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Normative data on cardiovascular autonomic function in Greenlandic Inuit

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ABSTRACT

Introduction Diabetes is increasing among Greenlandic Inuit; however, the prevalence of cardiovascular autonomic neuropathy (CAN) is yet unknown. The assessment of CAN requires an ability to differentiate between normal and abnormal. The aim was to establish normative reference data of cardiovascular autonomic function in Greenlandic Inuit.

Research design and methods In this cross-sectional study, cardiovascular autonomic function was evaluated in participants without diabetes during the *Greenlandic Population Study 2018* and in the town Qasigiannguit in 2020. Assessment included cardiovascular autonomic reflex tests (CARTs) and power spectral analysis of heart rate variability (HRV). Normative reference limits were estimated by applying piecewise linear quantile regression models at the fifth percentile. Models were adjusted for age and sex.

Results Based on examinations of 472 participants (61.7% females), normative reference data was established for all outcomes. Mean age was 54 years (SD 13.1). Higher age was inversely associated with all outcomes of CARTs and HRV. A linear fall in cardiovascular autonomic function tended to level off beyond age of 60 or 70 years for supine-to-upright position ratio and low frequency power. However, the number of observations in subjects older than 60 or 70 years was limited, which may have caused a flattening of the curve around that age. No other associations were found.

Conclusions The general level of the CARTs and HRV for all age groups is notably lower than in previous studies from other nationalities. We speculate that sociodemographic and cultural aspects of the Greenlandic Inuit population including body mass index, smoking, physical activity and alcohol consumption may have affected the cardiovascular autonomic function.

Significance of this study

What is already known about this subject?

- Diabetes is increasing among Greenlandic Inuit; however, the prevalence of cardiovascular autonomic neuropathy (CAN) is yet unknown.

What are the new findings?

- This study provides normative reference data of cardiovascular autonomic function in Greenlandic Inuit. The identified cardiovascular autonomic reflex tests (CARTs) ratios are lower than previously reported CARTs ratios in healthy individuals from other nationalities.
- The study has established normative reference data on cardiovascular autonomic function in Greenlandic Inuit without diabetes, which can be applied to detect and prevent the occurrence of cardiovascular autonomic neuropathy in Greenlandic Inuit with diabetes.
- The identified CARTs ratios are lower than previously reported CARTs ratios in healthy individuals from other nationalities.
- The reason why cardiovascular autonomic function in Greenlandic Inuit without diabetes reacts differently on the physical stimuli compared with healthy individuals in other populations is yet unknown. However, lifestyle factors as smoking, obesity and lack of exercise may play a role.

How might these results change the focus of research or clinical practice?

- The normative reference data can be applied to detect and potentially prevent the occurrence of CAN in individuals with diabetes.

INTRODUCTION

Cardiovascular autonomic neuropathy (CAN) is a common and serious complication in diabetes.[1] CAN results from damage to the autonomic nerve fibres innervating the heart and blood vessels and may cause abnormalities in the heart rate control and vascular system. Clinical manifestations include impaired left ventricular function, impaired dilations of coronary resistance vessels, orthostatic hypotension, exercise intolerance and increased intraoperative cardiovascular risk. Furthermore, CAN is associated with silent myocardial ischemia and higher cardiovascular mortality.[2–5] The disorder is prevalent in 20% of the general diabetes population and may affect up to 60% of patients with type 2 diabetes after 15 years of disease duration.[3 6] Hyperglycemia, hypertension, dyslipidemia, smoking and obesity increase the risk of developing CAN in type 2 diabetes, while multifactorial lifestyle interventions may prevent and possibly revert CAN in both type 2 diabetes and pre-diabetes.[7–11]

Diabetes and pre-diabetes are increasing among Greenlandic Inuit. Prevalence depends on the applied diagnostic test and varies between 6.5%–16.5% for diabetes and 11.8%–29% for pre-diabetes.[12–14] The high occurrence of diabetes and pre-diabetes in Greenlandic Inuit could potentially lead to a rise in CAN prevalence; however, the prevalence is yet unknown.

Early detection of CAN by screening is now feasible and quick using a point-of-care device that assesses the changes in heart rate response to different standardized stimuli. It is internationally accepted to apply the three cardiovascular autonomic reflex tests (CARTs).[3 6 15] Moreover, spectral analysis of heart rate variability (HRV) provides useful quantitative markers of CAN. HRV measures are considered early and potentially reversible indicators of cardiovascular autonomic dysfunction and should be included in the assessment of CAN.[4 9]

It requires an ability to differentiate between normal and abnormal to correctly diagnose CAN and to use CAN indices in research. Normative reference thresholds of CARTs have been reported but not in Greenlandic Inuit.[16–22] Age-specific and sex-specific differences in HRV has previously been presented, and ethnicity may also play a role.[15 23–27] The Greenlandic Inuit population is characterized by unique sociodemographic and cultural aspects why normative reference data should be derived from the specific or similar population. The aim of this study was to establish normative reference data for CARTs and HRV in Greenlandic Inuit without diabetes, which can be used to estimate the prevalence of CAN in Greenlandic Inuit with diabetes.

RESEARCH DESIGN AND METHODS

Study population

In this cross-sectional study, data were collected during two projects: the *Greenland population Study 2018*[14] and the normative reference study *Qasigiannguit 2020*.

Inclusion criteria were age above 18 years, no current pregnancy, no diagnosis of diabetes or atrial fibrillation and no implanted pacemaker.

The *Greenland Population Study 2018* was a follow-up study of previous cohort studies from 1999 to 2001, 2005–2010 and 2014 with the overall aim to assess the status of public health in Greenland. The study was conducted from August 2017 to January 2019 in 12 towns and 8 villages, representing Greenland as a whole. The participant rate was 52%, and 5.8% of the entire Greenlandic population participated (n=2539).[14] Adult Greenlanders including previous participants and a random sample of the population were invited to undergo an interview and health survey. If participants were identified with dysglycemia in the current or previous cohort study, they were invited for further examination of CAN. Some of the participants did not meet the criteria for diabetes despite previous signs of dysglycemia, and they were included in the normative reference study population.

Second round of enrollment took place from January to April 2020 in the Greenlandic town Qasigiannguit located in western Greenland on the southern shore of the Disko Bay. With its 1081 inhabitants, Qasigiannguit is the 13th largest town in Greenland.[28] Previous participants from the *Greenland Population Study 2018* without any prior diagnosis of diabetes, and who were not previously examined for complications, were invited by phone to undergo the CARTs and examination of HRV. Moreover, subjects were recruited outside the local supermarket by a team from the project group including a local translator, who provided oral and written information about the study. Status of diabetes was evaluated prior examination.

Measures of cardiovascular autonomic function

The three standard CARTs and measures of resting HRV were obtained once and performed by trained operators.[3 15] Data were generated and registered by using the handheld device Vagus (Medicus Engineering, Aarhus, Denmark).[7]

HRV measures were obtained after 5 min of supine rest and subsequently by recording an ECG standard lead I for 5 min. Autoregressive modelling was used in the spectral analysis of HRV. HRV indices were analysed in time domain and frequency domain. Time domain analyses included the root mean square of successive differences between normal-to-normal heartbeats (RMSSD) and SD of normal-to-normal intervals (SDNN). Frequency domain analyses included low-frequency power band (LF) (0.04–0.15 Hz) and high-frequency power band (HF) (0.15–0.4 Hz) in both absolute and normalized units and total frequency power.[3 29]

Following the 5 min resting HRV, the three CARTs were performed: *supine-to-upright position*, *deep breathing* and *Valsalva maneuver*. The results of the three CARTs are expressed as ratios of the minimum to the maximum interval between successive heartbeats (RR) during the tests.

Supine-to-upright position

The test was performed after the 5 min recording of HRV. The participants were asked to stand up quickly after which the ECG recording started. The ratio of the minimal (around the 15th heartbeat) to the maximum (around the 30th heartbeat) RR interval was calculated.

Deep breathing

Deep breathing was defined as six breaths per minute. The test was performed in an upright position, and participants were breathing in intervals of 5 s for 1 min. The ratio between the average of the longest RR intervals during expiration and the average of the shortest RR intervals during inspiration was calculated.

Valsalva maneuver

The participants forcefully exhaled against a closed windpipe for 15 s. The device registered if an intrathoracic strain pressure of 35–40 mm Hg was maintained.[30 31] Subsequently, they breathed normally for 45 s. The ratio between the shortest RR interval during the forced expiration and the longest RR interval after pressure release was measured.

Participants from the *Greenland Population Study 2018* who were above age of 34 years and not diagnosed with diabetes performed an oral glucose tolerance test (OGTT) and were fasting and avoided smoking prior examination. The rest of the participants including the participants from *Qasigiannguit 2020* were asked to avoid intake of caffeine 3 hours prior examination. All were advised to restrain from doing moderate to vigorous exercise within 24 hours prior examination. No requirements about use of drugs before examination were applied.[15]

Recognition of data errors

RR interval errors were identified by assessing the level of SDNN and RMSSD. If SDNN was relatively high (arbitrarily set to >80 ms), however, lower than RMSSD, the specific measurement was visually inspected in the software *Vagus Cloud*. If a large spread on the RR intervals without a clear pattern was observed, indicating irregularities in the heart rhythm during the test, the specific measurement was excluded.

Biochemistry and diabetes diagnosis

Absence or presence of dysglycemia was assessed in accordance with recommendations from WHO: from fasting blood glucose (diabetes: ≥7.0 mmol/L, impaired fasting glucose (IFG): ≤6.1; 7.0> mmol/L) or OGTT (diabetes: ≥11.1 mmol/L, impaired glucose tolerance (IGT): ≤7.8; 11.1> mmol/L) or glycated hemoglobin (HbA1c) (diabetes: ≥48 mmol/mol, pre-diabetes: ≤42; 48>mmol/mol).[32 33] Presence of pre-diabetes was assessed from HbA1c, IFG or IGT.

In the *Greenlandic Population Study 2018*, HbA1c was evaluated in all participants. Participants with unknown status of diabetes and age of or above 35 years performed a standard 75 g OGTT. Venous baseline blood samples were drawn fasting and 30 and 120 min after the OGTT. All blood samples were frozen and sent to the laboratory of Steno Diabetes Center Copenhagen for analyses. Blood glucose was evaluated by standard enzymatic colorimetry techniques. HbA1c was evaluated immediately by apparatus DCA Vantage.

During data collection in *Qasigiannguit in 2020*, HbA1c was measured and analysed by DCA Vantage. Due to lack of measuring equipment, diabetes status relied on self-reporting during the first half data collection period.

Clinical characteristics

Body mass index (BMI), physical activity, smoking status and alcohol consumption were assessed during the *Greenlandic Population Study 2018* but not in *Qasigiannguit 2020*, and the measures are therefore not descriptive for the study population as a whole.

BMI was calculated from weight divided by height squared. Height and weight were measured using a fixed rigid stadiometer (Seca, Chino, USA) and an electronic scale (Mettler Toledo, Glostrup, Denmark), respectively.

Physical activity energy expenditure (PAEE) was estimated based on the International Physical Activity Questionnaire (long version), which has previously been modified to arctic living conditions in Greenland with moderate validity.[34] The scoring system and calculation of PAEE have been described elsewhere.[34 35]

Participants were questioned about their smoking status and alcohol consumption. Monthly binge episodes were used as a measure for alcohol intake.[36] To assess if participants had a possible alcohol problem to a greater or lesser degree, the verified and internationally acknowledged measure Alcohol Use Disorders Identification Test was applied.[37]

Statistics

Model selection

A quantile regression model was applied on continuous outcomes from CARTs and HRV.[38 39] Threshold of normality was defined as the lower fifth percentile, representing a specificity of 95%, based on the accepted practice of a 5% false-positive rate in statistical testing. For both CARTs and HRV measures, it was not relevant to assess an upper threshold since no adverse effects of increasing levels have been reported.

We included age in the models to be able to estimate age-specific reference values and adjusted for sex. Since participants, who in previous population studies had been identified with dysglycemia, were invited to participate in the *Greenlandic Population Study 2018*, there was a marked oversampling of participants with pre-diabetes compared with the background population. Hence, we weighted status of pre-diabetes and normoglycemia according to the prevalences in the total population of the *Greenlandic Population Study 2018*, representing the Greenlandic background population. These prevalences were identified by assessing HbA1c, IGT and IFG in the age span 20–79 years (n=2354), (online supplemental appendix 1, section 2.3). To evaluate whether the assumption of a linear decrease in the outcome over age was justifiable, we visually compared a model with a linear term for age to a model with a cubic spline function of age (online supplemental appendix 1, section 2.4). For the outcomes, where the spline model differed markedly from the linear trajectory and implied a cessation in the decrease after age of 60 years, we fitted a piecewise linear function allowing the linear model to change slope after the specific threshold age (online supplemental appendix 1, section 2.5). For the remaining outcomes, a linear decrease over age was assumed.

In the final models, we tested for the potential effect of age and sex (online supplemental appendix 1, section 2.6).

When adjusting for covariates, we adopted the Huber sandwich estimate for estimating SEs).[40] We used two-sided statistically significance of 0.05 and adjusted for multiple tests by the Benjamini-Hochberg procedure.[41]

Reference table

For all the CARTs ratios and HRV measures, reference values with 95% CIs were derived for each 10-year age interval as the estimated value for the median age of that age interval. For example, the reference value for the 20–29 years age interval was the estimated fifth percentile value for age of 25 years (online supplemental appendix 1, section 3).

Statistical analyses were performed in R Studio V.3.6.0 and SAS Studio V.3.8.

RESULTS

Study population

A total of 493 participants were included in the study. Ten participants did not meet the inclusion criteria and were excluded. The observations (n=11) before and beyond age of 20 and 80 years, respectively, were excluded in order to avoid statistical noise in the models. Thus, the final study population consisted of 472 participants. Of these, 326 participants were from the *Greenland Population Study 2018* and 146 participants from *Qasigiannguit 2020* (online supplemental appendix 1, section 7). Females were represented with 61.7% (n=291). Mean age was 54 years (SD=13.1) (online supplemental appendix 1, section 5). Pre-diabetes was prevalent in 47.9% and normoglycemia in 39.8%. Presence of pre-diabetes or normoglycemia was not assessed in 12.3%. Distribution and overlap of diagnostic tests are described in table 1 and the online supplemental appendix 1, section 6. Median HbA1c was 41 mmol/mol (IQR=38–43); however, for 58 participants (12.3%), glycemic status relied solely on self-reporting (table 1). In the *Greenlandic Population Study 2018*, participants were overweight with a mean BMI of 28.2 (SD=5.6). Median PAEE was 36.8 kJ/kg/day (IQR 13.1–70.6). Prevalence of current smoking was 54.5% (n=177), and former smoking was 34.2% (n=111), while 11.3% (n=37) never smoked. In the same subpopulation, the median weekly consumption of alcohol was 1 unit (IQR 0.3–3.8); however, 30.8% (n=60) of the respondents stated to drink five or more units at least once a month, and 37.4% (n=73) were estimated to have a possible alcohol problem.

Associations to demographic variables

All CARTs and HRV outcomes except for heart rate showed a skewed distribution (table 2 and online supplemental appendix 1, section 9). Normalized values of HRV indicated a sympathetic dominance during the 5 min ECG recording (online supplemental appendix 1, section 9).

Table 1 Characteristics of the study population

Characteristics	Pooled		Greenland Population Study 2018		Qasigiannguit 2020	
Clinical	N		N		N	
N (%)	472	100	326	69.1	146	30.9
Females (%)	291	61.7	203	62.3	88	60.3
Age (years)	472	53.9 (13.1)	326	56.4 (11.8)	146	48.3 (14.1)
Pre-diabetes (%)	226	47.9	216	66.3	10	6.8
Distribution of diagnostic tests						
IGT (%)	51	NA	51	15.6	NA	NA
IFG (%)	74	NA	74	22.7	NA	NA
HbA1c (%)	186	39.4	176	54	10	6.8
Normoglycemic state (%)	188	39.8	110	33.7	78	53.4
BMI	323	28.2 (5.6)	323	28.2 (5.6)	NA	NA
Physical activity energy expenditure (kJ/kg/day)	290	36.8 (13.1–70.6)	290	36.8 (13.1–70.6)	NA	NA
Smoking status	325		325		NA	
Current (%)	177	54.5*	177	54.5*	NA	NA
Former (%)	111	34.2*	111	34.2*	NA	NA
Never (%)	37	11.3*	37	11.3*	NA	NA
Alcohol intake (unit/week)	195	1.0 (0.3–3.8)	195	1 (0.3–3.8)	NA	
Heavy episodic drinking (%)	60	30.8*	60	30.8*	NA	NA
Possible alcohol problem (%)	73	37.4*	73	37.4*	NA	NA
Biochemical						
HbA1c (mmol/mol)	414	41 (38–43)	326	42 (39–43)	88	37.8 (3.2)
HbA1c (%)	414	5.9 (5.6–6.1)	326	6.0 (5.7–6.1)	88	5.6 (0.6)

Data are given in means (SD), medians (IQR) or proportions %.
Heavy episodic drinking refers to an intake of five or more units at least once a month; possible alcohol problem: estimated from the Alcohol Use Disorder Identification Test score.
*Proportion of the total number of respondents of smoking status (N=325) and alcohol consumption (N=195).
BMI, body mass index; HbA1c, glycated hemoglobin; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

Table 2 Distribution of cardiovascular autonomic function measures

Cardiovascular autonomic function	Pooled		Population study 2018		Qasigiannguit 2020	
CARTs	N		N		N	
Supine-to-upright position ratio	464	1.1 (1.0–1.2)	322	1.1 (1.0–1.2)	142	1.1 (1.1–1.2)
Deep-breathing ratio	462	1.2 (1.1; 1.3)	322	1.2 (1.1; 1.3)	140	1.2 (1.1; 1.2)
Valsalva maneuver ratio	377	1.4 (1.2; 1.5)	279	1.3 (1.2; 1.5)	98	1.4 (1.3; 1.6)
HRV measures						
Heart rate (bpm)	470	75.2 (12.1)	326	75.5 (12.2)	144	74.4 (11.9)
SDNN (ms)	465	26.7 (18.6–38.2)	323	25.5 (17.0–37.2)	142	30.7 (23.3–41.2)
RMSSD (ms)	465	16.9 (10.9; 27.4)	323	15.6 (9.9; 23.3)	142	21.1 (12.3; 33.4)
LF (ms^2)	465	56.6 (22.8; 124.5)	323	46.4 (18.1; 109.6)	142	80.7 (34.9; 166.7)
HF (ms^2)	465	37.8 (15.1; 100.8)	323	29.1 (12.3; 83.8)	142	68.3 (25.8; 154.2)
Total (ms^2)	465	234.9 (115.4; 498.0)	323	203.9 (91.3; 448.0)	142	319.9 (174.2; 607.1)

Data are given in means (SD), medians (IQR).
CARTs, Cardiovascular autonomic reflex tests; HRV, heart rate variability; LF and HF power, low-frequency power high-frequency power; RMSSD, root mean square of the sum of the squares of differences between consecutive R–R intervals; SDNN, SD of normal-to-normal intervals.

Cardiovascular autonomic reflex tests

Valsalva maneuver is a strenuous exercise and may be difficult to complete for especially elderly and physically weak individuals. Out of 472 participants, 95 were not able to perform the test. Median age of the 95 participants was 61 years (IQR: 51–69). Ten participants did not perform the deep-breathing test and eight participants failed to complete the supine-to-upright position test (table 2).

Higher age was inversely associated with supine-to-upright position ratio (p=1.4e-6), deep-breathing ratio (p=1.7e-8) and Valsalva maneuver ratio (p=0.047). Sex was associated with deep-breathing ratio (p=0.01), but significance was lost after adjustment for multiple test (online supplemental appendix 1, section 2.6).

HRV measures

Data on HRV were missing in seven participants due to data errors during recordings.

Higher age was significantly associated with lower values of all HRV outcomes (online supplemental appendix 1, section 2.6). No significant association was found between sex and the outcomes.

Final models

Cardiovascular autonomic reflex tests

For the outcomes supine-to-upright position ratio, a piecewise linear model allowing the slope to change to zero beyond age of 60 years provided the best fit (figure 1A). The best fit for deep-breathing and Valsalva maneuver ratio was obtained by applying a model with a linear decrease in the outcome over age (figure 1B,C).

HRV outcomes

To provide the best fit for LF, a piecewise linear model with a horizontal trajectory beyond age of 70 years was applied (figure 1F). For the remaining outcomes, no horizontal change in the slope was observed; thus, the final models had a linear approach (figure 1D–E,G–H).

Outcome specific model formulas are stated in the online supplemental appendix 1, section 2.7. The generic models for all outcomes are presented below:

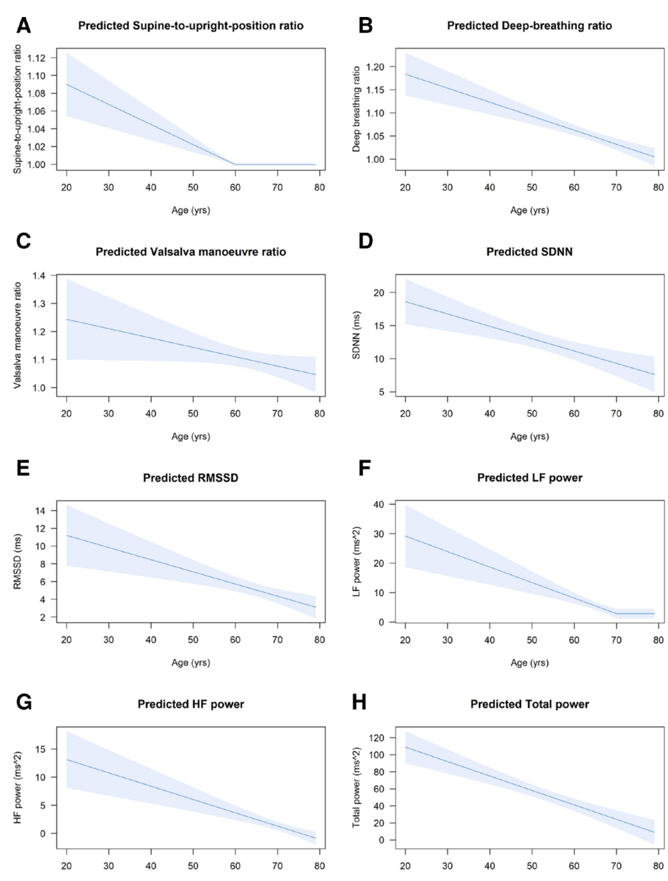

Figure 1 Visual presentation of the final models for each measure of cardiovascular autonomic function in Greenlandic Inuit without diabetes. HF power, high-frequency power; LF power, low-frequency power; RMSSD, root mean square of the sum of the squares of differences between consecutive R–R intervals; SDNN, SD of normal-to-normal intervals.

Table 3 Age-specific reference data for the CARTs

	Supine-to-upright position ratio n=464		Deep-breathing ratio n=462		Valsalva maneuver ratio n=377	
Age (years)	N	Estimate (95% CI)	N	Estimate (95% CI)	N	Estimate (95% CI)
20–29	23	1.08 (1.05 to 1.11)	22	1.17 (1.13 to 1.21)	17	1.23 (1.10 to 1.35)
30–39	53	1.06 (1.03 to 1.08)	52	1.14 (1.11 to 1.17)	47	1.19 (1.10 to 1.29)
40–49	79	1.03 (1.02 to 1.05)	77	1.11 (1.09 to 1.13)	68	1.16 (1.09 to 1.23)
50–59	138	1.01 (1.01 to 1.02)	140	1.08 (1.06 to 1.09)	119	1.13 (1.09 to 1.17)
60–69	124	1.00 (1.00 to 1.00)	125	1.05 (1.04 to 1.06)	97	1.09 (1.06 to 1.13)
70–79	47	1.00 (1.00 to 1.00)	46	1.02 (1.00 to 1.03)	29	1.06 (1.01 to 1.11)

The results are estimated from the median age of the specific age spans and are presented as ratios with 95% CI.
CARTs, cardiovascular autonomic reflex tests.

Piecewise linear model

$$Q(\tau|Age) = \begin{cases} \beta_0(\tau) + \beta_1(\tau)\cdot age, 20 \leq age < 60|70 \\ \beta_0(\tau), 60|70 \leq age \end{cases}$$

Linear model

$$Q(\tau|Age) = \beta_0(\tau) + \beta_1(\tau)\cdot \text{age}$$

Age-specific estimates

A table with CARTs and HRV reference data inclusive 95% CI are presented for ages between 20 and 80 years divided into intervals of 10 years (tables 3–4).

DISCUSSION

In this cross-sectional study comprising 472 participants, we found that higher age was inversely associated with all outcomes of CARTs and HRV. A linear fall in cardiovascular autonomic function over age was observed in all outcomes except for supine-to-upright position ratio and LF where the decrease tended to level off beyond age of 60 and 70 years of age. Sex was not significantly associated with the outcomes.

Final models

Cardiovascular autonomic reflex tests

The ratios of all three CARTs declined with age. That is in line with findings in most previous studies, in which associations are either linear or logarithmic.[16–22] For the supine-to-upright position ratio, the inverse and linear relationship with age tended to flatten around 60 years of age. The phenomenon has been demonstrated previously.[17] However, in the present study, the number of participants older than 60 years were few which, combined with modelling a low percentile, may have lower associated precision. The horizontal shift of the curve at age 60 years was identified by visual inspection of the plotted spline model and may be reconsidered in later studies with more participants.

Previous studies do not convincingly indicate that sex is related to cardiovascular autonomic function, nor was an association found in this study.[15]

Heart rate variability

In the present study, HRV measures decreased linearly with age. For LF, the association with age stabilized around age of 70 years. Again, the number of participants above age of 70 years as limited. No association between sex and HRV outcomes was found. Conclusions on the effect of age and sex on HRV in previous studies are mixed.[23–26 42–44] However, studies examining HRV measures similar to the measures in the present study in a broad age range do find decreasing HRV with increasing age.[23 25 26 44]

Reference data

Cardiovascular autonomic reflex tests

Normal limits for CARTs have been assessed previously. Spallone *et al*[15] presents the results of four studies, three Italian and one German, reporting age-related normal limits of CARTs. The reference ratios of the present study are notably lower compared with the studies. The reference ratios closest to our findings are presented by Ziegler *et al*.[22] Despite that the ratios are presented at the lower 2.3rd percentile, they are still markedly higher. Three CARTs like ours were performed on 120 healthy individuals from Germany; however, different measuring devices were applied.

Heart rate variability

Normative reference data on spectral analysis of HRV in the existing literature is scarce; however, there are studies reporting the mean or median of HRV measures. Nunan *et al*[45] presented a systematic review of normal values for short-term HRV in adults counting 44 papers with a sample size of 21 438 participants. The presented level of the cross study medians of *SDNN, RMSSD, LF and HF* are like the CARTs notably higher than the levels reported in the present study (table 2). For instance, Nunan *et al* identified a cross-study median SDNN of 51 ms (IQR=32–93), which is markedly higher than 27 ms (IQR=19–38) identified in the present study. The difference in median RMSSD is also substantial, 42 ms (IQR=19–75) versus 17 ms (IQR=11–27). As to frequency domain, Nunan *et al* reports median LF and HF of 458 ms^2 (IQR=193–1009) and 385 ms^2

Table 4 Age-specific reference data for HRV

Age (years)	SDNN (ms) n=465		RMSSD (ms) n=465		LF (ms²) n=465		HF (ms²) n=465		Total power (ms²) n=465	
	N	Estimate (95% CI)	N	Estimate (95% CI)	N	Estimate (95% CI)	N	Estimate (95% CI)	N	Estimate (95% CI)
20–29	23	17.7 (14.7 to 20.7)	23	10.5 (7.5 to 13.6)	23	26.6 (17.1 to 36.0)	23	11.9 (7.4 to 16.5)	23	100.4 (84.1 to 116.7)
30–39	53	15.8 (13.6 to 18.0)	53	9.2 (6.8 to 11.5)	53	21.3 (14.2 to 28.5)	53	9.6 (6.0 to 13.2)	53	83.4 (71.6 to 95.3)
40–49	79	14.0 (12.5 to 15.5)	79	7.8 (6.1 to 9.4)	79	16.0 (11.1 to 20.9)	79	7.2 (4.6 to 9.8)	79	66.5 (58.4 to 74.7)
50–59	138	12.1 (10.9 to 13.3)	138	6.4 (5.4 to 7.5)	138	10.8 (8.0 to 13.5)	138	4.8 (3.1 to 6.6)	138	49.6 (43.0 to 56.3)
60–69	125	10.2 (8.6 to 11.8)	125	5.0 (4.3 to 5.8)	125	5.5 (4.2 to 6.8)	125	2.5 (1.5 to 3.4)	125	32.7 (24.1 to 41.3)
70–79	47	8.4 (6.1 to 10.7)	47	3.7 (2.6 to 4.7)	47	2.9 (1.2 to 4.5)	47	0.1 (-0.8 to 1.0)	47	15.8 (3.3 to 28.2)

The results are estimated from the median age of the specific age spans and presented with 95% CI.
HF power, high-frequency power; HRV, heart rate variability; LF power, low-frequency power; RMSSD, root mean square of the sum of the squares of differences between consecutive R–R intervals; SDNN, SD of normal-to-normal intervals.

(IQR=82–3630), respectively, whereas we found a median LF of 56.6 ms² (IQR=22.8–124.5) and HF of 37.8 ms² (IQR=15.1–100.8).

Valera *et al* presented two studies concerning HRV assessed in Inuit from Nunavik (Northern Quebec).[46 47] Despite the geographically and culturally similarities with Greenlandic Inuit also these studies presented levels of mean HRV measures that are substantially higher compared with our results.

The cause of the relatively low levels of ratios and HRV measures identified in the present study is yet unknown. We may just speculate on why the cardiovascular autonomic function in Greenlandic Inuit without diabetes reacts differently compared with healthy individuals in other populations.

It is well known that CAN in pre-diabetes is more common than in subjects with normal glucose tolerance.[48] Subjects with pre-diabetes were oversampled in our study that could explain the relatively low level of CART ratios and HRV measures. However, we did take this aspect in to account and weighted the presence of pre-diabetes and normoglycemia according to prevalences in the background population. Another explanation could be that sociodemographic and cultural aspects such as smoking, alcohol consumption, diet, contaminant exposure, level of physical activity and BMI characterize the Greenlandic population and affect the cardiovascular autonomic function. The participants from the *Greenlandic Population Study 2018* were overweight, and more than half of them were smoking (table 1). The median weekly intake of alcohol was 1 unit (IQR=0.3–3.8); however, of the respondents, 30.8% had an alcohol intake of five or more units at least once a month and 37.4% had a possible alcohol problem indicating an episodic drinking pattern. Median PAEE was 36.8 kJ/kg/day (IQR: 13.1–70.6), which reflects a low level of physical activity when compared with previous assessment of physical activity in Greenlandic Inuit where median PAEE was 51.7 and 47.3 kJ/kg/day for men and women, respectively.[34] These characteristics correspond more or less to the background population in which approximately half of the population are smoking, around one-third are severely overweight and 34% drink five or more units at least once a month.[14] CAN may be prevalent in those who consume large quantities of alcohol over a prolonged period of time,[49] and both smoking and obesity have been identified as risk factors of CAN in diabetes.[7 8] However, it is unclear if the same is applicable on individuals without diabetes. Another consideration is that mercury accumulated in in marine mammals and predator fish, which are essential components in traditional Inuit diet, may have a deleterious impact on HRV measures.[47] In contrast, some of the same authors have suggested that n-3 polyunsaturated fatty acids, commonly found in marine oils, have a beneficial effect on HRV measures among Nanuvik Inuit women.[46]

Strengths and limitations

A notable strength is that examinations were conducted nationwide during the *Greenland Population Study 2018* thus representing Greenland as a whole. Moreover, suitable and recommended statistical methods for reference data studies were applied.

Regarding limitations, there may occur selection bias when people are invited to participate in a survey. Volunteering participants may be more resourceful and come from higher socioeconomic backgrounds, resulting in a less representative study population. Additionally, we had no information on medication such as use of beta blockers that could have affected the results. Restraining from eating and smoking prior examinations is recommended practice.[15] Participants with age above 34 years without known diabetes from the *Greenland Population Study 2018* were fasting prior the survey due to various blood samples; however, the rest of the participants including the population of *Qasigiannguit 2020* did not meet these recommendations.

Due to lack of measuring equipment in *Qasigiannguit 2020*, diabetes status relied solely on self-reporting for 58 participants. A confirmatory blood sample would have been appropriate for all participants in order to exclude participants with diabetes and take status of pre-diabetes into account. We weighted status of pre-diabetes and normoglycemia so both corresponded to the prevalences in the background population. The 58 participants with unknown glycemic status were not weighted and may have affected the final models. However, the models and estimates did not change notably when the 58 participants were excluded.

The lack of breathing control during the 5 min ECG recording may be a limitation of the study. Controlled breathing may enable easier separation of the LF and HF power band. However, controlled breathing may also increase the HF power band and push the sympathovagal balance toward a parasympathetic predominance in the HRV.[50]

Valsalva maneuver is a strenuous exercise that elderly and physically weak individuals may have difficulties in performing. A substantial number of participants (n=95) with a median age of 61 years (IQR=51–69) were not able to complete Valsalva maneuver in the present study (online supplemental appendix 1, section 6). Elderly and physically weak individuals may be unintentionally excluded from the test, which indicates the presence of selection bias. Thus, the results of the higher age groups may be based on a performance of healthier and stronger individuals compared with the background population. It may be considered if Valsalva maneuver is ideal in order to assess the cardiovascular autonomic function in elderly and physically weak individuals.

It is also a limitation that we do not have information about smoking, BMI, exercise and alcohol consumption of the entire study population including *Qasigiannguit 2020*, which could have been used to assess the possible causes of the identified low levels of CARTs and HRV. The clinical information from the *Greenlandic Population Study 2018* (table 1) may just give an indication of the health status of the participants.

CONCLUSIONS

By applying a recommended and acknowledged statistical method, we established reference equations and data for cardiovascular autonomic function in Greenlandic Inuit without diabetes. These data are highly relevant to diagnose CAN in Greenlandic Inuit with diabetes. Surprisingly, estimated reference values for CARTs and HRV for all age groups were low compared with previous studies from other nationalities suggesting that cardiovascular autonomic function is different in Greenlandic Inuit without diabetes compared with healthy individuals of other ethnicities. Lifestyle factors such as smoking, high BMI, alcohol consumption, contaminant exposure and lack of exercise may affect the cardiovascular autonomic function in Greenlandic Inuit; however, more studies examining the associations between measures of CAN and potential covariates are needed to draw definite conclusions.

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