Appendix

Normative data on cardiovascular autonomic function in Greenlandic Inuit

Abbrevations:

CARTs – cardiovascular autonomic reflex tests

HRV – heart rate variability

bpm_hr - heart rate

ei - deep breathing-ratio

rs - supine to upright position-ratio

vm - valsalva manoeuvre

sdnn_hr - standard deviation of normal-to-normal intervals, SDNN

rmssd_hr - root mean square of the sum of the squares of differences between consecutive R–R intervals, RMSSD

lf_hr - low frequency power, LF

hf_hr - high frequency power, HF

total_hr - total power

1 Quantile regression and model selection

Normal limits of cardiovascular autonomic function were evaluated by applying quantile regression. Quantile regression is an extension of linear regression and is a method suitable for establishing normative thresholds since the method fits conditional quantile(s) of the response with a general linear model that assumes no parametric form for the distribution of the response. Regression coefficients are a function of the chosen percentile and do not apply to the entire data range. Thus, the method is ideal when estimates of specific low or high percentiles are computed which often is the approach in reference studies. The fact that quantile regression requires fewer restrictive assumptions about normality offers greater flexibility compared to linear regression models.

We chose to define the normal threshold as the lower 5th percentile. This was based on the accepted practice of a 5% false-positive rate in statistical testing. For heart variability measures it is not relevant to assess an upper limit since there is no theory describing the adverse effects of exceeding high levels.

The presence of prediabetes was oversampled in the study population. Hence, we weighted prediabetes and normoglycaemia according to prevalence in the Greenlandic Inuit background population.

We initially applied a quantile regression model adjusted for age and sex with a linear and more flexible spline function at the lower 5th percentile. We performed a visual inspection of the two models and fitted data to either a linear or piecewise linear function. In the final models, we tested if age and sex were significantly associated to the outcomes. A two-sided statistical significance of 0.05 was applied. For the heart rate variability (HRV) measures, we additionally included adjustments for resting heart rate in the models. To ensure that the estimated thresholds remained positive when adjusting for resting heart rate, as it is not biologically plausible for HRV measures to exhibit values below zero, we applied a log-transformation of the outcome variables of HRV and subsequently back-transformed the coefficients.

2 Data analysis

2.1 The following packages and libraries were applied

```
library(dplyr)
library(ggpubr)
library(psych)
library(foreign)
library(stringr)
library(withr)
library(quantreg)
library(splines)
library(Epi)
library(splines2)
library(pander)
library(knitr)
library(tidyr)
library(readxl)
library(sas7bdat)
library(ggplot2)
library(tinytex)
library(VennDiagram)
library(RColorBrewer)
library(grDevices)
library(gridExtra)
library(flextable)
library(gtsummary)
```

2.2 Data is loaded and defined

```
data <- ReadRDS(dataframe)
```

2.3 Weighted status of prediabetes and normoglycaemia

Calculation of weight factors for prediabetes and normoglycemia. Background population: Prediabetes= 20.3%, normoglycaemia= 70% (diabetes= 9.7%)

The presence of prediabetes and normoglycemia is weighted according to prevalence in background population. A new variable is defined.Total population: N=383 (prediab=217, normogl=110, NA=56).

```
data$wgt <-
  ifelse(data$prediab_all == 1, (20.3 / (20.3 + 70)) / (217 / (217 + 110)), (70 /
(20.3 + 70)) / (110 / (217 + 110)))
```

Observations with missing status of prediabetes or normoglycemia (N=56) are not weighted hence, weight factor is set to 1.

```
data$wgt <-
  ifelse(is.na(data$prediab_all), 1, data$wgt)
```

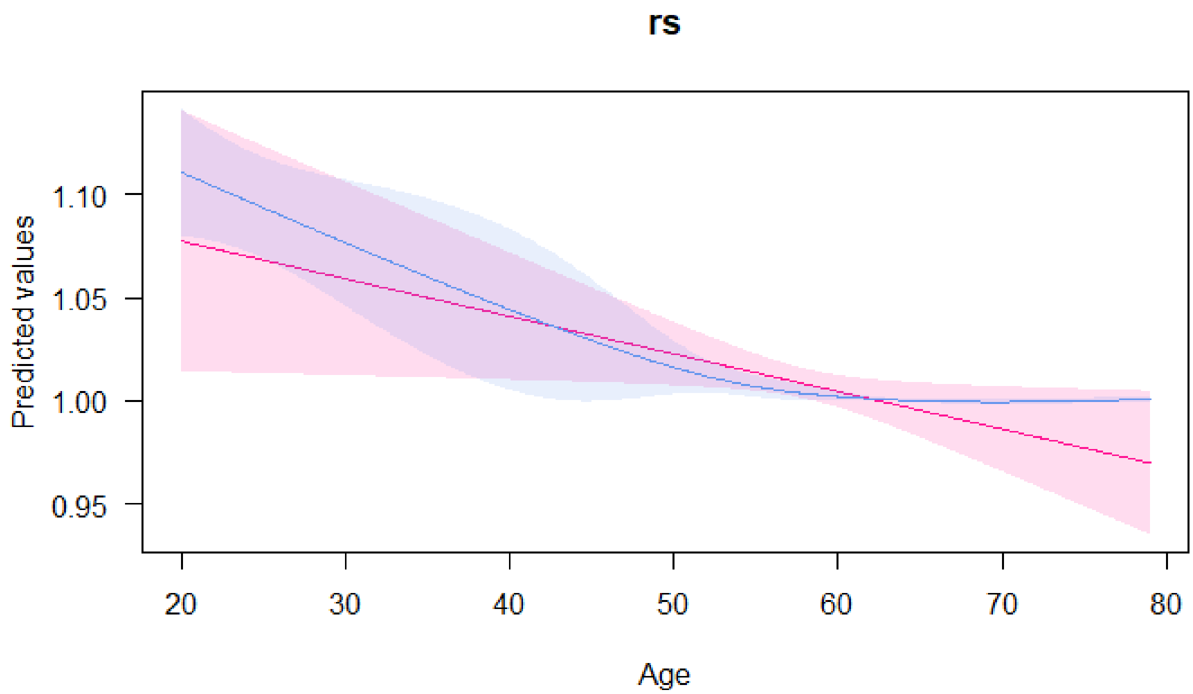
The sum of the weight variable must give 383 observations:

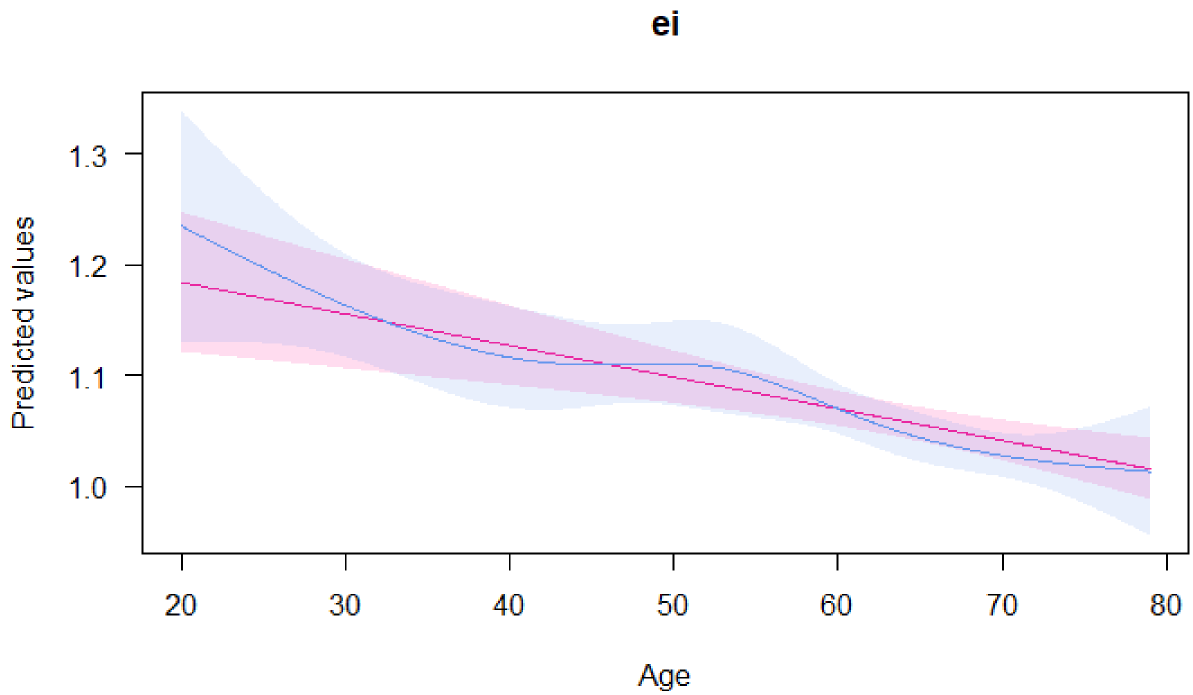
```
sum(data$wgt)
 [1] 383
```

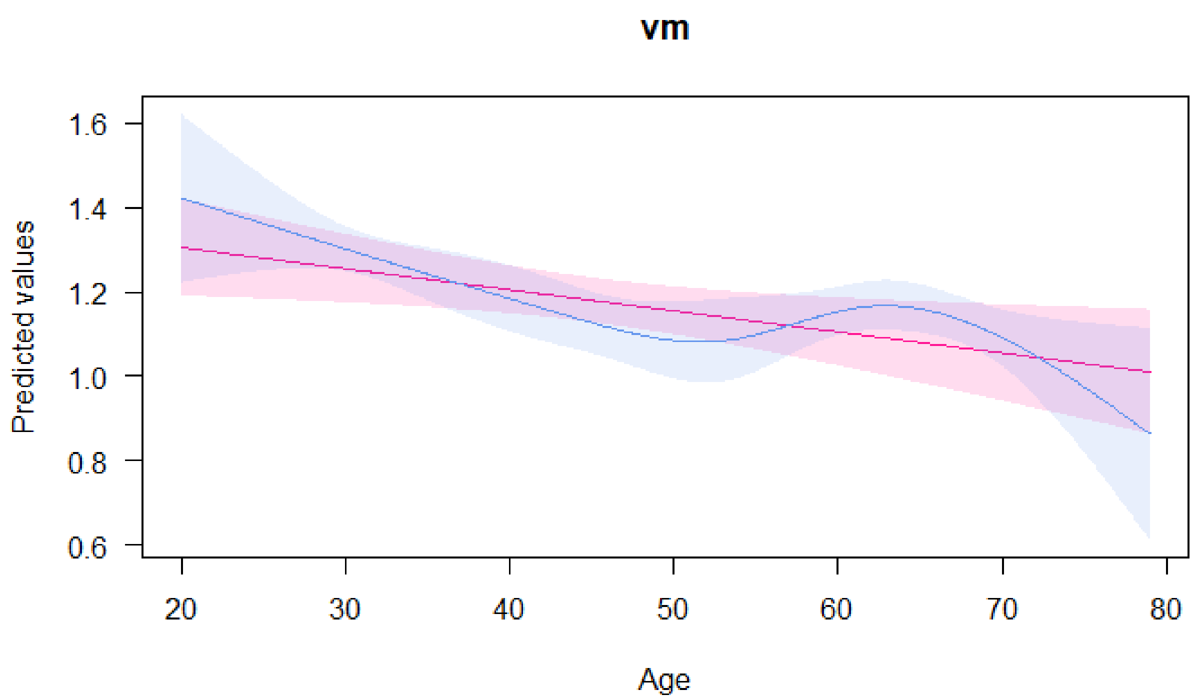
2.4 Visual inspection of linear and spline functions

Models including age

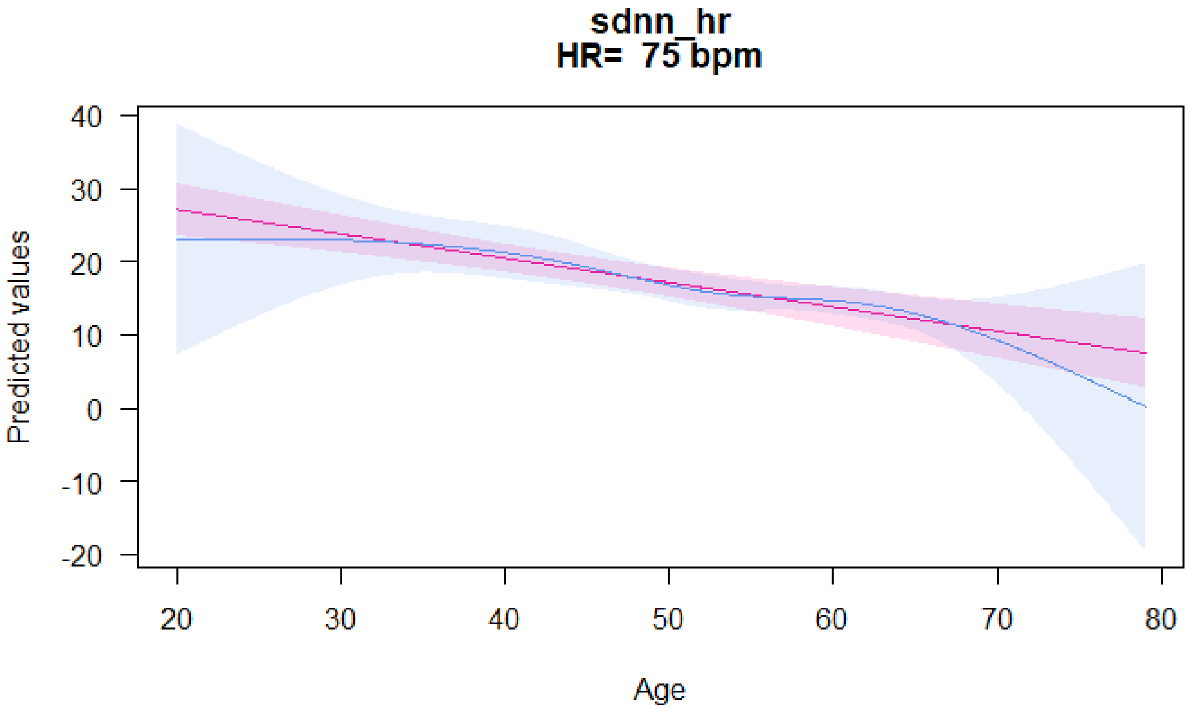
CARTs

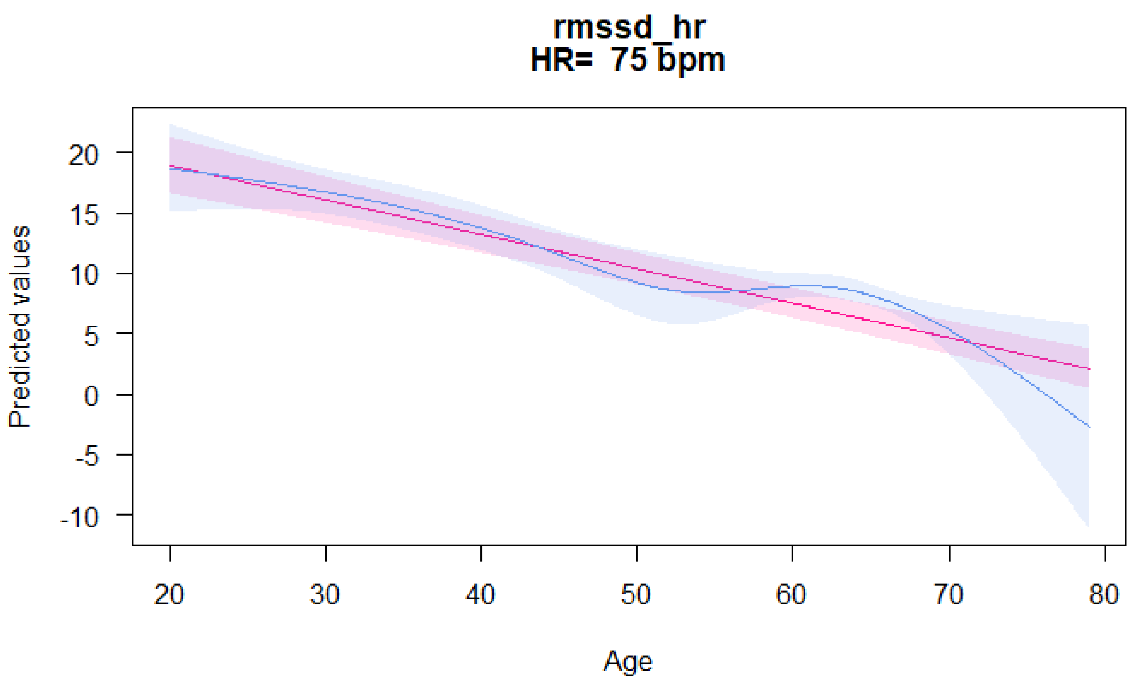


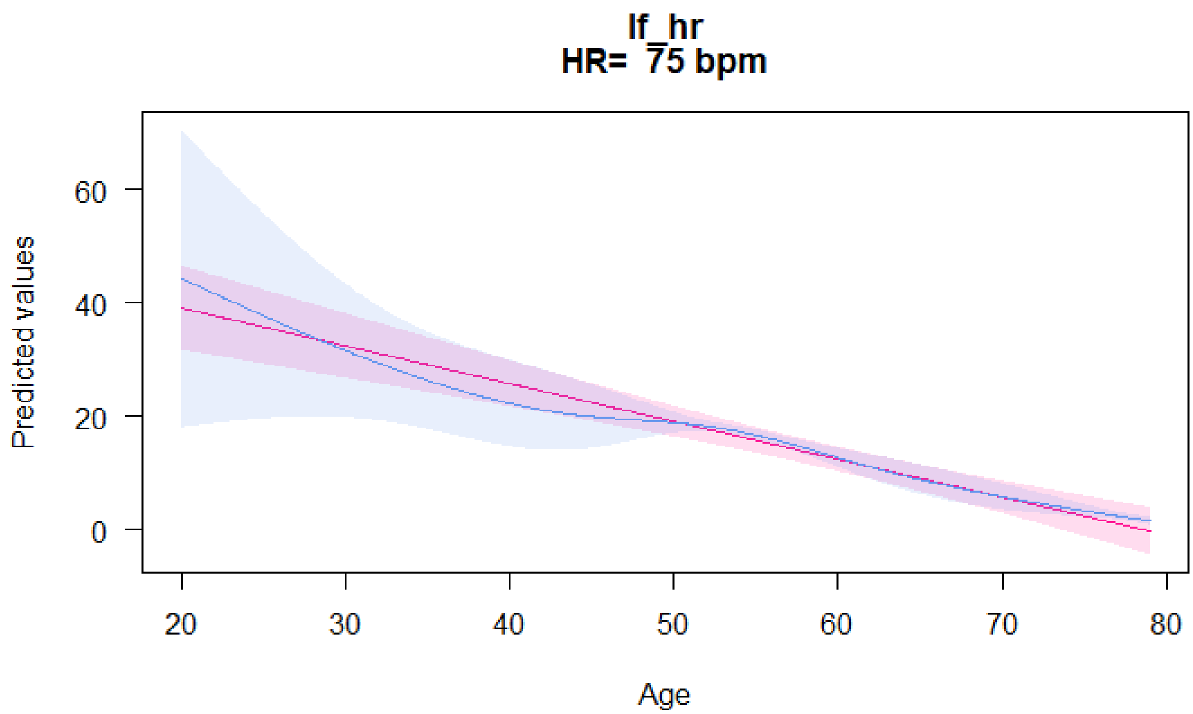

ei
Predicted values
1.3
1.2
1.1
1.0
20
30
40
50
60
70
80
Age

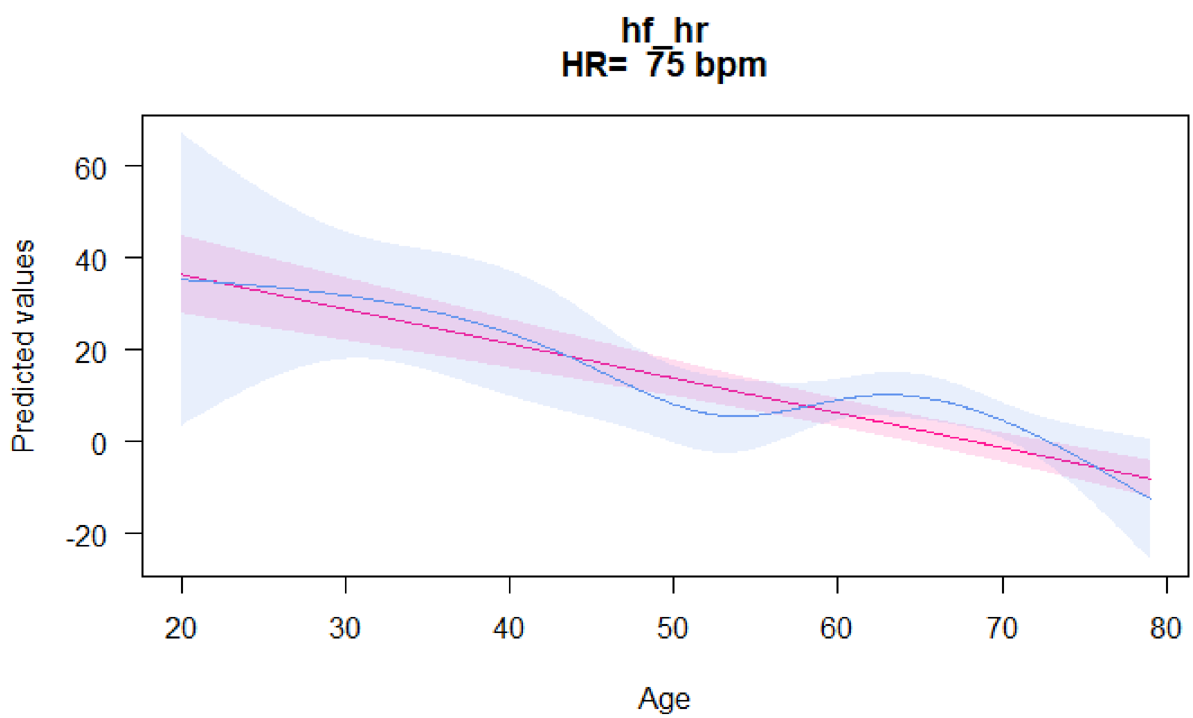

vm
Predicted values
1.6
1.4
1.2
1.0
0.8
0.6
20
30
40
50
60
70
80
Age

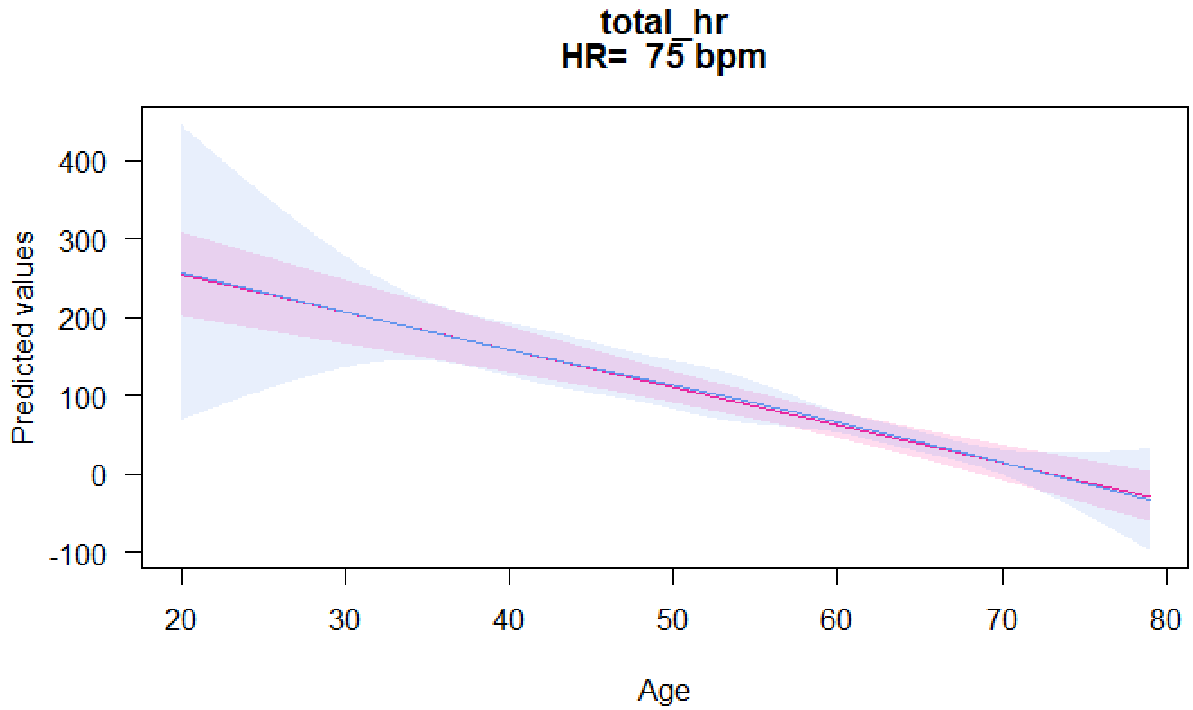
HRV


sdnn_hr
HR= 75 bpm
Predicted values
40
30
20
10
0
-10
-20
20
30
40
50
60
70
80
Age


rmssd_hr
HR= 75 bpm
Predicted values
20
15
10
5
0
-5
-10
20
30
40
50
60
70
80
Age

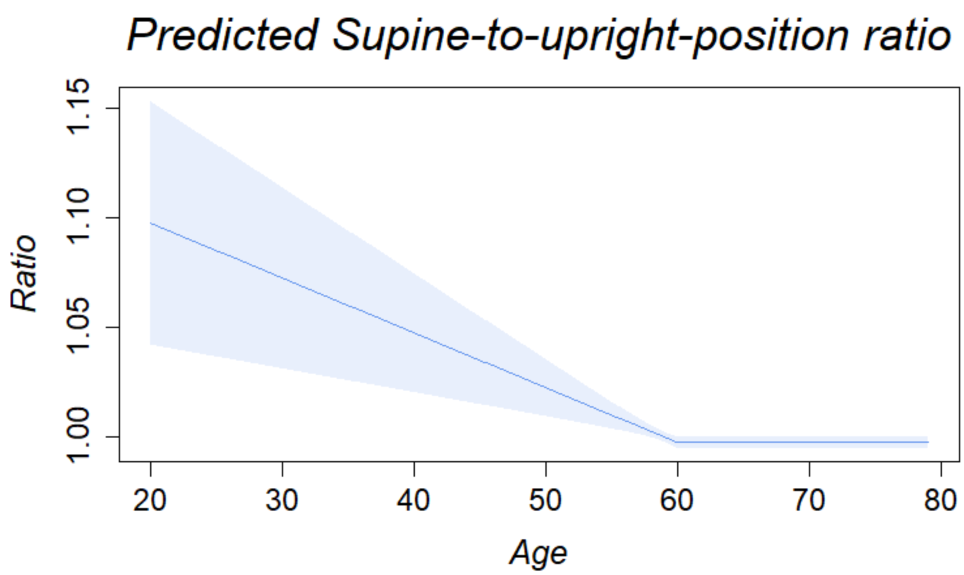

lf_hr
HR= 75 bpm
Predicted values
60
40
20
0
20
30
40
50
60
70
80
Age


hf_hr
HR= 75 bpm
Predicted values
60
40
20
0
-20
20
30
40
50
60
70
80
Age

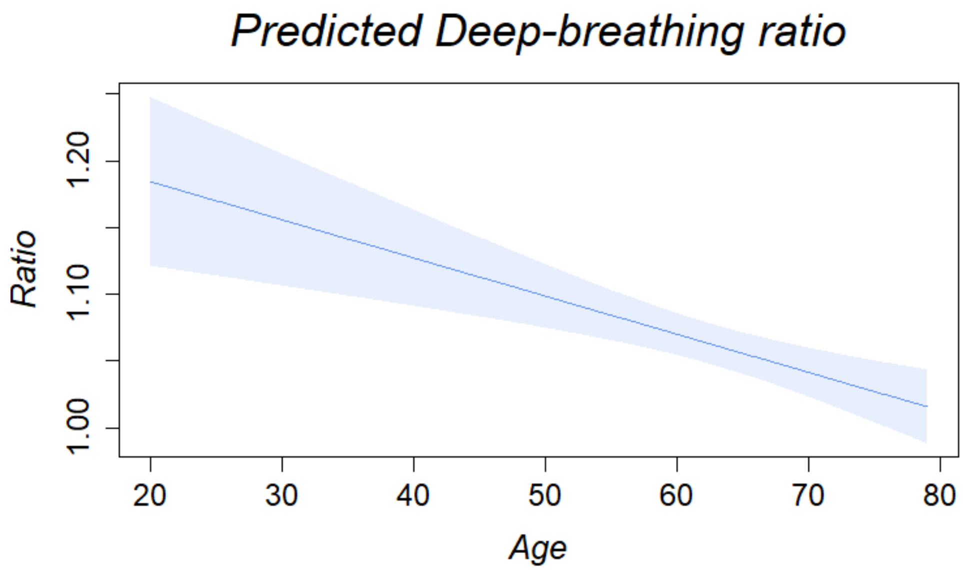


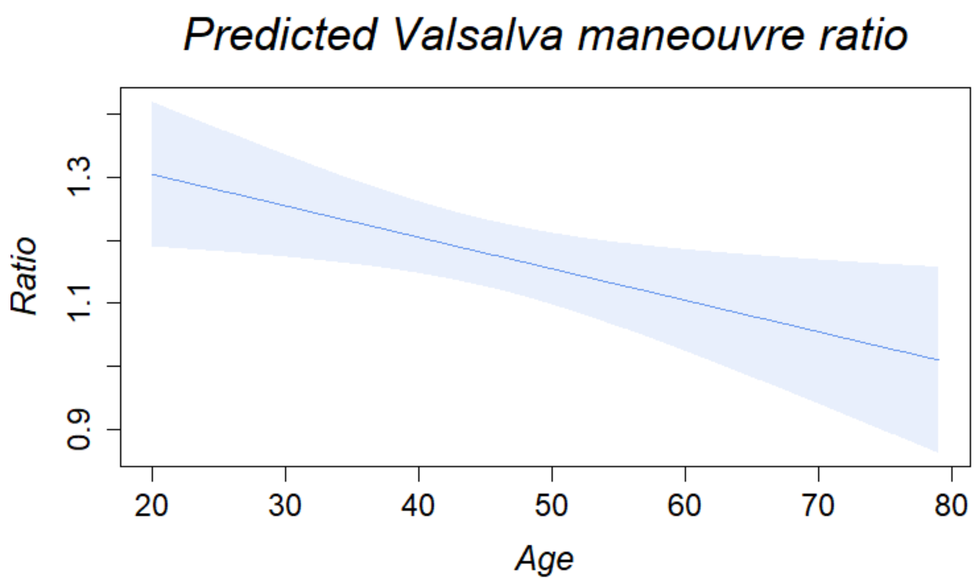
2.5 Final models

Piecewise linear models, linear fall untill age of 60 years where the slope is going towards 0

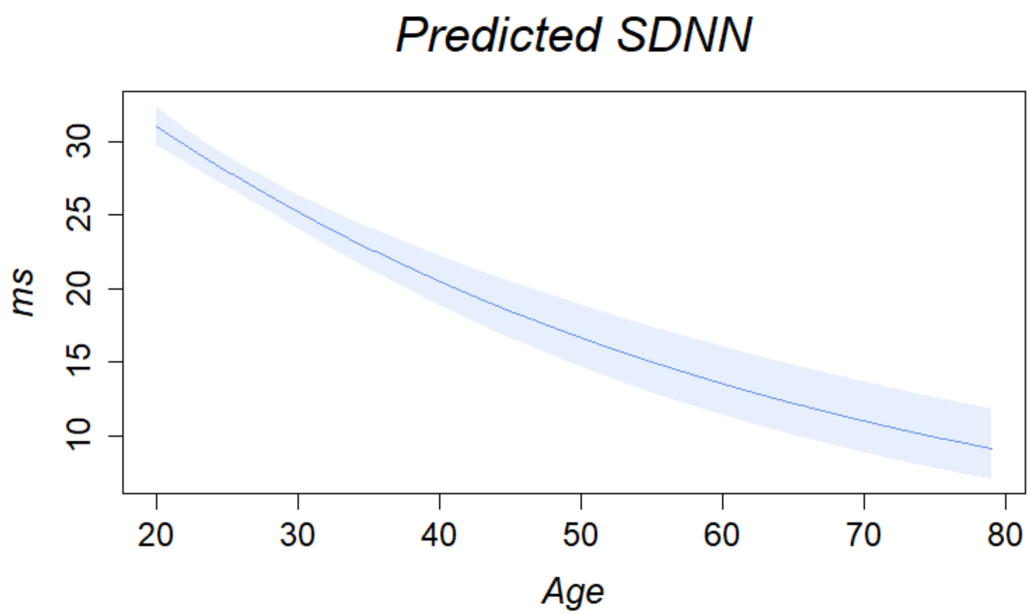


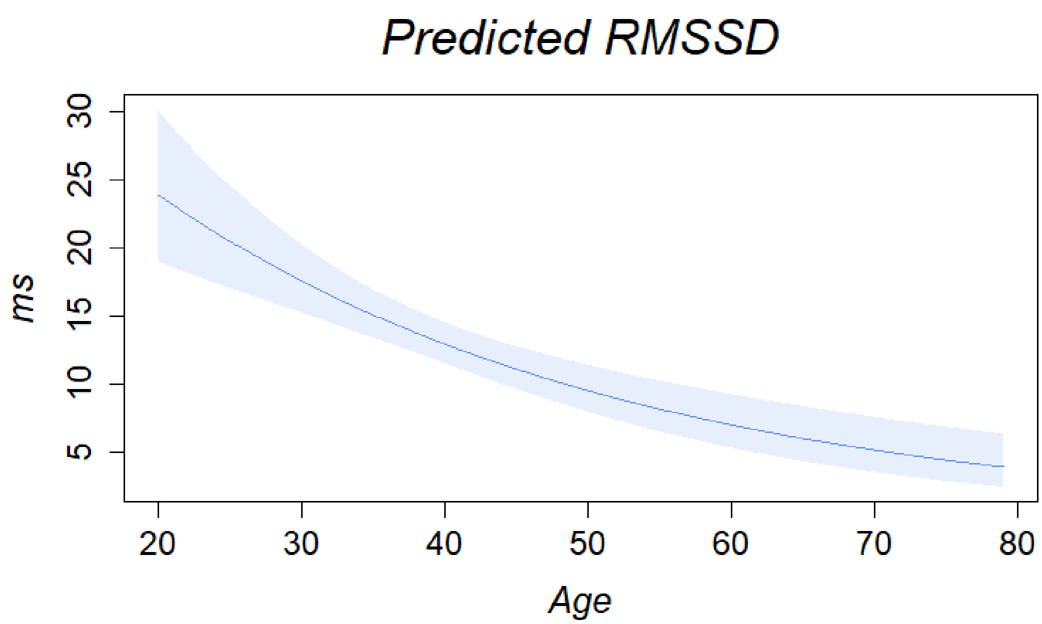
Linear models



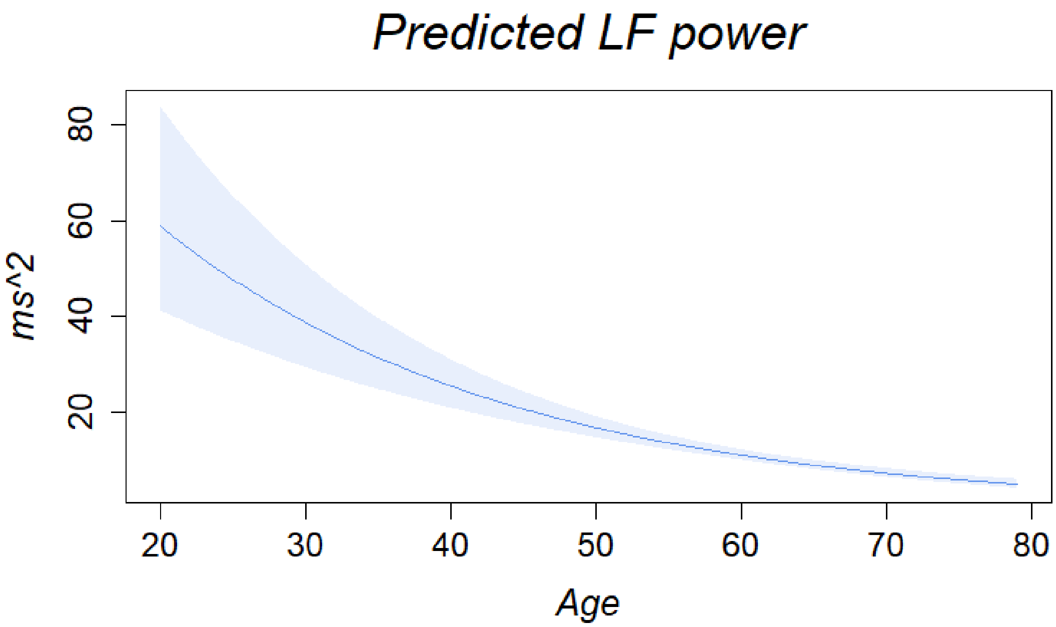


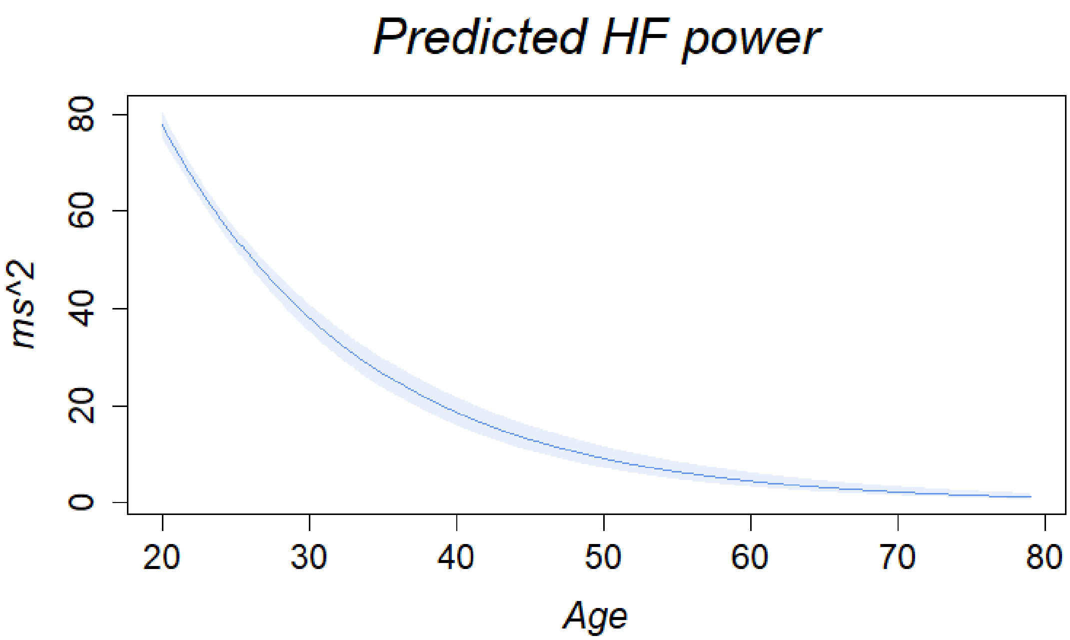
Log-transformed models adjusted for HR, presented for the median heart rate 75 bpm.



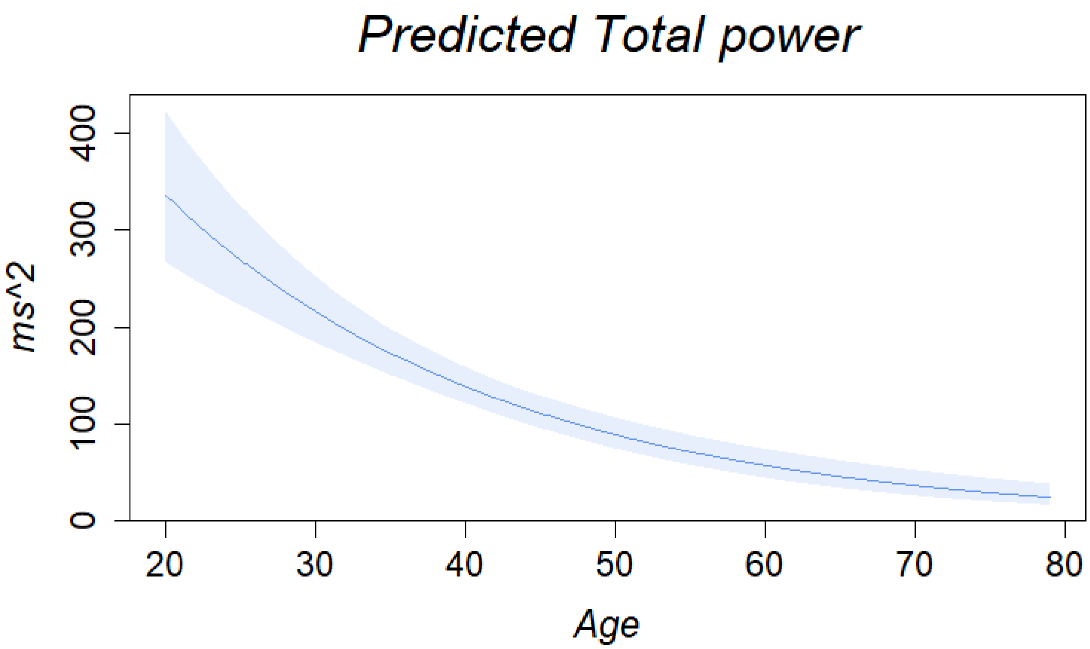


Christensen MMB, *et al. BMJ Open Diab Res Care* 2024; 9:e002121. doi: 10.1136/bmjdrc-2021-002121





Christensen MMB, *et al. BMJ Open Diab Res Care* 2024; 9:e002121. doi: 10.1136/bmjdrc-2021-002121



2.6 Testing dependency of age and sex in final models

Piecewise linear models:

Piecewise linear spline function, RS

```
                  Estimate   2.5% 97.5%     P
(Intercept)          0.998  0.988 1.009 0.000
pmax(y - age, 0)     0.002  0.001 0.004 0.001
sexmale              0.002 -0.013 0.016 0.833
```

Linear function, EI

```
             Estimate   2.5%  97.5%     P
(Intercept)     1.227  1.185  1.270 0.000
age            -0.002 -0.003 -0.002 0.000
sexmale        -0.033 -0.060 -0.005 0.022
```

Linear function, VM

```
            Estimate   2.5% 97.5%     P
(Intercept)    1.474  1.161 1.787 0.000
age           -0.006 -0.012 0.000 0.047
sexmale       -0.038 -0.184 0.108 0.613
```

Models adjusted for HR. Outcomes variables of HRV were log-transformed.

Log-transformed function, back-transformed coefficients, SDNN

```
            exp(Est.)    2.5%   97.5%     P
(Intercept)   333.011 190.519 582.078 0.000
age             0.978   0.971   0.985 0.000
sexmale         1.047   0.859   1.276 0.651
bpm_hr          0.975   0.970   0.980 0.000
```

Log-transformed function, back-transformed coefficients, RMSSD

```
            exp(Est.)    2.5%    97.5%     P
(Intercept)   501.534 215.223 1168.720 0.000
age             0.977   0.969    0.985 0.000
sexmale         0.812   0.630    1.045 0.106
bpm_hr          0.965   0.956    0.974 0.000
```

Log-transformed function, back-transformed coefficients, LF power

```
            exp(Est.)    2.5%    97.5%     P
(Intercept)  1723.552 607.614 4889.010 0.000
age             0.959   0.949    0.969 0.000
sexmale         1.293   0.983    1.700 0.066
bpm_hr          0.966   0.957    0.974 0.000
```

Log-transformed function, back-transformed coefficients, HF power

```
            exp(Est.)      2.5%      97.5%     P
(Intercept) 90552.516 22411.494 365872.902 0.000
age             0.931     0.918      0.945 0.000
sexmale         0.915     0.549      1.526 0.733
bpm_hr          0.929     0.912      0.945 0.000
```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Log-transformed function, back-transformed coefficients, Total power

```
            exp(Est.)      2.5%      97.5%     P
(Intercept) 38876.964 12428.056 121613.419 0.000
age             0.958     0.946      0.971 0.000
sexmale         0.965     0.696      1.339 0.833
bpm_hr          0.949     0.937      0.961 0.000
```

Sex was significantly associated to Deep breathing (EI) (P= 0.02) however, not after correcting for multiple testing.

Lastly, we tested solely if age was significantly associated to the outcomes:

Piecewise linear models

Piecewise linear spline function, RS

```
                 Estimate  2.5% 97.5%     P
(Intercept)         0.998 0.995 1.000 0.000
pmax(y - age, 0)    0.002 0.001 0.004 0.001
```

Linear models

Linear function, EI

```
            Estimate   2.5%  97.5% P
(Intercept)    1.241  1.150  1.332 0
age           -0.003 -0.004 -0.001 0
```

Linear function, VM

```
            Estimate   2.5%  97.5%     P
(Intercept)    1.405  1.215  1.595 0.000
age           -0.005 -0.009 -0.001 0.015
```

Models adjusted for HR. Outcomes variables of HRV were log-transformed.

Log-transformed function, back-transformed coefficients, SDNN

```
            exp(Est.)    2.5%   97.5% P
(Intercept)   301.430 169.143 537.177 0
```

```
age             0.979   0.975   0.984 0
bpm_hr          0.976   0.968   0.983 0
```

Log-transformed function, back-transformed coefficients, RMSSD

```
            exp(Est.)    2.5%    97.5% P
(Intercept)   769.403 261.583 2263.069 0
age             0.970   0.959    0.981 0
bpm_hr          0.963   0.952    0.973 0
```

Log-transformed function, back-transformed coefficients, LF power

```
            exp(Est.)     2.5%    97.5% P
(Intercept)  2812.372 1268.545 6235.042 0
age             0.959    0.951    0.967 0
bpm_hr          0.960    0.952    0.969 0
```

Log-transformed function, back-transformed coefficients, HF power

```
            exp(Est.)      2.5%      97.5% P
(Intercept) 74043.207 31988.999 171383.807 0
age             0.931     0.923      0.939 0
bpm_hr          0.930     0.920      0.940 0
```

Log-transformed function, back-transformed coefficients, Total power

```
            exp(Est.)      2.5%      97.5% P
(Intercept) 42876.683 14644.552 125535.418 0
age             0.957     0.947      0.967 0
bpm_hr          0.949     0.937      0.961 0
```

Age was significantly associated with all outcomes.

3 Predicted estimates for the reference limit at the 5th percentile

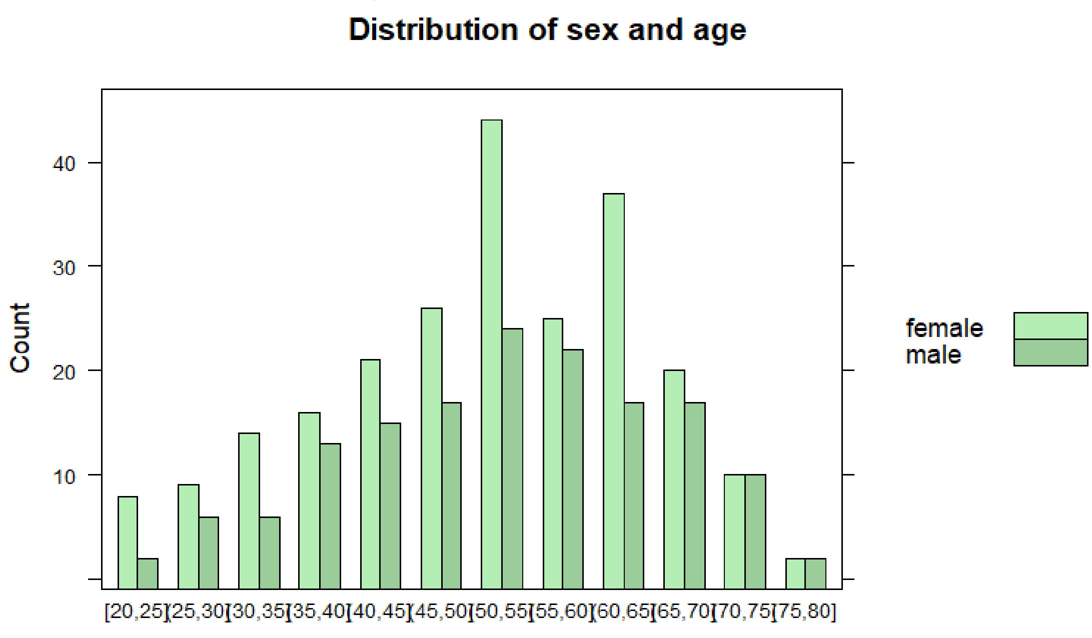
Piecewise linear models

```
tab1(x = data$rs, y = 60)

      fit     lower   higher
 1 1.0850 1.0367393 1.133261
 2 1.0600 1.0258722 1.094128
 3 1.0350 1.0149726 1.055027
 4 1.0100 1.0038139 1.016186
 5 0.9975 0.9948271 1.000173
 6 0.9975 0.9948271 1.000173
```

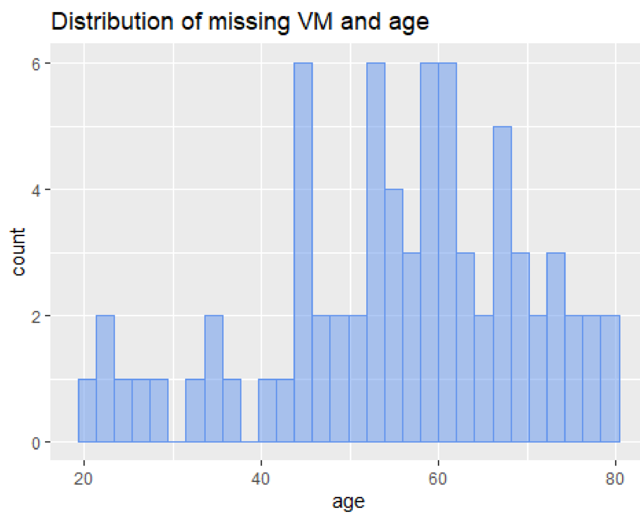
Linear models

```
tab2(data$ei)

        fit    lower   higher
 1 1.170000 1.113685 1.226315
 2 1.141429 1.098717 1.184140
 3 1.112857 1.083080 1.142634
 4 1.084286 1.065346 1.103226
 5 1.055714 1.040210 1.071219
 6 1.027143 1.004085 1.050201

tab2(data$vm)

    fit     lower   higher
 1 1.28 1.1824855 1.377515
 2 1.23 1.1627594 1.297241
 3 1.18 1.1267875 1.233212
 4 1.13 1.0633674 1.196633
 5 1.08 0.9833238 1.176676
 6 1.03 0.8975207 1.162479
```

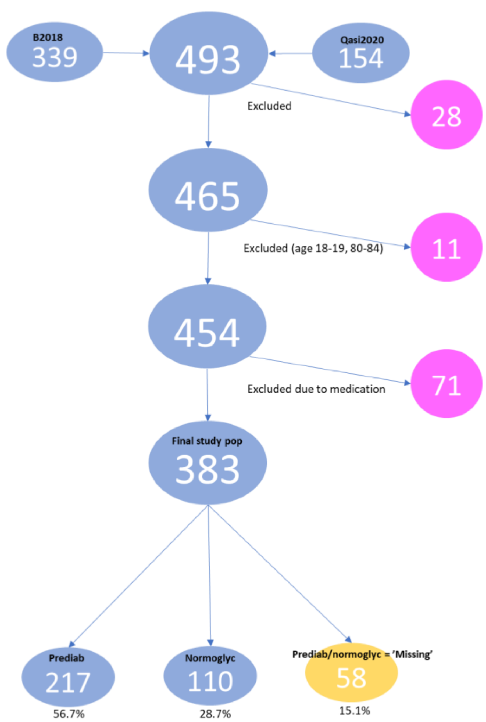
4 Distribution of sex and age



5 Distribution of missing VM over age



6 Flow chart of recruitment of participants and distribution of prediabetes / normoglycaemia



7 Normalized units of LF and HF power

The normalized units of LF (LF nu) and HF power (HF nu) are calculated from the short-term frequency band LF or HF and divided by the total power (LF + HF). LF nu and HF nu may be used to quantify the proportional sympathetic and parasympathetic activity, respectively.

	Pooled		Population Study 2018		Qasigiannguit 2020	
	N		N		N	
LF nu (%)	378	56.6 [40.8;75.4]	241	57.0 [40.8;75.6]	137	55.3 [41.2;73.7]
HF nu (%)	378	43.4 [24.6;59.2]	241	43.0 [24.4;59.2]	137	44.7 [26.3;58.8]

Data is given in medians [IQR]. LF nu: normalized unit of low-frequency power, HF nu: normalized unit of high-frequency power.

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