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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 quantile regression models at the lowest fifth percentile, with age as the exposure variable and adjusted for sex. HRV models were additionally adjusted for resting heart rate.

Results Based on examinations of 383 participants (60.6% females), normative reference data was established for all outcomes. Mean age was 52 years (SD 12.9). 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 years for supine-to-upright position ratio. However, the number of observations in subjects older than 60 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 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 CAN in Greenlandic Inuit with diabetes.

⇒ The identified cardiovascular autonomic reflex test ratios (CARTs) 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]

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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 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 have 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 and no diagnosis of diabetes. Exclusion criteria were pregnancy, known atrial fibrillation or ischemic heart disease, pacemaker, and treatment with medication that may affect the heart rate (betablockers, tricyclic antidepessants, opioids etc.).

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.

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 before the 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 sitting 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

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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 participants from *Qasigiannguit 2020* were asked to avoid intake of caffeine 3 hours prior to the examination and 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. Models including HRV-measures were furthermore adjusted for resting heart rate. 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, 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, 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, section 2.5). For the remaining outcomes, a linear decrease ovcer age was assumed. However, if the predicted limits exhibited negative values, which is not biologically plausible, a log-transformation was applied to the specific outcome variables and coefficients were subsequently back-transformed to the original scale.

For the final models for the CARTs, we tested for the potential effect of age and sex. For the models including

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HRV measures, we tested for the effect of age, sex and resting heart rate (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, 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, 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. Twenty-eight participants did not meet the inclusion criteria, 18 of whom had diabetes. A total of 71 participants were excluded due to treatment with a medication that may affect the resting heart rate. 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 383 participants. Of these, 242 participants were from the *Greenland Population Study 2018* and 141 participants from *Qasigiannguit 2020*. Females were represented with 60.6% (n=232). Mean age was 52 years (SD=12.9) (online supplemental appendix, section 4). Pre-diabetes was prevalent in 56.7% and normoglycemia in 28.7%. Presence of pre-diabetes or normoglycemia was not assessed in 14.6%. Median HbA1c was 40 mmol/mol (IQR=37-43); however, for 56 participants (14.6%), glycemic status relied solely on self-reporting (table 1). In the *Greenlandic Population Study 2018*, mean BMI was 27.1 kg/m2 (IQR=23.4-31.4). Median PAEE was 36.8 kJ/kg/day (IQR=14.4-71.3). Prevalence of current smoking was 59.3% (n=143), and former smoking was 29.9% (n=72), while 10.8% (n=26) never smoked. In the same subpopulation, the median weekly consumption of alcohol was 1.3 unit (IQR=0.3-3.8); however, 19.8% (n=48) of the respondents stated to drink five or more units at least once a month, and 22.7% (n=48) were estimated to have a possible alcohol problem.

Table 1 Characteristics of the study population

Characteristics	Pooled		Greenland Populat. Study 2018		Qasigiannguit 2020	
Clinical	**N**		**N**		**N**	
N (%)	383	100	242	63.2	141	36.8
Females (%)	232	60.6	147	60.7	85	60.3
Age (yrs)	383	52.3 (12.9)	242	54.7 (11.5)	146	48 (14)
Glycemic status						
Pre-diabetes (%) Distribution of diagnostic tests:	217	56.7	208	86	9	6.4
Normoglycaemic state (%)	188	28.7	34	14	76	53.9
Unknown (%)	56	14.6	0	0	56	39.7
BMI	240	27.1 (23.4–31.4)	240	27.1 (23.4–31.4)	NA	NA
Physical activity energy expenditure (kJ/kg/day)	217	36.8 (14.4–71.3)	217	36.8 (14.4–71.3)	NA	NA
Smoking status	241		241		NA	
Current (%)	143	59.3*	143	59.3*	NA	NA
Former (%)	72	29.9*	72	29.9*	NA	NA
Never (%)	26	10.8*	26	10.8*	NA	NA
Alcohol intake (unit/week)	156	1.3 (0.3–3.8)	156	1.3 (0.3–3.8)	NA	
Heavy episodic drinking (%)	48	19.8*	48	19.8*	NA	NA
Possible alcohol problem (%)	55	22.7*	55	22.7*	NA	NA
Biochemical						
HbA1c (mmol/mol)	318	40 (37–43)	242	42 (39–43)	85	37.0 (36–39)
HbA1c (%)	318	5.8 (5.5–6.1)	242	6.0 (5.7–6.1)	85	5.5 (5.4–5.7)

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=241) and alcohol consumption (N=156).
BMI, body mass index; HbA1c, glycated hemoglobin.

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Table 2 Distribution of cardiovascular autonomic function measures

Cardiovascular autonomic function	Pooled		Population Study 2018		Qasigiannguit 2020	
CARTs	N	383	N	242	N	141
Supine-to-upright-position ratio	377	1.1 (1.0–1.2)	240	1.1 (1.0–1.2)	139	1.1 (1.1–1.2)
Deep-breathing ratio	373	1.2 (1.1; 1.3)	238	1.2 (1.1; 1.3)	137	1.2 (1.1; 1.3)
Valsalva manoeuvre ratio	310	1.4 (1.2; 1.5)	216	1.3 (1.2; 1.5)	94	1.3 (1.2; 1.5)
HRV measures						
Heart rate (bpm)	381	75.4 (12.3)	242	76.0 (12.5)	139	74.3 (11.9)
SDNN (ms)	378	27.8 (20.1; 39.1)	241	26.3 (17.8; 37.2)	137	30.9 (23.5; 41.5]
RMSSD (ms)	378	17.4 (11.3; 28.0)	241	15.7 (10.4; 24.4)	137	21.3 (12.8; 33.9)
LF power (ms^2)	378	65.1 (26.0; 132.3)	241	50.0 (20.9; 119.1)	137	82.6 (37.2; 176.3)
HF power (ms^2)	378	42.4 (16.8; 109.3)	241	30.1 (13.7; 83.8)	137	69.0 (26.6; 154.2)
Total power (ms^2)	378	255.0 (125.5; 501.2)	241	211.7 (97.9; 448.0)	137	340.0 (175.0; 607.4)

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.

Associations to demographic variables

All CARTs and HRV outcomes except for heart rate showed a skewed distribution (table 2). Normalized values of HRV indicated a sympathetic dominance during the 5 min ECG recording (online supplemental appendix, section 7).

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 383 participants, 73 were not able to perform the test. Median age of the 73 participants was 57 years (IQR=46;67). Ten participants did not perform the deep-breathing test and six participants failed to complete the supine-to-upright position test (table 2).

Higher age was inversely associated with supine-to-upright position ratio (p=2.9e-2), deep-breathing ratio (p=1.03e-4) and Valsalva maneuver ratio (p=1.6e-2). Sex was associated with deep-breathing ratio (p=2.2e-2), but significance was lost after adjustment for multiple test (online supplemental appendix, section 2.6).

HRV measures

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

Higher age and higher resting heart rate were significantly associated with lower values of all HRV measures (online supplemental appendix, 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

A log-transformed model provided the best fit for alle HRV measures to ensure that the predicted limits would not exhibit values below zero when adjusting for resting heart rate (figure 1D–H).

Outcome specific model formulas are presented below:
Normative threshold formulas for CARTS:

$$Supine\ upright\ position\ ratio_{\ Normal\ limit} = 1.15 - 0.0025 \cdot age, 20 \leq age < 60\ 1.00, 60 \leq age$$

$$Deep\ breathing\ ratio_{\ Normal\ limit} = 1.24 - 0.003 \cdot age, 20 \leq age$$

$$Valsalva\ maneouvre\ ratio_{\ Normal\ limit} = 1.41 - 0.005 \cdot age, 20 \leq age$$

Normative threshold formulas for HRV:

$$SDNN_{\ Normal\ limit} = exp(5.71 - 0.021 age - 0.025 \cdot heart\ rate)\ 20 \leq age$$

$$RMSSD_{\ Normal\ limit} = exp(6.65 - 0.031 age - 0.038 \cdot heart\ rate), 20 \leq age$$

$$LF\ power_{\ Normal\ limit} = exp(7.94 - 0.042 age - 0.040 \cdot heart\ rate), 20 \leq age$$

$$HF\ power_{\ Normal\ limit} = exp(11.21 - 0.072 age - 0.072 \cdot heart\ rate), 20 \leq age$$

$$Total\ power_{\ Normal\ limit} = exp(10.67 - 0.044 \cdot age - 0.053 \cdot heart\ rate), 20 \leq age$$

Age-specific estimates

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

DISCUSSION

In this cross-sectional study comprising 383 participants, we found that higher age was inversely associated with all outcomes of CARTs and HRV. Moreover, higher resting heart rate was associated with lower

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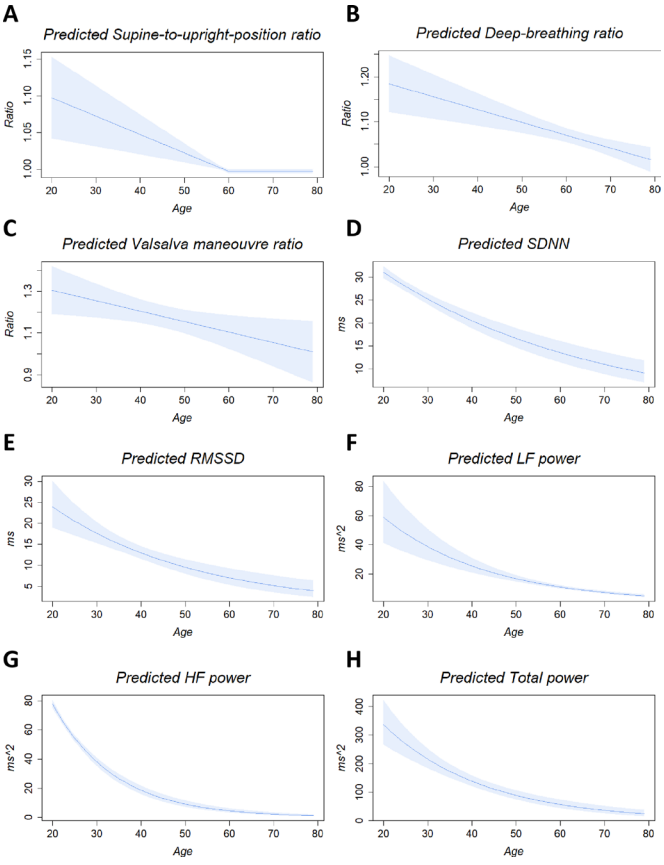

Figure 1 Visual presentation of the final models for each measure of cardiovascular autonomic function in Greenlandic Inuit without diabetes. The estimated thresholds of HRV measures (SDNN, RMSSD, LF power, HF power and Total power) are presented at mean heart rate of 75 bpm. The estimates displayed at the y-axis have been back-transformed to the original scale. 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.

levels of all HRV measures. Sex was not significantly associated with the outcomes. A piecewise linear model with a linear fall with higher age till age 60 years, where the decrease leveled off was observed for the supine-to-upright-position ratio, whereas a linear fall over age was noted for the other CARTs. Log-transformed models provided the best fit for HRV measures.

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, higher age and resting heart rate were associated with lower HRV measures. Models with log-transformed outcomes provided the best fit, as the predicted limits were not allowed to exhibit values below zero. 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

Table 3 Age-specific reference data for the CARTs

	Supine-to-upright-position ratio n=377		Deep-breathing ratio n=373		Valsalva manoeuvre ratio n=310	
Age (years)	N	Estimate (95% CI)	N	Estimate (95% CI)	N	Estimate (95% CI)
20–29	23	1.09 (1.04 to 1.13)	22	1.17 (1.11 to 1.23)	17	1.28 (1.18 to 1.38)
30–39	47	1.06 (1.03 to 1.09)	46	1.14 (1.10 to 1.18)	43	1.23 (1.16 to 1.30)
40–49	69	1.04 (1.01 to 1.06)	67	1.11 (1.08 to 1.14)	58	1.18 (1.13 to 1.23)
50–59	117	1.01 (1.00 to 1.02)	119	1.08 (1.07 to 1.10)	101	1.13 (1.06 to 1.20)
60–69	96	1.00 (1.00 to 1.00)	95	1.06 (1.04 to 1.07)	77	1.08 (1.00 to 1.18)
70–79	25	1.00 (1.00 to 1.00)	24	1.03 (1.00 to 1.05)	14	1.03 (1.00 to 1.16)

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.

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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 28 ms (IQR=20-39) identified in the present study. The difference in median RMSSD is also substantial, 42 ms (IQR=19–75) versus 17 ms (IQR=11-28). As to frequency domain, Nunan *et al* reports median LF and HF of 458 ms² (IQR=193–1009) and 385 ms² (IQR=82–3630), respectively, whereas we found a median LF of 65 ms² (IQR=26-132) and HF of 42 ms² (IQR=17-109).

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 1.3 unit (IQR=0.3-3.8); however, of the respondents, 20% had an alcohol intake of five or more units at least once a month and 23% had a possible alcohol problem indicating an episodic drinking pattern. Median PAEE was 36.8 kJ/kg/day (IQR=14-71), 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] Some of the 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 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. Restraining from eating and smoking before the examination of the cardiovascular autonomic nervous system 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 to align with 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

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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=73) with a median age of 57 years (IQR=46-67) were not able to complete Valsalva maneuver in the present study (online supplemental appendix, section 5). 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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Data availability statement Data are available upon reasonable request. Data that support the findings of this study are available from the corresponding author on reasonable request. Data analyses are presented in the appendix.

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REFERENCES

1 Kuehl M, Stevens MJ. Cardiovascular autonomic neuropathies as complications of diabetes mellitus. *Nat Rev Endocrinol* 2012;8:405–16.
2 Maser RE, Lenhard MJ. Cardiovascular autonomic neuropathy due to diabetes mellitus: clinical manifestations, consequences, and treatment. *J Clin Endocrinol Metab* 2005;90:5896–903.
3 Spallone V. Update on the impact, diagnosis and management of cardiovascular autonomic neuropathy in diabetes: what is defined, what is new, and what is unmet. *Diabetes Metab J* 2019;43:3–30.
4 Vinik AI, Maser RE, Mitchell BD, *et al*. Diabetic autonomic neuropathy. *Diabetes Care* 2003;26:1553–79.
5 Maser RE, Mitchell BD, Vinik AI, *et al*. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. *Diabetes Care* 2003;26:1895–901.
6 Pop-Busui R, Boulton AJM, Feldman EL, *et al*. Diabetic neuropathy: a position statement by the American diabetes association. *Diabetes Care* 2017;40:136–54.

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7 Andersen ST, Witte DR, Fleischer J, *et al*. Risk factors for the presence and progression of cardiovascular autonomic neuropathy in type 2 diabetes: ADDITION-Denmark. *Diabetes Care* 2018;41:2586–94.
8 Fleischer J, Yderstraede K, Gulichsen E, *et al*. Cardiovascular autonomic neuropathy is associated with macrovascular risk factors in type 2 diabetes: new technology used for routine large-scale screening adds new insight. *J Diabetes Sci Technol* 2014;8:874–80.
9 Carnethon MR, Prineas RJ, Temprosa M, *et al*. The association among autonomic nervous system function, incident diabetes, and intervention arm in the diabetes prevention program. *Diabetes Care* 2006;29:914–9.
10 Gaede P, Lund-Andersen H, Parving H-H, *et al*. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* 2008;358:580–91.
11 Spallone V, Ziegler D, Freeman R, *et al*. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev* 2011;27:639–53.
12 Jørgensen ME, Borch-Johnsen K, Witte DR, *et al*. Diabetes in Greenland and its relationship with urbanization. *Diabet Med* 2012;29:755–60.
13 Jørgensen ME, Bjeregaard P, Borch-Johnsen K. Diabetes and impaired glucose tolerance among the Inuit population of Greenland. *Diabetes Care* 2002;25:1766–71.
14 Larsen CVL, Hansen CB, Ingemann C. *Befolkningsundersøgelsen I grønland 2018. levevilkår, livsstil og helbred: oversigt over indikatorer for folkesundhed*. Copenhagen: Syddansk Universitet. Statens Institut for Folkesundhed, 2019. https://www.sdu.dk/da/sif/rapporter/2019/befolkningsundersoegelsen_i_groenland
15 Spallone V, Bellavere F, Scionti L, *et al*. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. *Nutr Metab Cardiovasc Dis* 2011;21:69–78.
16 Braune S, Auer A, Schulte-Mönting J, *et al*. Cardiovascular parameters: sensitivity to detect autonomic dysfunction and influence of age and sex in normal subjects. *Clin Auton Res* 1996;6:3–15.
17 Low PA, Denq JC, Opfer-Gehrking TL, *et al*. Effect of age and gender on sudomotor and cardiovagal function and blood pressure response to tilt in normal subjects. *Muscle Nerve* 1997;20:1561–8.
18 Ndayisaba JP, Fanciulli A, Granata R. Cardiovascular responses to various autonomic tests in males and females. *Clincal Autonomic Research* 2015;25:317–26.
19 O'Brien IA, O'Hare P, Corrall RJ. Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. *Br Heart J* 1986;55:348–54.
20 Vita G, Princi P, Calabro R, *et al*. Cardiovascular reflex tests. assessment of age-adjusted normal range. *J Neurol Sci* 1986;75:263–74.
21 Cardone C. I test che valutano La risposta riflessa cardiovascolare. *Neuropatia Diabetica: rassegnabibliografica* 1990;2:151–60.
22 Ziegler D, Laux G, Dannehl K, *et al*. Assessment of cardiovascular autonomic function: age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. *Diabet Med* 1992;9:166–75.
23 Antelmi I, de Paula RS, Shinzato AR, *et al*. Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. *Am J Cardiol* 2004;93:381–5.
24 Britton A, Shipley M, Malik M, *et al*. Changes in heart rate and heart rate variability over time in middle-aged men and women in the general population (from the Whitehall II cohort study). *Am J Cardiol* 2007;100:524–7.
25 Jandackova VK, Scholes S, Britton A, *et al*. Are changes in heart rate variability in middle-aged and older people normative or caused by pathological conditions? findings from a large population-based longitudinal cohort study. *J Am Heart Assoc* 2016;5. doi:10.1161/JAHA.115.002365. [Epub ahead of print: 12 Feb 2016].
26 Umetani K, Singer DH, McCraty R, *et al*. Twenty-Four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J Am Coll Cardiol* 1998;31:593–601.
27 Hill LK, Hu DD, Koenig J, *et al*. Ethnic differences in resting heart rate variability: a systematic review and meta-analysis. *Psychosom Med* 2015;77:16–25.
28 Greenland S. Population in localities January 1st 1977-2020, 2020. Available: https://bank.stat.gl/pxweb/en/Greenland/Greenland__BE__BE01__BE0120/BEXST4.PX/?rxid=a1551b20-d1d5-4bcf-a80c-de114c001595 [Accessed 03 Nov 2020].
29 Malik M, Bigger JT, Camm AJ, *et al*. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* 1996;17:354–81.
30 Charles M, Fleischer J, Witte DR, *et al*. Impact of early detection and treatment of diabetes on the 6-year prevalence of cardiac autonomic neuropathy in people with screen-detected diabetes: addition-Denmark, a cluster-randomised study. *Diabetologia* 2013;56:101–8.
31 Looga R. The Valsalva manoeuvre--cardiovascular effects and performance technique: a critical review. *Respir Physiol Neurobiol* 2005;147:39–49.
32 World Health Organization. *Classification of diabetes mellitus*. 13, 2019. https://www.who.int/publications/i/item/classification-of-diabetes-mellitus
33 World Health Organization. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia*. 3, 2006.
34 Dahl-Petersen IK, Hansen AW, Bjerregaard P, *et al*. Validity of the International physical activity questionnaire in the Arctic. *Med Sci Sports Exerc* 2013;45:728–36.
35 The IPAQ group: guidelines for data processing and analysis of IPAQ—short and long forms, 2005. Available: https://docs.google.com/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnx0aGVpcGFxfGd4OjE0NDgxMDk3NDU1YWRlZTM [Accessed 29 May 2021].
36 Bjerregaard P, Becker U. Validation of survey information on smoking and alcohol consumption against import statistics, Greenland 1993-2010. *Int J Circumpolar Health* 2013;72. doi:10.3402/ijch.v72i0.20314. [Epub ahead of print: 05 03 2013].
37 Saunders JB, Aasland OG, Babor TF, *et al*. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption--II. *Addiction* 1993;88:791–804.
38 Peng L, Wuu J, Benatar M. Developing reference data for nerve conduction studies: an application of quantile regression. *Muscle Nerve* 2009;40:763–71.
39 Koenker R. *Quantile regression*. Cambridge University Press, 2005.
40 Koenker R, Bassett G. Regression quantiles. *Econometrica* 1978;46:33–50.
41 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Statistical Society: Series B* 1995;57:289–300.
42 Schroeder EB, Liao D, Chambless LE, *et al*. Hypertension, blood pressure, and heart rate variability: the atherosclerosis risk in communities (ARIC) study. *Hypertension* 2003;42:1106–11.
43 Mølgaard H, Hermansen K, Bjerregaard P. Spectral components of short-term RR interval variability in healthy subjects and effects of risk factors. *Eur Heart J* 1994;15:1174–83.
44 Greiser KH, Kluttig A, Schumann B, *et al*. Cardiovascular diseases, risk factors and short-term heart rate variability in an elderly general population: the Carla study 2002-2006. *Eur J Epidemiol* 2009;24:123–42.
45 Nunan D, Sandercock GRH, Brodie DA. A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing Clin Electrophysiol* 2010;33:1407–17.
46 Valera B, Dewailly E, Anassour-Laouan-Sidi E, *et al*. Influence of n-3 fatty acids on cardiac autonomic activity among Nunavik Inuit adults. *Int J Circumpolar Health* 2011;70:6–18.
47 Valera B, Dewailly E, Poirier P. Cardiac autonomic activity and blood pressure among nunavik Inuit adults exposed to environmental mercury: a cross-sectional study. *Environ Health* 2008;7:29.
48 Ziegler D, Voss A, Rathmann W, *et al*. Increased prevalence of cardiac autonomic dysfunction at different degrees of glucose intolerance in the general population: the KorA S4 survey. *Diabetologia* 2015;58:1118–28.
49 Julian TH, Syeed R, Glascow N, *et al*. Alcohol-Induced autonomic dysfunction: a systematic review. *Clin Auton Res* 2020;30:29–41.
50 Montano N, Porta A, Cogliati C, *et al*. Heart rate variability explored in the frequency domain: a tool to investigate the link between heart and behavior. *Neurosci Biobehav Rev* 2009;33:71–80.

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Correction: *Normative data on cardiovascular autonomic function in Greenlandic Inuit*

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This article was previously published with an error.

The original paper presented normative thresholds of cardiovascular autonomic function in Greenlanders without diabetes. The levels of the normative thresholds were relatively low, and authors discussed in the paper, that they could only speculate on the reason for the seemingly reduced level of cardiovascular autonomic function in healthy Greenlanders. The thresholds were derived from analyses based on a Greenlandic study population for which they lacked extensive descriptive details. Meanwhile, authors have received information indicating that some of the participants previously have been identified with diabetes in a different study. Likewise, authors obtained information on medication taken by some of the participants when they were examined. This new information has led to substantial amendments in the study population, requiring the exclusion of 99 individuals. These adjustments have impacted the normative thresholds, causing them to rise despite remaining relatively modest. It is highly relevant to adjust the normative thresholds as they may be applied to diagnose cardiovascular autonomic dysfunction in Greenlanders.

The adjustments have required corrections in the main paper, the tables, the figures and the supplemental appendix.

The specific corrections by the authors are described as follows:

RESEARCH DESIGN AND METHODS

Study Population

As we have acquired additional information about the participants, we have expanded the exclusion criteria. In addition to pregnancy, atrial fibrillation or the presence of an implanted pacemaker, participants were excluded if they had a history of ischemic heart disease or were undergoing treatment with medications (such as beta-blockers, tricyclic antidepressants, opioids, etc) that could potentially influence heart rate during the examinations.

Statistics

In our models predicting heart rate variability (HRV) outcomes (SDNN, RMSSD, low-frequency power, high-frequency power and total power), with age as an independent determinant and with adjustment for sex, we now also adjust for resting heart rate (HR). To avoid the estimated normative limits to exhibit negative values when adjusting for HR, as it is not biologically plausible, we applied a log-transformation to the outcome variables of the HRV measures. Subsequently, we back-transformed the coefficients to the original scale. Therefore we are presenting age- and HR-specific normative thresholds for the HRV outcomes. Consequently, due to this added dimension, visualizing the HRV measure thresholds in a two-dimensional table is no longer feasible however, instead, we are providing the equations to calculate the specific normative thresholds for each outcome in the main paper.

Results

Study population

In the updated analyses, instead of excluding only 10 participants, a total of 99 participants were excluded. Twenty-eight participants did not meet the inclusion

criteria of whom 18 participants were categorised with diabetes. An additional 71 participants were excluded due to medication that could affect HR. Like in the original analyses, 11 participants were excluded due to their age being below 20 years or above 80 years. Therefore, the final study population comprised 383 participants, with 242 participants from the Greenlandic Population Study 2018 and 141 participants from Qasigiannguit 2020. The additional participant information also included a prior evaluation of diabetes and pre-diabetes diagnoses, allowing us to categorize participants differently according to their glycemic status. After the adjustments, 56.7% were categorized as having pre-diabetes and 28.1% as having normoglycemia. Glycemic status was not evaluated in 14.6%. These amendments in the study population have affected the characteristics reported in the text, table 1 and table 2.

Associations to demographic variables

In the revised analyses, despite changes in the p-values, age continued to exhibit a significant and inverse association with the three cardiovascular autonomic reflex tests (CARTs) and HRV measures. Furthermore, higher HR was significantly associated with lower HRV measures. Notably, there remained no significant association between sex and any of the outcomes.

Final models

The levels of the updated predicted age-dependent normative thresholds for all outcomes generally trended higher when compared with the original thresholds presented. Besides that, there were no modifications to the final trajectories of the CARTs. Before adjusting for HR, linear models and piecewise linear models yielded the best fit for the final trajectories of HRV outcomes. Following the adjustment for HR, the predicted normative limits for the HRV measures, tended to assume negative values in instances where both age and HR were elevated. Thus, models with log-transformed outcome variables demonstrated the best fit for the HRV measures (figure 1).

We have chosen to present the outcome-specific formulas in the main paper. In that way, it is convenient for the reader to calculate the age-specific normative thresholds for the CARTs and age- and HR-specific normative thresholds for the HRV measures.

Discussion and conclusion

Despite changes in the study population leading to a general increase in normative thresholds, the presented thresholds, when compared with similar studies based on other nationalities, remain relatively low. Thus, no substantial corrections have been made to the Discussion and Conclusion sections.

Tables and supplemental appendix

There have been revisions to the information in tables 1–3 due to the alterations in the study population. For example, in table 1, which outlines the characteristics of the study population, the mean age has been modified from 53.9 years (SD 13.1) to 52.3 years (SD 12.9). The percentage of current smokers, previously at 54.5%, has been updated to 59.3% after the revision. Additionally, HbA1c has shifted from 41 mmol/mol (IQR 38;43) to 40 mmol/mol (IQR 37;43). More characteristics can be found in table 1.

The updated median values of the outcomes presented in table 2 are not notably different from the previous. For instance, the median values for the CARTs remain consistent with the values before the revision. However, the revised medians of the HRV measures exhibit a slight increase compared with the previous values.

Table 4, which initially contained normative thresholds for HRV measures, has been removed because the models predicting these thresholds have been adjusted for HR. This introduces an additional dimension to the data, making it impractical to present in a two-dimensional table. Instead, we provide the equations for calculating age- and HR-specific normative thresholds in the main paper.

The appendix has been edited to remove sections of coding in order to offer readers a clearer overview.

We are no longer able to present the distribution and overlap of diagnostic tests as information related to glycemic status is obtained from various assessments. Therefore, the Venn Diagram in the supplemental appendix 1, section 6, has been eliminated and information concerning diagnostic tests in table 1 has also been withdrawn.

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