCH DESIGN AND METHODS

This study was conducted in the cohort of the Atherosclerosis Risk in Communities (ARIC) study. (25) ARIC is a longitudinal study of cardiovascular and pulmonary diseases, sponsored by the National Heart, Lung and Blood Institute, that includes community surveillance and cohort components. The ARIC cohort was selected as a probability sample of 15,792 men and women between the ages of 45 and 64 years at four study centers in the United States, three of which enumerated and enrolled all age-eligible residents sampled from geographically defined areas (Washington County, MD; Forsyth County, NC; and selected suburbs of Minneapolis, MN). The fourth quarter of the ARIC cohort was sampled from black residents of Jackson, MS. Details of sampling, study design, and cohort examination procedures have been published (25). Eligible participants were interviewed at home and then invited to a baseline clinical examination conducted in 1987–1989, followed by triennial follow-up clinical examinations. Overall, 75% of eligible individuals responded to the home interview, and 65% of eligible individuals participated in the baseline clinical examination (45% from the Jackson center and >85% from other three centers). Annual telephone contacts with the cohort participants and a community surveillance system have been in place since 1987 to support the identification and classification of cardiovascular disease incidence and other mortality.

All black and white examinees who were free of CHD at baseline were eligible for the analyses of incident CHD in this report (n = 14,075). A total of 110 participants were excluded from this report because of incomplete data to define their diabetic status at baseline, or their heart rate variability measurements (n = 2,941). Of the latter, 95% were due to equipment failures in the first several months of the baseline examination and 5% were due to artifacts in the R-R interval data. Thus, this report is based on 11,654 individuals who were free of CHD at the intake examination. The cohort was followed up for an average of 8 years for cardiovascular disease morbidity, mortality, and non-CHD mortality.

Assessment of cardiac autonomic control. The resting, supine, 2-min beat-to-beat R-R interval data were collected and analyzed according to standardized protocols and methods (26–29). The short-term intraparticipant reliability coefficients were 0.82, 0.56, 0.64, and 0.70 for high frequency (HF), low frequency (LF), LF/HF ratio, and standard deviation of all normal R-R intervals (SDNN), respectively, and the intra- and interdata operator reliability coefficients for the HF, LF, LF/HF ratio, and SDNN were >0.95 (28). For this study, the total power (TP), the HF spectral power component, the LF spectral power component, and the very low frequency (VLF) spectral power component were calculated. TP was defined as the total area (0.00–0.50 Hz) under the power spectral density (PSD) curve, whereas HF, LF, and VLF were defined as the power (area) between 0.15 and 0.40 Hz, 0.04 and 0.15 Hz, and 0.00 and 0.04 Hz bands under the PSD curve, respectively. Following the recommendation by the Task Force on HRV research (18), normalized HF [HFnu = HF/(TP − VLF) × 100], normalized LF [LFnu = LF/(TP − VLF) × 100], and LF/HF ratio were calculated. The SDNN and the mean of the sum of squared differences (MSSD) between adjacent normal R-R intervals were calculated from the time domain data. The mean heart rate from the 2-min R-R interval data were also calculated.

Classification of cardiovascular disease morbidity, mortality, and other causes of death. The ARIC study participants have been contacted yearly by telephone (97% complete follow-up) to ascertain vital status, hospitalizations, and visits to medical practitioners. Cardiovascular and cerebrovascular disease end points are identified and validated through abstraction of hospital records and death certificates, physician questionnaires, interviews of next of kin, and classification of end points by a panel of reviewers, as described elsewhere (30). The monitored cardiovascular disease events include hospitalized MI, fatal CHD, revascularization procedures (coronary artery bypass graft or percutaneous transluminal coronary angioplasty), and electrocardiogram (ECG) detected silent MI on follow-up cohort examinations. ECG detected silent MI was defined as significant Q wave, or borderline Q wave with significant ST-segment or T-wave abnormalities, in the absence of ventricular conduction defects that interfere with Q-wave coding. Cohort event investigation is supported by community surveillance of all individuals who are aged 35–74 years and reside in the study communities. This report includes all events that occurred before 1 January 1997, with an average length of follow-up of 8 years. Overall, <4% of cohort members have been lost to follow-up.

Covariables. Type 2 diabetes was defined as a fasting (minimum of 8 h) serum glucose ≥127 mg/dl, or glucose ≥200 mg/dl if fasting <8 h, or self-reported physician-diagnosed diabetes, or self-reported use of an oral hypoglycemic agent or insulin. Hypertension was defined as diastolic blood pressure ≥90 mmHg, or systolic blood pressure ≥140 mmHg, or the self-reported use of antihypertensive medications.

Sitting blood pressure was measured three times on each participant with a random zero sphygmomanometer, after a 5-min rest, by trained technicians following a standardized protocol. The average of the second and the third readings of systolic and fifth-phase diastolic blood pressure were used in this report.

Fasting blood was drawn to obtain serum and plasma, which were then shipped on dry ice to the central laboratories for assay within 1 week of their collection. All assays were performed at a central laboratory. Glucose was measured by a hexokinase/glucose-6 phosphate dehydrogenase method. Insulin was measured by radioimmunoassay ($_{125}$Insulin Kit; Cambridge Medical Diagnosis, Billerica, MA). Total cholesterol, triglycerides, and HDL cholesterol were measured by enzymatic procedures using a Cobas analyzer (Hoffman-La Roche, Basel, Switzerland).

Other demographic variables and risk factors for CHD were assessed according to standardized protocols common to all ARIC study sites and were subject to regular quality control checks (27). Briefly, age, race, sex, education level, and cigarette smoking status were determined during a home interview with the use of a standardized questionnaire by trained and certified interviewers. BMI was calculated as the measured weight (kg)/height (m)^2. Clinical manifestations of CHD at baseline was defined by history of a hospitalized MI, history of cardiac revascularization procedures, or prevalent MI documented by ECG.

Statistical analysis. Following the recommendation by the Task Force on HRV research (18), a natural logarithmic transformation was used to normalize the distribution of the HRV frequency domain HRV indexes (HF and LF) when used as continuous variables. Pearson correlation coefficients between HRV indexes were calculated. The means and SE of HRV indexes were calculated comparing people with different events with people who remained event-free through the end of 1996, stratified by diabetes status. Cox proportional hazards models were used to estimate the relative risk of each type of event associated with lower HRV indexes. In our primary models, HRV indexes were entered into the models as "low HRV" versus "normal HRV" status based on the 25th percentile cut point for HRV indexes. This cut point was based on our previous experience of threshold effects of HRV indexes when used as predictor and/or outcome variables and was supported by the preliminary analysis showing consistent pattern of threshold effects at the same cut point in this population. Interaction between HRV indexes and other potential effect modifiers (age, race, sex, smoking, hypertension, and diabetes) were tested using the log-likelihood ratio test at $P < 0.10$.

RESULTS

The characteristics of the study population are presented for the entire cohort in Table 1 and stratified by the major event categories (incident CHD, incident MI, fatal CHD, and non-CHD death), as well as by quartiles of SDNN (SDNN was chosen because it can be considered as an overall measure of beat-to-beat variation of R-R intervals from the 2-min record). The pattern of association between the risk factors and lower HRV remains unchanged regardless of which HRV indexes were used. The mean age at the baseline examination was 54 years, 42% of study participants were men, and 74% were white. Of the original 11,654 CHD-free study participants, 635 developed incident CHD (5.4%); among them, 346 had incident MI (3.0%), 82 had fatal CHD (0.70%), and the rest underwent revascularization procedures or exhibited a silent MI documented by a centrally read ECG. A total of 623 participants died of non-CHD causes (5.3%). As expected, cardiovascular risk factors and comorbidity profiles of people who developed events during follow-up were higher than the average of the study population, particularly the prevalence of diabetes, hypertension, and current smoking. Men

TABLE 1
Mean (SD) or proportion of baseline characteristics of the study population and stratified by major event categories and quartiles of HRV*: the ARIC Study

Variable	All (n = 11,654)	Outcome categories				2-min SDNN quartile	
		Incident CHD (n = 635)	Incident MI (n = 346)	Fatal CHD (n = 82)	Non-CHD death (n = 623)	Lowest (n = 2,916)	Upper three (n = 8,736)
Age (years)	54 (5.7)	56 (5.4)	56 (5.4)	57 (5.52)	57 (5.4)	55 (5.7)	53 (5.6)
Sex (% male)	42	67	62	66	50	35	44
Race (% white)	74	78	75	51	59	72	75
Education (% > high school)	78	70	67	62	62	74	79
Current smoker (%)	26	37	41	37	42	25	26
BMI (kg/m^2)	27.52 (5.31)	28.18 (4.96)	28.39 (5.30)	28.52 (5.96)	27.33 (6.06)	28.25 (5.87)	27.27 (5.09)
Glucose (mg/dl)	108 (40)	127 (65)	129 (64)	152 (98)	126 (76)	118 (57)	105 (31)
Insulin (pmol/l)	98 (196)	176 (415)	182 (421)	271 (628)	157 (491)	137 (326)	85 (123)
Total cholesterol (mg/dl)	214 (41)	226 (42)	227 (42)	223 (53)	216 (47)	219 (43)	213 (41)
HDL cholesterol (mg/dl)	52 (17)	43 (13)	44 (14)	45 (15)	51 (18)	52 (18)	52 (17)
Triglycerides (mg/dl)	129 (87)	161 (102)	162 (104)	162 (123)	144 (121)	145 (107)	124 (79)
Systolic BP (mmHg)	121 (19)	129 (21)	130 (22)	138 (24)	129 (24)	125 (20)	120 (18)
Diastolic BP (mmHg)	73 (11)	76 (13)	76 (14)	78 (14)	76 (14)	75 (12)	73 (11)
Hypertension (%)	33	51	51	73	46	44	30
Diabetes (%)	11	25	27	38	23	18	8
Duration of diabetes*							
<1 year or unknown (%)	70	68	65	93	90	68	72
1–5 years (%)	12	9	10	0	4	11	12
5–10 years (%)	8	9	12	0	4	8	8
>10 years (%)	10	14	13	6.5	2	13	9
Treated diabetes (%)*	46	58	61	71	65	54	40
Mean heart rate (beat/min)	68 (10)	68 (12)	68 (12)	73 (15)	71 (12)	74 (12)	66 (9)
Incident CHD (%)	5.5	—	—	—	—	7.3	4.8

*Among diabetes. BP, blood pressure.

were more likely to experience cardiovascular events and non-CHD mortality than the women. Although white individuals were slightly more likely to develop incident CHD and MI, black individuals were much more likely to experience fatal events such as fatal CHD or non–CHD-related death. As summarized in Table 1, comparison of the main covariables between the lowest quartile and the upper three quartiles of HF revealed the relationship of HRV to specific cardiovascular risk factors: a lower HRV was associated with an unfavorable cardiovascular risk profile with respect to age, education, BMI, fasting glucose and insulin, total cholesterol, triglycerides, mean heart rate, systolic and diastolic blood pressures, hypertension, and diabetes. The cumulative incident CHD rate was 7.3% in the lower HF group compared with 4.8% in the remainder of the HF distribution.

The Pearson correlation coefficients between the time and frequency domain HRV indexes derived from 2-min R-R interval data collected in the supine position after 20 min of rest are presented in Table 2. As expected, when used as absolute values, the HRV indexes (HF, LF, SDNN, and MSSD) were highly to moderately correlated to each other. Comparing the correlation between time and frequency domain indexes, it can be noted that HF was equally correlated with SDNN and MSSD, but LF correlated better with SDNN than with MSSD. The time domain indexes (SDNN and MSSD) were moderately correlated with mean heart rate but not the frequency domain indexes. When used as a ratio or proportion, the frequency domain indexes (LF/HF ratio, normalized HF and LF) were highly correlated with each other in the direction predicted from the formulation of these indexes, but they are either not correlated or only weakly correlated with time domain indexes and heart rate.

The interaction between HRV indexes and major covariables in the prediction of outcomes was tested, and diabetes was consistently found to be the only significant effect modifier ($P < 0.05$). Therefore, nested models were fitted to account for the interaction terms, and results were reported according to diabetes status.

TABLE 2
Pearson correlation coefficients of HRV indices: the ARIC Study baseline examination (1987–1989)

	LnHF (ms^2)	LnLF (ms^2)	LF/HF	HFnu	LFnu	2-min SDNN (ms)	MSSD (ms)	Mean heart rate (beat/min)
LnHF [Ln (ms^2)]	1.00	0.76	−0.27	0.26	−0.31	0.54	0.60	−0.16
LnLF [Ln (ms^2)]		1.00	0.28	−0.39	0.34	0.53	0.38	−0.18
LF/HF			1.00	−0.72	0.70	0.05	−0.18	−0.07
HFnu				1.00	−0.84	0.00	0.24	−0.05
LFnu					1.00	0.05	−0.27	−0.12
SDNN (ms)						1.00	0.82	−0.41
MSSD (ms^2)							1.00	−0.40

TABLE 3
Mean (SE) of HRV indexes comparing different categories of outcome, stratified by diabetes status: the ARIC Study

HRV indexes	Diabetic subjects				Nondiabetic subjects				Non-CHD death
	Event-free	Incident CHD	Incident MI	Fatal CHD	Non-CHD death	Event-free	Incident CHD	Incident MI	Fatal CHD	
LnHF (Ln [ms^2)]	1.87 (0.045)	1.26 (0.123)	1.24 (0.163)	1.02 (0.331)	1.37 (0.141)	2.16 (0.013)	1.99 (0.062)	2.16 (0.081)	1.89 (0.194)	1.85 (0.064)
LnLF [Ln (ms^2)]	2.33 (0.047)	1.74 (0.137)	1.73 (0.180)	1.21 (0.298)	1.65 (0.157)	2.77 (0.014)	2.65 (0.059)	2.73 (0.076)	2.21 (0.208)	2.36 (0.067)
LF/HF ratio	2.34 (0.071)	2.47 (0.195)	2.47 (0.253)	1.79 (0.285)	1.96 (0.156)	2.75 (0.030)	2.99 (0.170)	2.63 (0.166)	2.63 (0.480)	2.56 (0.117)
HFnu	38 (0.56)	38 (1.44)	38 (1.96)	41 (2.45)	40 (1.38)	37 (0.18)	36 (0.79)	37 (1.10)	41 (2.95)	37 (0.83)
LFnu	60 (0.71)	60 (1.89)	60 (2.34)	55 (5.01)	55 (2.04)	64 (0.22)	65 (1.01)	63 (1.38)	57 (4.06)	61 (1.1)
2-min SDNN (ms)	32 (0.574)	28 (1.446)	27 (1.752)	25 (4.40)	27 (1.372)	38 (0.204)	36 (0.770)	36 (1.096)	31 (2.04)	35 (0.82)
MSSD (ms^2)	25 (0.635)	21 (1.876)	21 (2.016)	23 (7.21)	22 (1.53)	30 (0.240)	28 (0.923)	30 (1.377)	28 (2.81)	28 (1.04)

The mean values (and their SE) of HRV indexes comparing people with different categories of events with people who remained event-free at the end of follow-up (1996) are presented in Table 3. Similar to our previous reports (23,24), individuals with diabetes had significantly lower HRV indexes than people without diabetes across each stratum of the outcome categories, except for the ratio and the proportion measures of HRV. The absolute measures of the HRV indexes were statistically significantly lower among people who developed cardiovascular events and non-CHD death than in people who remained event-free. This relationship was more pronounced and more consistent among individuals with diabetes than among individuals without diabetes. No consistent relationships were found for the ratio/proportion measures of HRV.

Table 4 presents hazard ratios (and 95% CI) by quartile of HRV indexes for each of the outcome categories according to diabetes status, adjusting for age, sex, ethnicity-center, smoking, and mean heart rate. The hazard ratios estimated from these models indicated consistent and significant associations between quartiles of HRV and cardiovascular and noncardiovascular outcomes among individuals who had diabetes at baseline. Moreover, the pattern of associations suggests a threshold effect of HRV, in that only people in the lowest quartile were at significantly higher risk of events. As a result, all primary models were formulated to contrast the lowest quartile with the upper three quartiles of the HRV indexes. By contrast, no consistent association was found among individuals without diabetes between quartiles of HRV and cardiovascular and noncardiovascular outcomes. None of ratio/proportion measures of HRV (HFnu, LFnu, and LF/HF ratio) was statistically significantly associated with the development of any of the outcomes under investigation.

Table 5 presents hazard ratios (and their 95% CI) for each outcome category contrasting HRV in the lowest quartile to the remainder of the population, estimated from nested multivariable Cox's proportional models to account for diabetes status. The covariables adjusted for in these models include baseline age, sex, ethnicity-center, smoking, mean heart rate, BMI, fasting glucose, triglycerides, HDL cholesterol, and hypertension. The hazard ratios estimated from these models indicated that individuals with diabetes with baseline HRV below the 25th percentile of the population distribution were at statistically significantly increased risk of various outcomes than those with their HRV above this cut point, particularly for incident CHD and incident MI. The hazard ratio estimates for fatal CHD were similar to those for incident CHD, but their 95% CI included 1.00, ostensibly because of the small number of fatal CHD events (n = 31). Associations were less consistent for non-CHD mortality: of the four measures of HRV, only lower LF was statistically significant (HR = 1.58, 95% CI 1.12–2.23). None of the ratio/proportion measures of HRV was significantly associated with the development of any of the outcomes under investigation (data not shown). These associations were considerably weaker and/or inconsistent among individuals without diabetes.

For minimizing potential confounding by the duration and severity of diabetes, Cox's models were fitted for

TABLE 4
Hazards ratios* (95% CI) of cardiovascular events and non-CHD mortality associated quartiles of baseline HRV indexes according to diabetes status: 8 years of follow-up of the ARIC cohort

Quartiles of HRV	Incident CHD	Incident MI	Fatal CHD	Non-CHD death
Diabetic subjects (n = 1,275)	(n = 161)	(n = 94)	(n = 31)	(n = 142)
HF				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	0.90 (0.50–1.59)	0.92 (0.42–1.99)	0.42 (0.10–1.78)	0.64 (0.37–1.12)
Q2	1.07 (0.62–1.86)	1.15 (0.55–2.39)	0.59 (0.16–2.21)	0.71 (0.41–1.25)
Q1 (Lowest)	1.87 (1.15–3.03)	2.27 (1.20–4.30)	1.29 (0.45–3.69)	1.23 (0.77–1.97)
LF				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	0.75 (0.42–1.35)	0.53 (0.24–1.17)	1.26 (0.21–7.58)	0.90 (0.48–1.69)
Q2	0.98 (0.58–1.67)	0.82 (0.41–1.63)	1.60 (0.32–8.08)	0.88 (0.49–1.58)
Q1 (Lowest)	1.65 (1.02–2.68)	1.72 (0.94–3.13)	3.49 (0.79–15.50)	1.57 (0.92–2.69)
2-min SDNN				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	0.85 (0.47–1.55)	0.85 (0.38–1.91)	1.54 (0.28–8.47)	0.93 (0.48–1.79)
Q2	1.00 (0.58–1.75)	1.09 (0.51–2.32)	1.72 (0.34–8.65)	1.27 (0.69–2.37)
Q1 (Lowest)	1.75 (1.04–2.96)	2.28 (1.13–1.91)	2.54 (0.54–11.98)	1.48 (0.81–2.69)
MSSD				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	1.16 (0.65–2.05)	1.25 (0.61–2.58)	1.66 (0.41–6.69)	1.01 (0.56–1.82)
Q2	0.89 (0.48–1.66)	0.77 (0.33–1.77)	0.21 (0.02–2.10)	1.06 (0.58–1.95)
Q1 (Lowest)	1.78 (1.03–3.10)	1.93 (0.94–3.97)	1.64 (0.42–6.43)	1.35 (0.76–2.43)
Nondiabetic subjects (n = 10,372)	(n = 474)	(n = 252)	(n = 51)	(n = 480)
HF				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	1.03 (0.79–1.33)	1.05 (0.74–1.47)	0.83 (0.35–1.99)	1.34 (1.02–1.76)
Q2	0.83 (0.63–1.08)	0.86 (0.60–1.22)	1.16 (0.53–2.51)	1.12 (0.85–1.48)
Q1 (Lowest)	0.93 (0.71–1.20)	0.71 (0.49–1.03)	1.00 (0.45–2.22)	1.38 (1.05–1.80)
LF				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	1.07 (0.83–1.38)	1.02 (0.72–1.44)	0.88 (0.34–2.28)	0.94 (0.71–1.25)
Q2	1.12 (0.87–1.44)	1.03 (0.72–1.45)	1.50 (0.65–3.49)	1.07 (0.82–1.40)
Q1 (Lowest)	1.01 (0.78–1.32)	0.90 (0.62–1.29)	1.92 (0.85–4.31)	1.33 (1.03–1.73)
2-min SDNN				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	1.02 (0.78–1.31)	1.08 (0.75–1.54)	1.50 (0.61–3.68)	1.05 (0.83–1.37)
Q2	1.05 (0.80–1.36)	1.15 (0.80–1.66)	1.48 (0.59–3.68)	0.91 (0.69–1.20)
Q1 (Lowest)	1.26 (0.96–1.67)	1.38 (0.94–2.03)	2.29 (0.93–5.62)	1.12 (0.85–1.48)
MSSD				
Q4 (Highest)	1.00	1.00	1.00	1.00
Q3	0.82 (0.62–1.07)	0.63 (0.43–0.91)	0.73 (0.31–1.72)	1.03 (0.79–1.35)
Q2	0.87 (0.67–1.14)	0.85 (0.60–1.21)	1.75 (0.32–1.74)	0.91 (0.69–1.19)
Q1 (Lowest)	1.09 (0.81–1.46)	0.89 (0.60–1.32)	1.13 (0.48–2.68)	0.97 (0.72–1.31)

*All models were fitted with quartile of HRV, diabetes status, and HRV-diabetes interaction terms as dependent variables, simultaneously adjusted for baseline age, sex, ethnicity-center, cigarette smoking status, and mean heart rate.

people with diabetes to also include estimated diabetes duration and treatment status as covariables, in addition to a set of variables considered to be associated with diabetes severity and glycemic control among individuals with diabetes. These results, presented in Fig. 1, were similar to those for individuals with diabetes presented in Table 5.

DISCUSSION

Traditionally, HRV analyses have been based on heart rate data recorded over several hours. This provides a wealth of data but is costly and logistically complex for population-based, epidemiological research. In this report, we applied spectral analysis to 2-min beat-to-beat heart rate data collected according to a standardized protocol, by trained and certified technicians, subject to well-established quality control monitoring. We were able to maintain 90% of the R-R interval records "artifact free" and maintain high degrees of short-term intraparticipant reliability coefficients and intrareader and interreader reliability coefficients for all HRV indexes used in this study. Caution should be exercised when estimating HRV indexes, particularly the LF component of HRV, from short-term heart rate data. Published recommendations indicated that a 5-min record is sufficient for short-term HRV analysis (18). For frequency domain analysis, Bigger (2) recommended that estimates of HF power centered around 0.25 Hz should be based on records of at least 1 min duration and that LF power estimates (0.04–0.15 Hz) require ~2.5 min of beat-to-beat data. Additional findings supportive of using short records of R-R intervals have been reported by other investigators (8,31) and in our

TABLE 5
Multivariable adjusted* proportional hazard ratios (95% CI) of cardiovascular events and non-CHD mortality comparing the lowest and upper three quartiles of HRV indexes, according to diabetes status: 8 years of follow-up of the ARIC study

	Lowest quartile of frequency domain HRV indexes		Lowest quartile of time domain HRV indexes	
	HF	LF	2-min SDNN	MSSD
Diabetic subjects (n = 1,275)				
Incident CHD (n = 161)	1.55 (1.13–2.13)	1.47 (1.07–2.02)	1.49 (1.07–2.07)	1.51 (1.08–2.11)
Incident MI (n = 94)	1.75 (1.15–2.65)	1.79 (1.18–2.72)	1.80 (1.16–2.77)	1.59 (1.02–2.47)
Fatal CHD (n = 31)	1.61 (0.75–3.46)	1.94 (0.90–4.18)	1.31 (0.59–2.90)	1.65 (0.71–3.80)
Non-CHD death (n = 42)	1.37 (0.97–1.94)	1.58 (1.12–2.23)	1.20 (0.84–1.71)	1.08 (0.75–1.55)
Nondiabetic subjects (n = 10,379)				
Incident CHD (n = 474)	0.95 (0.78–1.17)	0.86 (0.69–1.08)	1.17 (0.94–1.45)	1.22 (0.98–1.52)
Incident MI (n = 252)	0.74 (0.54–1.00)	0.81 (0.60–1.10)	1.22 (0.91–1.64)	1.11 (0.81–1.51)
Fatal CHD (n = 51)	0.83 (0.44–1.57)	1.50 (0.84–2.70)	1.52 (0.82–2.80)	1.30 (0.68–2.46)
Non-CHD death (n = 481)	1.21 (0.99–1.48)	1.35 (1.11–1.64)	1.14 (0.93–1.41)	1.03 (0.82–1.29)

*Adjusted for baseline age, sex, ethnicity-center, cigarette smoking status, heart rate, BMI, fasting glucose, triglycerides, HDL cholesterol, and hypertension.

previous reports (16,17,24,32). Regardless of the limitation of the mathematical imprecision in estimating LF from 2-min records, LF in these data performed well in predicting CHD events.

The correlation between various HRV indexes estimated from our 2-min supine R-R interval data suggests that *1*) the HF and LF are highly correlated, indicating that both HF and LF are predominantly under the influence of parasympathetic control under our study conditions; *2*) frequency domain measures (HF and LF) are highly to moderately correlated with time domain measures (SDNN and MSSD); *3*) SDNN is record length– and protocol-dependent, e.g., the mean SDNN in this population-based biethnic data is only 32 ms among apparently healthy individuals, which is smaller than the conventional cut point of SDNN <50 ms used to define lower HRV in clinical studies (10); and *4*) the frequency domain indexes (HF and LF) are less heart rate dependent than the time domain indexes. Future studies and clinical application of HRV analysis should take into account these observations.

In this data, lower HRV is significantly associated with increased risk of CHD events only among individuals with diabetes, not among individuals without diabetes. This differs from our previous reports (16,17) in which we did not find diabetes as a significant effect modifier for the HRV and CHD association. A possible explanation is that previously reported results were based on small sample sizes and/or shorter follow-up time and thus were insufficiently powered to detect an effect modification by diabetes status. According to this argument, previously reported associations between lower HRV and increased risk of CHD may reflect the average of the associations for people with and without diabetes. In our current multivariable models, adjustment of the duration and treatment of diabetes and several factors traditionally considered clustered with the severity of diabetes and metabolic syndrome (20,31–35), including BMI, fasting glucose, triglycerides, HDL cholesterol, and hypertension, did not change the pattern of associations. Thus, we consider the effect modification by diabetes to be real in these data.

Lower HRV has previously been found to be predictive of the development of CHD (15–17), indicating that reduced cardiac autonomic control as a result of various underlying pathophysiological factors represents a stage of increased risk of cardiac events. Our observation in this large population-based cohort is that lower HRV is associated with increased risk of developing CHD over an average of >8 years of follow-up among individuals who had diabetes at baseline examination but not among individuals without diabetes at baseline. From an etiologic

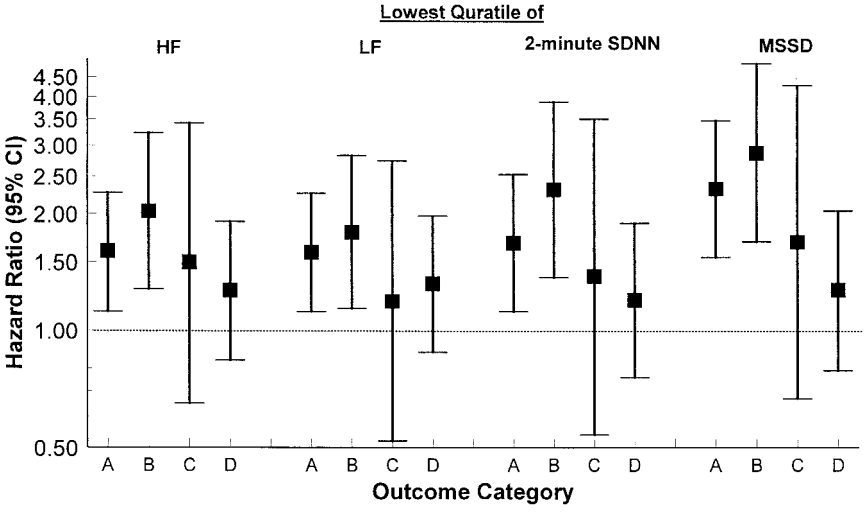

FIG. 1. Multivariate adjusted hazard ratios and 95% CIs of incident CHD and non-CHD mortality, comparing the lowest quartile with the upper three quartiles of HRV indexes among individuals with diabetes. Adjusted for age, sex, ethnicity-center, smoking status, heart rate, BMI, fasting glucose, HDL cholesterol, triglycerides, hypertension, and duration and treatment of diabetes. A = incident CHD; B = incident MI; C = fatal CHD; D = non-CHD death.

perspective, our findings suggest that asymptomatic cardiac autonomic impairment contributes to increased risk of cardiovascular events among individuals with diabetes. From a clinical and a public health perspective, our data suggest that measures that prevent cardiac autonomic impairment, such as long-term glycemic control, improved physical activity, and control of obesity, may reduce the risk of cardiovascular disease among individuals with diabetes.

Another noteworthy finding in these data is the apparent nonlinear relationships between various outcomes and HRV indexes. As summarized in Table 4, a significantly increased risk for various outcomes was identifiable only in individuals who had diabetes and whose HRV indexes were below the 25th percentile cut point derived from the entire study population. Furthermore, better fitting models were observed, allowing for piece-wise estimation of risk comparing the lowest quartile to the remainder of the distribution of the HRV indexes (Table 5) than in models in which measures of HRV were entered as continuous variables (data not shown). These observations, together with the previously reported threshold effects of HRV (16,32) at a cut point approximately placed at the 25th percentile, suggest a threshold effect of HRV at the population level, not only in the development of CHD but also in insulin resistance/glucose levels (23–24), and the development of hypertension (36).

Because of small numbers of fatal CHD, the association between lower HRV and fatal CHD was not statistically significant. It can be noted that the point estimates (hazard ratios) in Table 5 and Fig. 1 consistently pointed to a stronger association for HRV indexes and fatal CHD than that of non-CHD death, except for HF. Of the four measures of HRV, lower LF was consistently significantly associated with non-CHD death (Table 5) among individuals both with and without diabetes. This is consistent with previous observations from a subsample of this population-based cohort (17). The reasons for such an association are not fully understood. Perhaps impaired HRV is a marker of suboptimal health where lower HRV is a readily measurable variable.

In this study, none of the ratio/proportion measures (HFnu, LFnu, and LF/HF ratio) was significantly associated with increased risk of CHD events. This result is similar to that reported by Tsuji et al. (15) in a subset of the Framingham Heart Study cohort. This is not surprising because both HF and LF are highly and positively correlated and are mostly reflective of parasympathetic outflow under our study conditions of prolonged supine rest. Moreover, it would be unusual for a ratio of LF and HF to provide information additional to that contributed by each variable individually. Others (37) also argued against using short-term LF/HF ratio and the normalized powers as measures of sympathetic and parasympathetic balance.

In this study, we excluded 20% of the original cohort from the analyses because of inadequate or missing HRV data, as a result of equipment failure during the early months of the cohort examination. This could introduce bias and impose a modest limitation to the generalizability of our findings. However, the ARIC study assigned the baseline examination dates at random, making it unlikely that the early examinees are meaningfully different from those who were included in these analyses.

In summary, this study suggests that at the population level, lower HRV, reflective of impaired cardiac autonomic control, relates significantly to the development of CHD only among individuals with diabetes. This relationship is independent of CHD risk factors and of metabolic impairments typically associated with type 2 diabetes.

ACKNOWLEDGMENTS

Support provided by National Heart, Lung, and Blood Institute grant 5-R01-HL55669; contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022; and American Heart Association grant 9730175N.

We acknowledge the valuable contributions made by the ARIC study participants and the ARIC staff at the collaborating institutions.

REFERENCES

1. Pfeifer MA, Cook D, Brodsky J, Tice D, Reenan A, Swedine S, Halter JB, Porte D Jr: Quantitative evaluation of cardiac parasympathetic activity in normal and diabetic man. *Diabetes* 31:339–345, 1982
2. Bigger JT Jr: Spectral analysis of R-R variability to evaluate autonomic physiology and pharmacology and to predict cardiovascular outcomes in humans. In *Cardiac Electrophysiology: From the Cell to the Bedside*. 2nd ed. Zipes D, Jalife J, Eds. Philadelphia, PA, WB Saunders, 1995, p. 1151–1170
3. Akselrod S, Gordon D, Ubel FA, Shannon DC, Barger AC, Cohen RJ: Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 213:220–222, 1981
4. Pomeranz B, Macaulay RJB, Caudill MA, Kutz I, Adam D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, Benson H: Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 248: H151–H153, 1985
5. Hayano J, Sakakibara Y, Yamada A, Yamada M, Mukai S, Fujinami T, Yokoyama K, Watanabe Y, Takata K: Accuracy of assessment of cardiac parasympathetic tone by heart rate variability in normal subjects. *Am J Cardiol* 67:199–204, 1991
6. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Mulfatto G, Dellorto S, Piccaluga E: Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-parasympathetic interaction in man and conscious dog. *Circ Res* 59:178–193, 1986
7. Kamath MV, Ghista DN, Fallen EL, Fitchett D, Miller D, McKelvie R: Heart rate variability power spectrogram as a potential noninvasive signature of cardiac regulatory system response, mechanisms, and disorders. *Heart Vessels* 3:33–41, 1987
8. Malik M, Camm AJ: Heart rate variability. *Clin Cardiol* 13:570–576, 1990
9. Öri Z, Monir G, Weiss J, Sayhouni X, Singer DH: Heart rate variability: frequency domain analysis. *Cardiol Clin* 10:499–437, 1992
10. Kleiger RE, Miller JP, Bigger JT Jr: Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 59:256–262, 1987
11. Vaishnav S, Stevenson R, Marchant B, Lagi K, Ranjadayalan K, Timmis A: Relation between heart rate variability early after acute myocardial infarction and long-term mortality. *Am J Cardiol* 73:653–657, 1994
12. Lombardi F, Sandrone G, Pernpruder S, Sala R, Garimoldi M, Cerutti S, Baselli G, Pagani M, Malliani A: Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction. *Am J Cardiol* 60:1239–1245, 1987
13. Malik M, Farrell T, Camm AJ: Circadian rhythm of heart rate variability after acute myocardial infarction and its influence on the prognostic value of heart rate variability. *Am J Cardiol* 66:1049–1054, 1990
14. Martin GJ, Magid NM, Myers G, Barnett PS, Schaad JW, Weiss JS, Lesch M, Singer DH: Heart rate variability and sudden death secondary to coronary artery disease during ambulatory electrocardiographic monitoring. *Am J Cardiaol* 60:86–89, 1987
15. Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, Levy D: Impact of reduced heart rate variability on cardiac events: the Framingham Heart Study. *Circulation* 94:2850–2855, 1996
16. Liao D, Cai J, Rosamond W, Barnes RW, Hutchinson R, Whitsel E,

Rautaharju P, Heiss G: Cardiac autonomic function and incident CHD: a population-based case-cohort study. The ARIC Study. *Am J Epidemiol* 145:696–706, 1997
17. Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, Schouten EG: Low heart rate variability in a two minute rhythm strip predicts risk of coronary heart disease and mortality from several causes. The ARIC Study. *Circulation* 102:1239–1244, 2000
18. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93:1043–1065, 1996
19. Kannel WB, Neaton JD, Wentworth D, Thomas HE, Stamler J, Hulley SB, Kjelsberg MO: Overall and coronary heart disease mortality rates in relation to major risk factors in 325,348 men screened for MRFIT. *Am Heart J* 112:528–536, 1986
20. Pooling Project Research Group: Relationship of blood pressure, serum cholesterol, relative weight and ECG abnormalities to incidence of major coronary events: final report of the Pooling Project. *J Chron Dis* 31:201–306, 1978
21. Defronzo RA, Ferramini E: Insulin resistance: a multiple faceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173–194, 1991
22. Folsom AR, Szklo M, Stevens J, Liao F, Smith R, Eckfeldt JH: A prospective study of coronary heart disease in relation to fasting insulin, glucose, and diabetes. The Atherosclerosis Risk in Communities (ARIC) Study. *Diabetes Care* 20:935–942, 1997
23. Liao D, Cai J, Brancati F, Crow R, Barnes RW, Tyroler HA, Heiss G: Association of vagal tone with serum insulin, glucose and diabetes mellitus. The ARIC Study. *Diabetes Res Clin Pract* 30:211–221, 1995
24. Liao D, Sloan RP, Cascio WE, Folsom AR, Liese AD, Evans GW, Cai J, Sharrett AR: The multiple metabolic syndrome is associated with lower heart rate variability. The ARIC Study. *Diabetes Care* 21:2116–2122, 1998
25. ARIC investigators: The Atherosclerosis Risk in the Communities (ARIC) Study: design and objectives. *Am J Epidemiol* 129:687–602, 1989
26. Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C: Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. *Am J Epidemiol* 134:250–256, 1991
27. National Heart, Lung and Blood Institute: The ARIC Manuals of Operation: 2. Cohort Component Procedures; 3. Surveillance component procedures; 6. Ultrasound assessment; 11. Sitting blood pressure and postural changes in blood pressure and heart rate. Chapel, NC, ARIC Coordinating Center, School of Public Health, University of North Carolina at Chapel Hill, 1987
28. Liao D, Barnes RW, Chambless LE, Heiss G: A computer algorithm to impute interrupted heart rate data for the spectral analysis of heart rate variability. The ARIC Study. *Comput Biomed Res* 29:140–151, 1996
29. Liao D, Creason J, Shy C, Williams R, Watts R, Zweidinger R: Daily variation of particulate air pollution and poor cardiac autonomic control in the elderly. *Environ Health Perspect* 107:521–525, 1999
30. Rosamond WD, Chambless LE, Folsom AR, Cooper LS, Conwill DE, Clegg L, Wang CH, Heiss G: Trends in the incidence of myocardial infarction and in mortality due to coronary heart disease, 1987 to 1994. *N Engl J Med* 339:861–867, 1998
31. Freed LA, Stein KM, Gordon M, Urban M, Kligfield P: Reproducibility of power spectral measures of heart rate variability obtained from short-term sampling periods. *Am J Cardiol* 74:972–973, 1994
32. Liao D, Barnes RW, Chambless LE, Simpson RJ, Sorlie P, Heiss G: Population based study of heart rate variability and prevalent myocardial infraction. The ARIC Study. *J Electrocardiol* 29:189–198, 1996
33. Reaven GM, Lithell H, Landsberg L: Hypertension and associated metabolic abnormalities: the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* 334:374–381, 1996
34. Reaven GM: Syndrome X: 6 years later. *J Intern Med* 236:13–22, 1994
35. Liese AD, Mayer-Davis EJ, Haffner SM: The development of the multiple metabolic syndrome: an epidemiologic perspective. *Epidemiol Rev* 20: 157–172, 1998
36. Liao D, Cai J, Barnes RW, Tyroler HA, Rantaharju P, Holme I, Heiss G: Cardiac autonomic function and the development of hypertension. The ARIC Study. *Am J Hypertens* 9:1147–1156, 1996
37. Hϕjgaard MV, Holstein-Rathlou NH, Agner E, Kanters JK: Dynamics of spectral components of heart rate variability during changes in autonomic balance. *Am J Physiol* 275:H213–H219, 1998