Supplemental Material:

Cardiovascular autonomic dysfunction is linked with arterial stiffness across glucose metabolism: The Maastricht Study

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Removal of 24-hour HRV outliers

HRV indices are very sensitive to data error in the time-series collection of interbeat intervals. This can be due to missing heartbeat signals, arrhythmia, ectopic beats and more. These premature, skipped, or non-captured heartbeat recordings might not have been unfiltered from the IBI data and should have been removed, as it does not reflect autonomic activity. The time segment of frequency domain measures in the Maastricht Study was over the whole recording to capture very- and ultra-lower frequency components. Reference values of 24-hour HRV are done with small population size and with either time domain HRV or 5-min segments of frequency domain indices [1]. To determine cutoff for the exclusion of outliers, we visualize each HRV index value across ages and compare it to available reference values by age from studies [1]. Based on visually observing distribution and available reference material, we excluded time-domain indices according to reference material, and the upper 1^{th} percentile in frequency-domain measures.

Reference:

1. Sammito S, Böckelmann I. Reference values for time- and frequency-domain heart rate variability measures. Heart Rhythm. 2016;13(6):1309-16. doi: 10.1016/j.hrthm.2016.02.006.

Table S1: Descriptives of included and non-included population

	No, N = 5,514	**Yes**, N = 3,673
Sex		
Men	2,782 (50%)	1,789 (49%)
Women	2,732 (50%)	1,884 (51%)
Age (years)	61 (53, 67)	60 (53, 66)
Ethnicity		
White	5,418 (98%)	3,633 (99%)
Non-white	93 (1.7%)	40 (1.1%)
Education		
Low (No education, (un)completed primary education, or lower vocational education)	1,911 (35%)	1,094 (30%)
Middle (intermediate vocational education or higher secondary education)	1,449 (27%)	1,050 (29%)
High (Higher vocational education or university education)	2,028 (38%)	1,529 (42%)
Alcohol consumption		
None	1,083 (20%)	609 (17%)
Low (Women: ≤ 7, Men: ≤ 14)	3,237 (60%)	2,147 (58%)
High (Women: > 7, Men: > 14)	1,119 (21%)	917 (25%)
Smoking status		
Never	2,086 (38%)	1,417 (39%)
Former (quit > 6 months ago)	2,511 (46%)	1,733 (47%)
Former (quit < 6 months ago)	106 (1.9%)	62 (1.7%)
Current	740 (14%)	461 (13%)
Total physical activity (hours/week)	13 (8, 18)	13 (8, 19)
Moderate to vigorous physical activity (hours/week)	4.5 (1.8, 7.5)	4.5 (2.3, 7.8)
BMI (kg^2/m)	26.4 (23.9, 29.5)	26.0 (23.6, 28.8)
Waist (cm)	95 (86, 105)	93 (85, 102)
HbA1c (%)	5.54 (5.26, 5.99)	5.54 (5.26, 5.90)
Fasting plasma glucose (mmol/L)	5.40 (5.00, 6.20)	5.40 (4.90, 6.00)
LDL (mmol/L)	2.90 (2.30, 3.70)	3.10 (2.40, 3.80)
HDL (mmol/L)	1.50 (1.20, 1.80)	1.50 (1.20, 1.90)
Total cholesterol (mmol/L)	5.10 (4.30, 5.90)	5.30 (4.60, 6.10)
Triglycerides (mmol/L)	1.19 (0.87, 1.70)	1.18 (0.87, 1.65)
Glucose metabolism status		

	No, N = 5,514	**Yes**, N = 3,673
Normal glucose metabolism	3,358 (61%)	2,389 (65%)
Prediabetes	844 (15%)	538 (15%)
Type 2 Diabetes	1,259 (23%)	746 (20%)
Duration of type-2 diabetes (only for diagnosed participants)	4 (0, 10)	3 (0, 9)
Mean IBI (ms)	829 (765, 910)	828 (765, 904)
SDNN (ms)	134 (110, 161)	133 (110, 158)
RMSSD (ms)	29 (21, 49)	25 (20, 34)
SDANN (ms)	121 (98, 148)	119 (97, 143)
SDNNi (ms)	54 (43, 71)	52 (42, 63)
pNN50 (%)	8 (3, 20)	6 (3, 12)
TP (ms^2)	11,509 (7,706, 16,540)	11,566 (7,991, 16,394)
ULF (ms^2)	9,665 (6,310, 14,055)	9,788 (6,655, 14,183)
VLF (ms^2)	1,064 (722, 1,606)	1,105 (736, 1,571)
LF (ms^2)	370 (213, 625)	364 (222, 593)
HF (ms^2)	104 (55, 212)	84 (50, 149)
Systolic blood pressure (mmHg)	126 (117, 136)	126 (116, 136)
Diastolic blood pressure (mmHg)	75 (71, 80)	76 (71, 81)
Mean arterial pressure (mmHg)	96 (89, 103)	96 (89, 103)
Carotid artery distensibility (10-3/kPa)	13.8 (10.7, 17.5)	14.2 (11.0, 17.8)
Carotid-femoral pulse wave velocity (m/s)	8.72 (7.60, 10.16)	8.40 (7.44, 9.76)
Prior CVD	1,482 (27%)	0 (0%)
Hypertension (Yes)	3,056 (56%)	1,740 (47%)
Glucose lowering medication	973 (18%)	519 (14%)
Antihypertensive medication	2,192 (40%)	1,108 (30%)
Lipid-lowering medication	1,803 (33%)	905 (25%)

n (%); Median (IQR)

Table S2: Descriptives of participants with both CarDC and cf-PWV measured and participants with only cf-PWV measured

	CAD measured, N = 1,802	**Without CAD measurements**, N = 1,871
Sex		
Men	901 (50%)	888 (47%)
Women	901 (50%)	983 (53%)
Age (years)	60 (54, 66)	59 (52, 66)
Education		
Low (No education, (un)completed primary education, or lower vocational education)	506 (28%)	588 (31%)
Middle (intermediate vocational education or higher secondary education)	541 (30%)	509 (27%)
High (Higher vocational education or university education)	755 (42%)	774 (41%)
Smoking status		
Never	643 (36%)	774 (41%)
Former (quit > 6 months ago)	892 (50%)	841 (45%)
Former (quit < 6 months ago)	33 (1.8%)	29 (1.5%)
Current	234 (13%)	227 (12%)
BMI (kg^2/m)	26.2 (23.7, 29.2)	25.7 (23.5, 28.6)
Waist (cm)	94 (86, 103)	92 (83, 101)
HbA1c (%)	5.63 (5.35, 5.99)	5.44 (5.17, 5.72)
Fasting plasma glucose (mmol/L)	5.50 (5.00, 6.30)	5.30 (4.90, 5.80)
LDL (mmol/L)	3.10 (2.40, 3.90)	3.10 (2.40, 3.70)
HDL (mmol/L)	1.50 (1.20, 1.80)	1.60 (1.20, 1.90)
Total cholesterol (mmol/L)	5.40 (4.60, 6.10)	5.30 (4.60, 6.00)
Triglycerides (mmol/L)	1.22 (0.89, 1.73)	1.14 (0.84, 1.59)
Hypertension (Yes)	928 (52%)	812 (43%)
Diabetes status		
Normal glucose metabolism	1,049 (58%)	1,340 (72%)
Prediabetes	323 (18%)	215 (11%)
Type 2 Diabetes	430 (24%)	316 (17%)
Duration of type-2 diabetes (only for diagnosed participants)	3 (0, 8)	3 (0, 9)
Mean IBI (ms)	824 (759, 900)	832 (772, 908)
SDNN (ms)	132 (109, 157)	133 (111, 159)
RMSSD (ms)	25 (19, 34)	26 (20, 34)

	CAD measured, N = 1,802	**Without CAD measurements**, N = 1,871
SDANN (ms)	120 (96, 143)	118 (98, 144)
SDNNi (ms)	51 (42, 62)	53 (43, 64)
pNN50 (%)	6 (3, 12)	6 (3, 12)
TP (ms²)	11,551 (7,860, 16,410)	11,571 (8,088, 16,366)
ULF (ms²)	9,850 (6,503, 14,183)	9,673 (6,780, 14,194)
VLF (ms²)	1,065 (707, 1,520)	1,129 (767, 1,643)
LF (ms²)	350 (213, 578)	381 (233, 613)
HF (ms²)	83 (48, 150)	86 (52, 149)
Systolic blood pressure (mmHg)	126 (117, 136)	125 (115, 135)
Diastolic blood pressure (mmHg)	76 (71, 81)	75 (71, 81)
Mean arterial pressure (mmHg)	96 (90, 103)	96 (89, 103)
Carotid-femoral pulse wave velocity (m/s)	8.48 (7.44, 9.84)	8.32 (7.44, 9.60)
Glucose-lowering medication (Yes)	301 (17%)	218 (12%)
Antihypertensive medication (Yes)	601 (33%)	507 (27%)
Using beta-blockers (Yes)	239 (13%)	182 (9.7%)

n (%); Median (IQR)

Table S3: Study population characteristics by diabetes status

	Normal glucose metabolism, N = 2,389	**Prediabetes**, N = 538	**Type 2 Diabetes**, N = 746
Sex			
Men	1,028 (43%)	280 (52%)	481 (64%)
Women	1,361 (57%)	258 (48%)	265 (36%)
Age (years)	58 (51, 64)	62 (57, 68)	63 (57, 68)
Ethnicity			
White	2,368 (99%)	533 (99%)	732 (98%)
Non-white	21 (0.9%)	5 (0.9%)	14 (1.9%)
Education			
Low (No education, (un)completed primary education, or lower vocational education)	604 (25%)	192 (36%)	298 (40%)
Middle (intermediate vocational education or higher secondary education)	697 (29%)	145 (27%)	208 (28%)
High (Higher vocational education or university education)	1,088 (46%)	201 (37%)	240 (32%)
Alcohol consumption			
None	338 (14%)	83 (15%)	188 (25%)
Low (Women: ≤ 7, Men: ≤ 14)	1,437 (60%)	298 (55%)	412 (55%)
High (Women: > 7, Men: > 14)	614 (26%)	157 (29%)	146 (20%)
Smoking status			
Never	988 (41%)	185 (34%)	244 (33%)
Former (quit > 6 months ago)	1,070 (45%)	286 (53%)	377 (51%)
Former (quit < 6 months ago)	43 (1.8%)	3 (0.6%)	16 (2.1%)
Current	288 (12%)	64 (12%)	109 (15%)
Total physical activity (hours/week)	13 (9, 19)	13 (9, 19)	12 (7, 17)
Moderate to vigorous physical activity (hours/week)	5.3 (3.0, 8.3)	4.5 (2.3, 7.5)	3.8 (1.5, 6.8)
BMI (kg^2/m)	25.0 (22.9, 27.4)	27.2 (24.9, 30.1)	28.8 (26.0, 31.7)
Waist (cm)	89 (81, 97)	98 (90, 105)	103 (96, 112)
HbA1c (%)	5.35 (5.17, 5.63)	5.63 (5.35, 5.90)	6.54 (6.08, 7.09)

	Normal glucose metabolism, N = 2,389	**Prediabetes**, N = 538	**Type 2 Diabetes**, N = 746
Fasting plasma glucose (mmol/L)	5.10 (4.80, 5.40)	5.90 (5.40, 6.30)	7.40 (6.60, 8.50)
LDL (mmol/L)	3.20 (2.70, 3.90)	3.30 (2.60, 4.00)	2.40 (1.80, 3.10)
HDL (mmol/L)	1.60 (1.30, 2.00)	1.40 (1.20, 1.80)	1.30 (1.00, 1.50)
Total cholesterol (mmol/L)	5.50 (4.80, 6.20)	5.50 (4.80, 6.30)	4.50 (3.90, 5.20)
Triglycerides (mmol/L)	1.05 (0.80, 1.45)	1.39 (1.03, 1.90)	1.51 (1.08, 2.14)
Duration of type-2 diabetes (only for diagnosed participants)	NA (NA, NA)	NA (NA, NA)	3 (0, 9)
Mean IBI (ms)	838 (775, 907)	815 (760, 897)	806 (744, 889)
SDNN (ms)	138 (117, 164)	127 (106, 152)	116 (96, 139)
RMSSD (ms)	26 (21, 34)	24 (19, 33)	22 (17, 31)
SDANN (ms)	125 (103, 149)	113 (92, 139)	103 (84, 127)
SDNNi (ms)	55 (46, 65)	50 (41, 60)	44 (36, 54)
pNN50 (%)	7 (3, 13)	5 (2, 10)	4 (2, 9)
TP (ms^2)	12,596 (8,880, 17,498)	10,615 (7,134, 15,374)	8,880 (6,064, 12,722)
ULF (ms^2)	10,771 (7,392, 15,142)	8,948 (5,852, 13,374)	7,524 (5,036, 11,001)
VLF (ms^2)	1,198 (833, 1,692)	1,015 (685, 1,478)	816 (541, 1,267)
LF (ms^2)	421 (257, 651)	328 (200, 540)	261 (154, 422)
HF (ms^2)	94 (57, 158)	78 (47, 138)	63 (36, 117)
Systolic blood pressure (mmHg)	123 (114, 133)	129 (122, 140)	130 (122, 139)
Diastolic blood pressure (mmHg)	75 (71, 80)	78 (73, 83)	76 (72, 81)
Mean arterial pressure (mmHg)	95 (88, 102)	99 (93, 107)	98 (92, 105)
Carotid artery distensibility (10-3/kPa)	15.0 (11.8, 18.8)	13.5 (10.4, 16.9)	12.5 (9.9, 16.0)
Carotid-femoral pulse wave velocity (m/s)	8.08 (7.28, 9.16)	8.96 (7.84, 10.32)	9.36 (8.16, 10.80)
Hypertension (Yes)	833 (35%)	317 (59%)	590 (79%)
Glucose-lowering medication (Yes)	0 (0%)	0 (0%)	519 (70%)
Antihypertensive medication (Yes)	431 (18%)	199 (37%)	478 (64%)

	Normal glucose metabolism, N = 2,389	**Prediabetes**, N = 538	**Type 2 Diabetes**, N = 746
Using beta-blockers (Yes)	149 (6.2%)	77 (14%)	195 (26%)
Lipid-lowering medication	280 (12%)	141 (26%)	484 (65%)

[1]n (%); Median (IQR)

Table S4: Association between 24-hour HRV in the original unit and pulse wave velocity

	Model 1	Model 2
HRV index	**PWV % (95% CI)**	**PWV % (95% CI)**
Mean IBI (ms)	-0.02255 (-0.02769; -0.017)	-0.02249 (-0.02771; -0.017)
SDNN (ms)	-0.07141 (-0.08689; -0.056)	-0.07189 (-0.08769; -0.056)
SDANN (ms)	-0.06527 (-0.08119; -0.049)	-0.06548 (-0.08166; -0.049)
SDNNi (ms)	-0.16548 (-0.20319; -0.128)	-0.16403 (-0.20266; -0.125)
RMSSD (ms)	-0.09565 (-0.13802; -0.053)	-0.09239 (-0.13495; -0.05)
pNN50 (%)	-0.13984 (-0.20466; -0.075)	-0.13592 (-0.20105; -0.071)
TP (ms^2)	-0.00035 (-0.00043; 0.000)	-0.00035 (-0.00043; 0000)
HF (ms^2)	-0.00857 (-0.01357; -0.004)	-0.00826 (-0.01326; -0.003)
LF (ms^2)	-0.00445 (-0.00627; -0.003)	-0.00427 (-0.00612; -0.002)
VLF (ms^2)	-0.0034 (-0.00418; -0.003)	-0.00337 (-0.00416; -0.003)
ULF (ms^2)	-0.00035 (-0.00044; 0000)	-0.00035 (-0.00045; 0000)

Percentage CD per original unit increase in heart rate variability index and heart period intervals. Model 1: adjusted for sex, age, educational status, diabetes status, and mean arterial pressure. Model 2: Model 1 + physical activity, smoking behaviour, alcohol use, body mass index, hba1c, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.

Table S5: Association between 24-hour HRV in the original unit and carotid distensibility

	Model 1	Model 2
HRV index	**CD % (95% CI)**	**CD % (95% CI)**
Mean IBI (ms)	0.0409 (0.02745; 0.054)	0.04214 (0.02858; 0.056)
SDNN (ms)	0.08919 (0.04849; 0.130)	0.08917 (0.04786; 0.130)
SDANN (ms)	0.08196 (0.04018; 0.124)	0.0814 (0.0391; 0.124)
SDNNi (ms)	0.18749 (0.08769; 0.287)	0.19231 (0.09109; 0.294)
RMSSD (ms)	0.07784 (-0.02725; 0.183)	0.09135 (-0.01362; 0.196)
pNN50	0.10975 (-0.05103; 0.271)	0.12704 (-0.03355; 0.288)
TP (ms^2)	0.00041 (0.00019; 0.001)	0.00041 (0.00019; 0.001)
HF (ms^2)	0.00415 (-0.00829; 0.017)	0.00523 (-0.00716; 0.018)
LF (ms^2)	0.00575 (0.00088; 0.011)	0.00572 (0.00082; 0.011)
VLF (ms^2)	0.00327 (0.00118; 0.005)	0.00336 (0.00124; 0.005)
ULF (ms^2)	0.00043 (0.00019; 0.001)	0.00043 (0.00019; 0.001)

Percentage CD per original unit increase in heart rate variability index and heart period intervals. Model 1: adjusted for sex, age, educational status, diabetes status, and mean arterial pressure. Model 2: Model 1 + physical activity, smoking behaviour, alcohol use, body mass index, hba1c, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.

Table S6: Association between 24-hour standardized HRV and pulse wave velocity by diabetes status

	PWV % (95% CI)	Interaction p-value
Heart period (ms)		
Normal glucose metabolism	-2.542 (-3.213; -1.866)	ref.
Prediabetes	-1.85 (-3.163; -0.519)	0.35031
Type 2 Diabetes	-2.225 (-3.313; -1.126)	0.62224
SDNN (ms)		
Normal glucose metabolism	-1.745 (-2.406; -1.08)	ref.
Prediabetes	-4.397 (-5.763; -3.012)	0.00065
Type 2 Diabetes	-3.725 (-4.899; -2.537)	0.00402
SDANN (ms)		
Normal glucose metabolism	-1.491 (-2.147; -0.831)	ref.
Prediabetes	-4.012 (-5.361; -2.643)	0.00108
Type 2 Diabetes	-3.291 (-4.47; -2.097)	0.00916
SDNN index (ms)		
Normal glucose metabolism	-1.851 (-2.552; -1.146)	ref.
Prediabetes	-3.914 (-5.264; -2.544)	0.00692
Type 2 Diabetes	-3.272 (-4.43; -2.1)	0.03746
RMSSD (ms)		
Normal glucose metabolism	-0.967 (-1.651; -0.278)	ref.
Prediabetes	-2.011 (-3.269; -0.738)	0.15392
Type 2 Diabetes	-0.995 (-2.042; 0.063)	0.96501
pNN50 (%)		
Normal glucose metabolism	-0.974 (-1.647; -0.297)	ref.
Prediabetes	-1.68 (-2.944; -0.4)	0.33419
Type 2 Diabetes	-1.095 (-2.241; 0.063)	0.85863
Time-domain Z-score		
Normal glucose metabolism	-2.053 (-2.863; -1.236)	ref.
Prediabetes	-4.897 (-6.523; -3.243)	0.00222
Type 2 Diabetes	-3.467 (-4.83; -2.084)	0.08088
Total power (ms²)		
Normal glucose metabolism	-1.57 (-2.206; -0.93)	ref.
Prediabetes	-4.336 (-5.735; -2.916)	0.00046
Type 2 Diabetes	-3.451 (-4.753; -2.132)	0.01121
HF (ms²)		
Normal glucose metabolism	-0.538 (-1.205; 0.133)	ref.
Prediabetes	-1.882 (-3.234; -0.511)	0.08129
Type 2 Diabetes	-1.249 (-2.382; -0.103)	0.29055
LF (ms²)		
Normal glucose metabolism	-0.7 (-1.391; -0.004)	ref.
Prediabetes	-3.425 (-4.795; -2.035)	4e-04
Type 2 Diabetes	-2.144 (-3.415; -0.855)	0.04795
VLF (ms²)		
Normal glucose metabolism	-1.946 (-2.605; -1.282)	ref.
Prediabetes	-3.736 (-5.092; -2.36)	0.01869
Type 2 Diabetes	-3.243 (-4.537; -1.931)	0.07796

	PWV % (95% CI)	Interaction p-value
ULF (ms²)		
Normal glucose metabolism	-1.442 (-2.072; -0.807)	ref.
Prediabetes	-3.975 (-5.364; -2.566)	0.00125
Type 2 Diabetes	-3.273 (-4.582; -1.946)	0.01403
Frequency-domain Z-score		
Normal glucose metabolism	-1.968 (-2.778; -1.153)	ref.
Prediabetes	-5.652 (-7.367; -3.904)	0.00015
Type 2 Diabetes	-3.871 (-5.386; -2.333)	0.02979

Percentage PWV per SD increase in heart rate variability index and heart period intervals Adjustment from model 2 including age, sex, education, mean arterial pressure, physical activity, smoking, alcohol, body mass index, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.

Table S7: Association between 24-hour standardized HRV and carotid distensibility by diabetes status

	CD % (95% CI)	Interaction p-value
Heart period (ms)		
Normal glucose metabolism	3.755 (1.818; 5.729)	ref.
Prediabetes	5.513 (2.119; 9.021)	0.37357
Type 2 Diabetes	5.663 (2.848; 8.555)	0.26882
SDNN (ms)		
Normal glucose metabolism	1.223 (-0.643; 3.124)	ref.
Prediabetes	7.175 (3.577; 10.898)	0.00349
Type 2 Diabetes	5.358 (2.347; 8.458)	0.0214
SDANN (ms)		
Normal glucose metabolism	1.16 (-0.691; 3.044)	ref.
Prediabetes	5.728 (2.293; 9.277)	0.02074
Type 2 Diabetes	4.76 (1.749; 7.86)	0.04525
SDNN index (ms)		
Normal glucose metabolism	1.338 (-0.632; 3.348)	ref.
Prediabetes	6.916 (3.28; 10.681)	0.00651
Type 2 Diabetes	3.944 (0.981; 6.993)	0.14548
RMSSD (ms)		
Normal glucose metabolism	0.311 (-1.461; 2.115)	ref.
Prediabetes	3.05 (0.15; 6.034)	0.11407
Type 2 Diabetes	1.386 (-1.187; 4.026)	0.50357
pNN50 (%)		
Normal glucose metabolism	0.15 (-1.589; 1.919)	ref.
Prediabetes	2.933 (0.015; 5.936)	0.10757
Type 2 Diabetes	1.64 (-1.131; 4.489)	0.37422
Time-domain Z-score		
Normal glucose metabolism	1.223 (-1.033; 3.53)	ref.
Prediabetes	8.049 (3.778; 12.495)	0.00486
Type 2 Diabetes	4.808 (1.316; 8.421)	0.09046
Total power (ms^2)		
Normal glucose metabolism	1.087 (-0.708; 2.915)	ref.
Prediabetes	6.053 (2.429; 9.807)	0.015
Type 2 Diabetes	5.35 (2.026; 8.782)	0.0261
HF (ms^2)		
Normal glucose metabolism	-0.517 (-2.228; 1.223)	ref.
Prediabetes	3.06 (-0.032; 6.247)	0.04629
Type 2 Diabetes	1.397 (-1.375; 4.247)	0.25123
LF (ms^2)		
Normal glucose metabolism	0.892 (-1.027; 2.849)	ref.
Prediabetes	4.128 (0.516; 7.869)	0.11249
Type 2 Diabetes	2.689 (-0.613; 6.101)	0.34982
VLF (ms^2)		
Normal glucose metabolism	1.258 (-0.641; 3.193)	ref.
Prediabetes	5.66 (2.01; 9.442)	0.03227
Type 2 Diabetes	3.494 (0.206; 6.89)	0.24368

ULF (ms²)		
Normal glucose metabolism	0.996 (-0.778; 2.801)	ref.
Prediabetes	5.535 (1.98; 9.213)	0.0241
Type 2 Diabetes	5.373 (2.027; 8.828)	0.02287
Frequency-domain Z-score		
Normal glucose metabolism	1.186 (-1.098; 3.523)	ref.
Prediabetes	8.277 (3.61; 13.154)	0.0063
Type 2 Diabetes	5.313 (1.374; 9.405)	0.07353

Percentage CD per SD increase in heart rate variability index and heart period intervals Adjustment from model 2 including age, sex, education, mean arterial pressure, physical activity, smoking, alcohol, body mass index, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.

Table S8: Association between 24-hour standardized HRV and pulse wave velocity by sex

	PWV % (95% CI)	Interaction p-value
Heart period (ms)		
Men	-1.853 (-2.538; -1.162)	ref.
Women	-3.159 (-3.997; -2.314)	0.01635
SDNN (ms)		
Men	-2.377 (-3.109; -1.639)	ref.
Women	-2.646 (-3.398; -1.889)	0.60266
SDANN (ms)		
Men	-2.095 (-2.829; -1.355)	ref.
Women	-2.312 (-3.055; -1.562)	0.67666
SDNN index (ms)		
Men	-2.041 (-2.769; -1.306)	ref.
Women	-3.012 (-3.823; -2.194)	0.06509
RMSSD (ms)		
Men	-1.012 (-1.711; -0.308)	ref.
Women	-1.316 (-2.093; -0.534)	0.56598
pNN50 (%)		
Men	-1.059 (-1.797; -0.316)	ref.
Women	-1.18 (-1.924; -0.43)	0.81918
Time-domain Z-score		
Men	-2.501 (-3.372; -1.622)	ref.
Women	-3.062 (-3.98; -2.134)	0.37062
Total power (ms²)		
Men	-2.044 (-2.777; -1.305)	ref.
Women	-2.432 (-3.18; -1.678)	0.45651
HF (ms²)		
Men	-0.717 (-1.476; 0.048)	ref.
Women	-1.049 (-1.782; -0.312)	0.532
LF (ms²)		
Men	-0.941 (-1.637; -0.24)	ref.
Women	-2.126 (-3.034; -1.209)	0.03287
VLF (ms²)		
Men	-2.04 (-2.729; -1.345)	ref.
Women	-3.027 (-3.884; -2.164)	0.06757
ULF (ms²)		
Men	-1.903 (-2.639; -1.162)	ref.
Women	-2.232 (-2.971; -1.486)	0.52879
Frequency-domain Z-score		
Men	-2.407 (-3.293; -1.512)	ref.
Women	-3.266 (-4.226; -2.297)	0.17941

Percentage PWV per SD increase in heart rate variability index and heart period intervals. Adjustment from model 2 including age, education, diabetes status, mean arterial pressure, physical activity, smoking, alcohol, body mass index, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.

Table S9: Association between 24-hour standardized HRV and carotid distensibility by sex

	CD % (95% CI)	Interaction p-value
Heart period (ms)		
Men	5.87 (3.977; 7.797)	ref.
Women	2.489 (0.177; 4.853)	0.02581
SDNN (ms)		
Men	4.006 (2.021; 6.03)	ref.
Women	2.23 (0.136; 4.368)	0.21613
SDANN (ms)		
Men	3.228 (1.262; 5.232)	ref.
Women	2.293 (0.227; 4.402)	0.51292
SDNN index (ms)		
Men	4.253 (2.26; 6.285)	ref.
Women	1.113 (-1.13; 3.407)	0.03247
RMSSD (ms)		
Men	2.788 (1.024; 4.582)	ref.
Women	-0.922 (-2.846; 1.04)	0.00546
pNN50 (%)		
Men	2.707 (0.848; 4.6)	ref.
Women	-0.65 (-2.496; 1.232)	0.01171
Time-domain Z-score		
Men	5.021 (2.662; 7.436)	ref.
Women	1.141 (-1.365; 3.711)	0.02393
Total power (ms²)		
Men	3.186 (1.209; 5.2)	ref.
Women	2.147 (0.045; 4.292)	0.47122
HF (ms²)		
Men	2.407 (0.523; 4.325)	ref.
Women	-1.254 (-3.089; 0.616)	0.00618
LF (ms²)		
Men	2.606 (0.698; 4.55)	ref.
Women	0.298 (-2.171; 2.83)	0.13317
VLF (ms²)		
Men	3.274 (1.366; 5.218)	ref.
Women	0.974 (-1.466; 3.473)	0.13568
ULF (ms²)		
Men	2.881 (0.907; 4.893)	ref.
Women	2.198 (0.134; 4.305)	0.6343
Frequency-domain Z-score		
Men	4.491 (2.058; 6.981)	ref.
Women	1.389 (-1.31; 4.162)	0.0849

Percentage CD per SD increase in heart rate variability index and heart period intervals. *Adjustment from model 2 including age, education, diabetes status, mean arterial pressure, physical activity, smoking, alcohol, body mass index, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.*

Table S10: Sensitivity analysis: Association between 24-hour HRV and pulse wave velocity

Sub-group	Population size	PWV %	5%	95%
Heart period (ms)				
Main	3673	-0.0225	-0.0277	-0.0173
No beta-blocker medication	3252	-0.0208	-0.0265	-0.0151
No antihypertension medication	2565	-0.0189	-0.0250	-0.0127
No diabetes and antihypertension medication	1958	-0.0199	-0.0266	-0.0133
SDNN (ms)				
Main	3673	-0.0719	-0.0877	-0.0561
No beta-blocker medication	3252	-0.0701	-0.0864	-0.0537
No antihypertension medication	2565	-0.0642	-0.0820	-0.0465
No diabetes and antihypertension medication	1958	-0.0509	-0.0700	-0.0319
SDANN (ms)				
Main	3673	-0.0655	-0.0816	-0.0493
No beta-blocker medication	3252	-0.0641	-0.0808	-0.0474
No antihypertension medication	2565	-0.0586	-0.0767	-0.0404
No diabetes and antihypertension medication	1958	-0.0443	-0.0638	-0.0248
SDNN index (ms)				
Main	3673	-0.1639	-0.2026	-0.1253
No beta-blocker medication	3252	-0.1657	-0.2063	-0.1250
No antihypertension medication	2565	-0.1476	-0.1914	-0.1038
No diabetes and antihypertension medication	1958	-0.1311	-0.1786	-0.0836
RMSSD (ms)				
Main	3673	-0.0923	-0.1349	-0.0498
No beta-blocker medication	3252	-0.1111	-0.1564	-0.0657
No antihypertension medication	2565	-0.1045	-0.1546	-0.0542
No diabetes and antihypertension medication	1958	-0.0932	-0.1486	-0.0377

pNN50 (%)				
Main	3673	-0.1359	-0.2010	-0.0707
No beta-blocker medication	3252	-0.1546	-0.2233	-0.0859
No antihypertension medication	2565	-0.1520	-0.2264	-0.0776
No diabetes and antihypertension medication	1958	-0.1510	-0.2330	-0.0689
Total power (ms²)				
Main	3673	-0.0003	-0.0004	-0.0003
No beta-blocker medication	3252	-0.0003	-0.0004	-0.0003
No antihypertension medication	2565	-0.0003	-0.0004	-0.0002
No diabetes and antihypertension medication	1958	-0.0002	-0.0003	-0.0002
HF (ms²)				
Main	3673	-0.0083	-0.0133	-0.0033
No beta-blocker medication	3252	-0.0096	-0.0148	-0.0043
No antihypertension medication	2565	-0.0088	-0.0145	-0.0032
No diabetes and antihypertension medication	1958	-0.0066	-0.0128	-0.0004
LF (ms²)				
Main	3673	-0.0043	-0.0061	-0.0024
No beta-blocker medication	3252	-0.0046	-0.0065	-0.0026
No antihypertension medication	2565	-0.0040	-0.0061	-0.0020
No diabetes and antihypertension medication	1958	-0.0033	-0.0054	-0.0011
VLF (ms²)				
Main	3673	-0.0034	-0.0042	-0.0026
No beta-blocker medication	3252	-0.0033	-0.0042	-0.0025
No antihypertension medication	2565	-0.0031	-0.0039	-0.0022
No diabetes and antihypertension medication	1958	-0.0029	-0.0038	-0.0020
ULF (ms²)				
Main	3673	-0.0004	-0.0004	-0.0003
No beta-blocker medication	3252	-0.0003	-0.0004	-0.0003
No antihypertension medication	2565	-0.0003	-0.0004	-0.0002

No diabetes and antihypertension medication	1958	-0.0002	-0.0004	-0.0001

Percentage PWV per original unit increase in heart rate variability index and heart period intervals

Main: Model 2 (adjusted for antihypertensive medication)

No beta-blocker medication: people using betablockers were excluded

No antihypertensive medication: people with antihypertensive medication was excluded

No antihypertensive medication and without diabetes: people with antihypertensive medication and diabetes was excluded

Table S11: Sensitivity analysis: Association between 24-hour HRV (in original unit) and carotid distensibility

Sub-group	Population size	CD %	5%	95%
Heart period (ms)				
Main	1802	0.0420	0.0284	0.0555
No beta-blocker medication	1563	0.0402	0.0252	0.0553
No antihypertension medication	1201	0.0366	0.0201	0.0530
No diabetes and antihypertension medication	846	0.0289	0.0092	0.0485
SDNN (ms)				
Main	1802	0.0887	0.0474	0.1300
No beta-blocker medication	1563	0.0968	0.0529	0.1407
No antihypertension medication	1201	0.0874	0.0388	0.1361
No diabetes and antihypertension medication	846	0.0499	-0.0077	0.1076
SDANN (ms)				
Main	1802	0.0809	0.0387	0.1232
No beta-blocker medication	1563	0.0913	0.0465	0.1361
No antihypertension medication	1201	0.0852	0.0353	0.1352
No diabetes and antihypertension medication	846	0.0572	-0.0017	0.1161
SDNN index (ms)				
Main	1802	0.1902	0.0890	0.2915
No beta-blocker medication	1563	0.1772	0.0670	0.2876
No antihypertension medication	1201	0.1515	0.0317	0.2714
No diabetes and antihypertension medication	846	0.0695	-0.0728	0.2120
RMSSD (ms)				
Main	1802	0.0915	-0.0134	0.1965
No beta-blocker medication	1563	0.0712	-0.0444	0.1869
No antihypertension medication	1201	0.1012	-0.0289	0.2315
No diabetes and antihypertension medication	846	0.0436	-0.1123	0.1998
pNN50 (%)				
Main	1802	0.1274	-0.0330	0.2881
No beta-blocker medication	1563	0.1098	-0.0630	0.2829
No antihypertension medication	1201	0.1362	-0.0555	0.3283
No diabetes and antihypertension medication	846	0.0560	-0.1743	0.2868
Total power (ms^2)				
Main	1802	0.0004	0.0002	0.0006
No beta-blocker medication	1563	0.0004	0.0002	0.0007
No antihypertension medication	1201	0.0004	0.0002	0.0007
No diabetes and antihypertension medication	846	0.0003	0.0000	0.0006
HF (ms^2)				
Main	1802	0.0053	-0.0070	0.0177
No beta-blocker medication	1563	0.0045	-0.0087	0.0177
No antihypertension medication	1201	0.0099	-0.0047	0.0246
No diabetes and antihypertension medication	846	0.0038	-0.0138	0.0214

LF (ms²)				
Main	1802	0.0056	0.0007	0.0105
No beta-blocker medication	1563	0.0047	-0.0006	0.0100
No antihypertension medication	1201	0.0043	-0.0013	0.0099
No diabetes and antihypertension medication	846	0.0017	-0.0048	0.0081
VLF (ms²)				
Main	1802	0.0033	0.0012	0.0054
No beta-blocker medication	1563	0.0031	0.0008	0.0054
No antihypertension medication	1201	0.0026	0.0002	0.0050
No diabetes and antihypertension medication	846	0.0010	-0.0018	0.0038
ULF (ms²)				
Main	1802	0.0004	0.0002	0.0007
No beta-blocker medication	1563	0.0005	0.0002	0.0007
No antihypertension medication	1201	0.0004	0.0002	0.0007
No diabetes and antihypertension medication	846	0.0003	0.0000	0.0006

Percentage CD per original unit increase in heart rate variability index and mean heart period intervals

Main: model 2 (adjusted for antihypertensive medication)

No beta-blocker medication: people using betablockers were excluded

No antihypertensive medication: people with antihypertensive medication were excluded

No antihypertensive medication and without diabetes: people with antihypertensive medication and diabetes were excluded

Table S12: Association between 24-hour standardized HRV and pulse wave velocity

	Model 1	Model 2
HRV index	**PWV % (95% CI)**	**PWV % (95% CI)**
Mean IBI (ms)	-2.373 (-2.906; -1.838)	-2.366 (-2.908; -1.822)
SDNN (ms)	-2.492 (-3.024; -1.957)	-2.508 (-3.051; -1.962)
SDANN (ms)	-2.195 (-2.724; -1.664)	-2.202 (-2.739; -1.662)
SDNNi (ms)	-2.487 (-3.045; -1.925)	-2.465 (-3.037; -1.890)
RMSSD (ms)	-1.189 (-1.711; -0.664)	-1.148 (-1.673; -0.621)
pNN50	-1.151 (-1.681; -0.619)	-1.119 (-1.652; -0.584)
Time-domain Z-score	-2.787 (-3.431; -2.139)	-2.766 (-3.42; -2.106)
TP (ms^2)	-2.235 (-2.766; -1.701)	-2.234 (-2.773; -1.692)
HF (ms^2)	-0.922 (-1.456; -0.385)	-0.888 (-1.423; -0.35)
LF (ms^2)	-1.412 (-1.984; -0.836)	-1.357 (-1.938; -0.773)
VLF (ms^2)	-2.442 (-2.990; -1.890)	-2.416 (-2.975; -1.854)
ULF (ms^2)	-2.069 (-2.596; -1.539)	-2.067 (-2.601; -1.529)
Frequency-domain Z-score	-2.819 (-3.487; -2.146)	-2.798 (-3.477; -2.113)

Percentage PWV per SD increase in heart rate variability index and heart period intervals Model 1: adjusted for sex, age, educational status, diabetes status, and mean arterial pressure. Model 2: Model 1 + physical activity, smoking behaviour, alcohol use, body mass index, hba1c, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.

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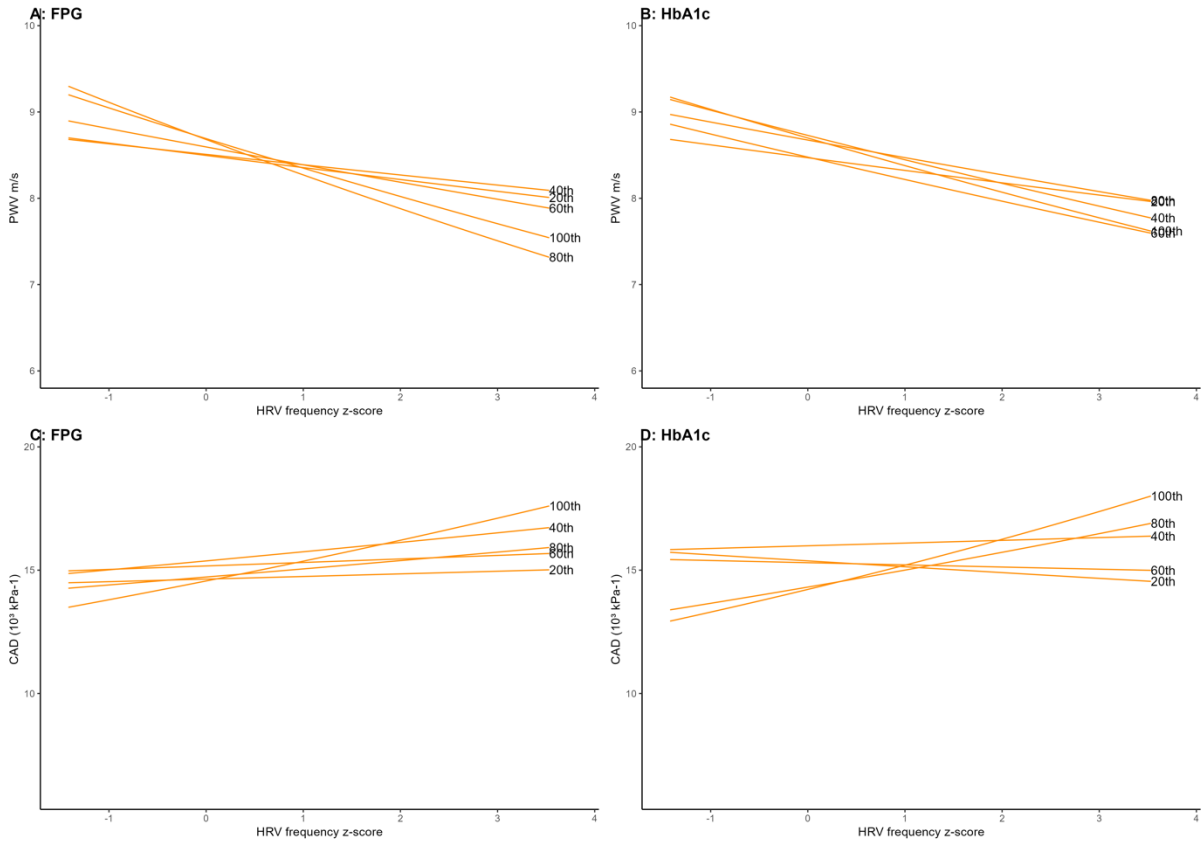
Table S13: Association between 24-hour standardized HRV and carotid distensibility

	Model 1	Model 2
HRV index	**CD % (95% CI)**	**CD % (95% CI)**
Mean IBI (ms)	4.45 (2.966; 5.956)	4.588 (3.09; 6.107)
SDNN (ms)	3.199 (1.727; 4.692)	3.198 (1.705; 4.714)
SDANN (ms)	2.824 (1.375; 4.294)	2.805 (1.338; 4.293)
SDNNi (ms)	2.889 (1.342; 4.46)	2.964 (1.394; 4.559)
RMSSD (ms)	0.977 (-0.34; 2.312)	1.148 (-0.17; 2.483)
pNN50	0.912 (-0.422; 2.263)	1.056 (-0.277; 2.407)
Time-domain Z-score	3.162 (1.397; 4.959)	3.284 (1.500; 5.100)
TP (ms^2)	2.696 (1.226; 4.187)	2.724 (1.237; 4.232)
HF (ms^2)	0.449 (-0.892; 1.809)	0.567 (-0.77; 1.922)
LF (ms^2)	1.857 (0.282; 3.457)	1.847 (0.263; 3.455)
VLF (ms^2)	2.405 (0.858; 3.976)	2.471 (0.908; 4.058)
ULF (ms^2)	2.564 (1.112; 4.037)	2.574 (1.108; 4.061)
Frequency-domain Z-score	3.098 (1.229; 5.001)	3.184 (1.295; 5.109)

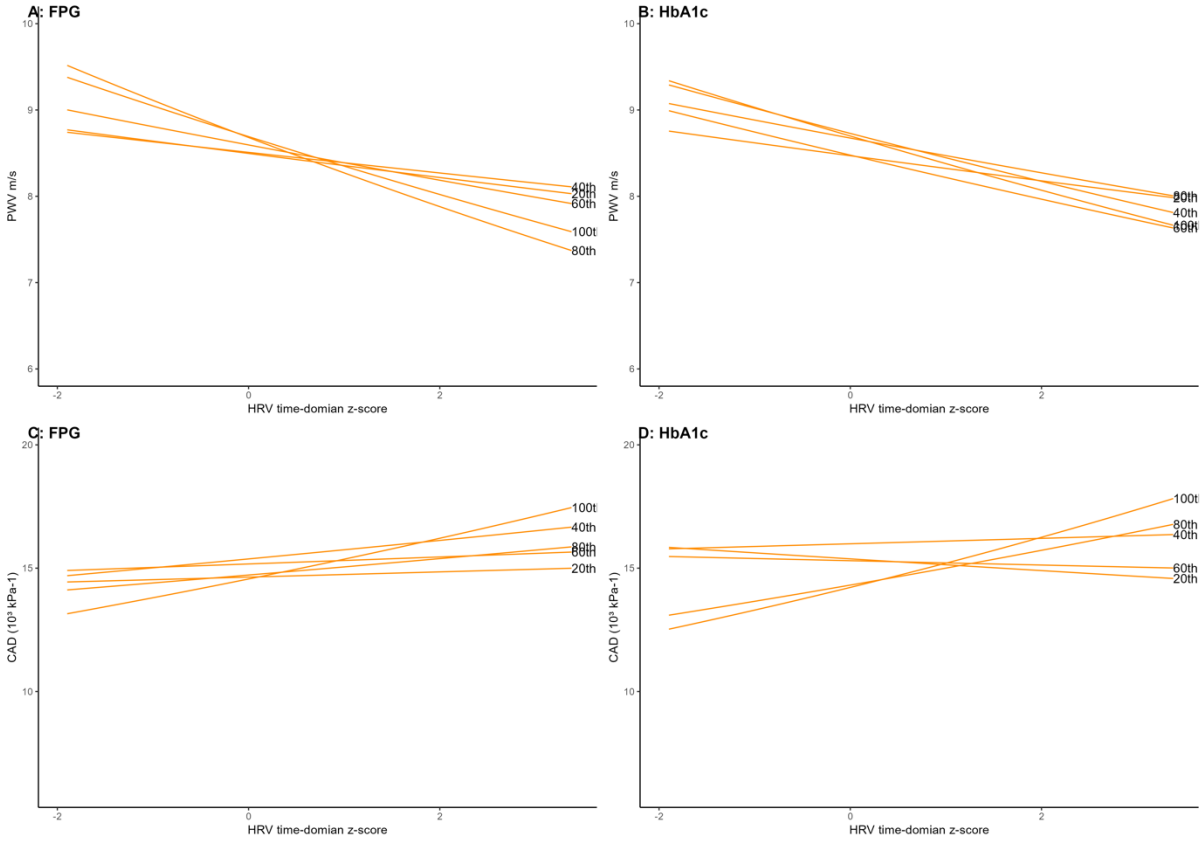
Percentage CD per SD increase in heart rate variability index and heart period intervals. Model 1: adjusted for sex, age, educational status, diabetes status, and mean arterial pressure. Model 2: Model 1 + physical activity, smoking behaviour, alcohol use, body mass index, hba1c, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication.

Figure S1: Association between 24-hour HRV frequency-domain Z-score and aortic (n= 3154) and carotid (n= 1653) stiffness stratified by glucose percentiles in a subpopulation without known type 2 diabetes



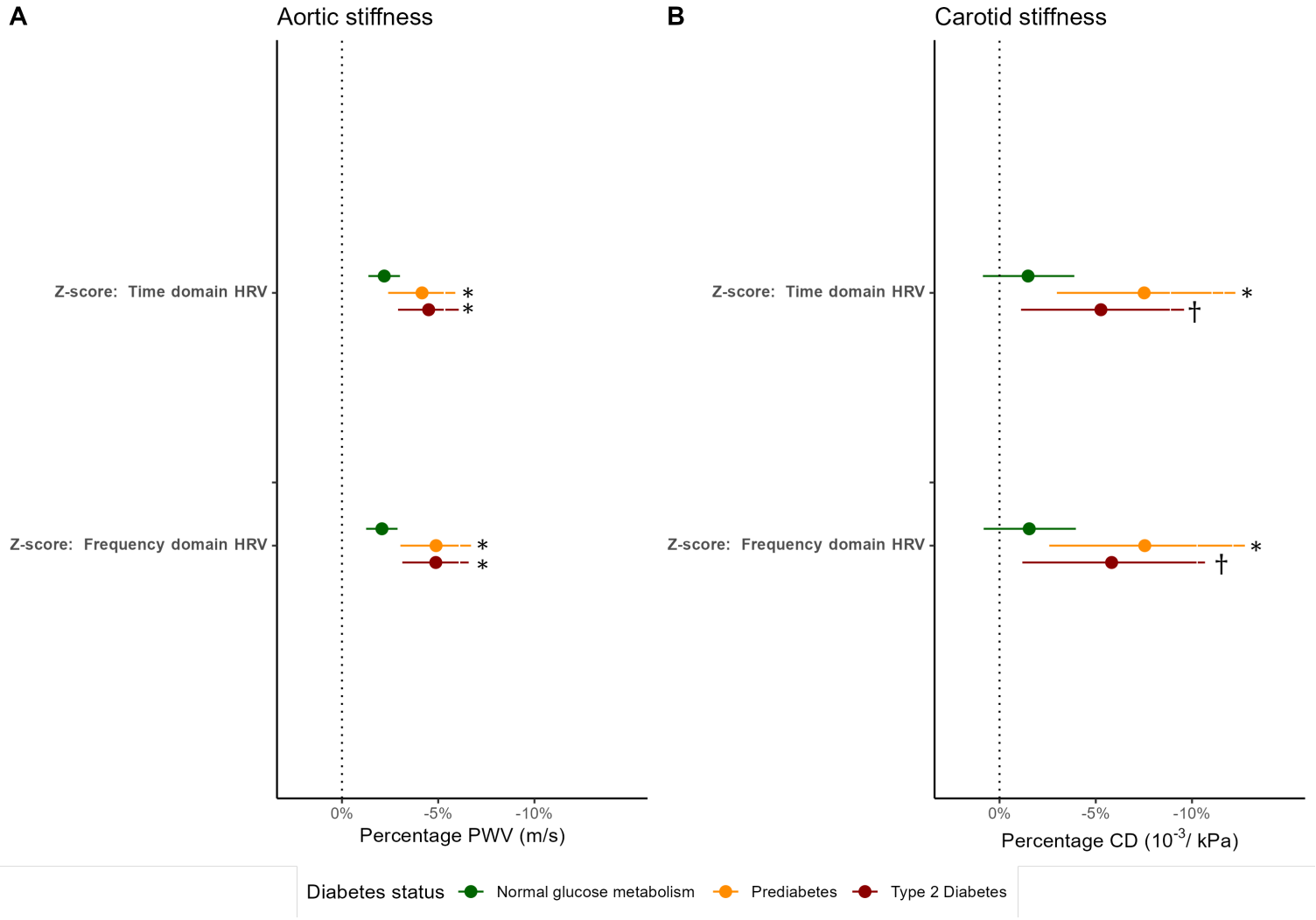
Adjusted for age, sex, and mean arterial pressure

Figure S2: Association between 24-hour time-domain Z-score and aortic (n= 3154 and carotid (n= 1653) stiffness stratified by glucose percentiles in a subpopulation without known type 2 diabetes



Adjusted for age, sex, and mean arterial pressure

Figure S3: Association between 24-hour standardized HRV and arterial stiffness modified by diabetes status without users of beta-blockers



A: *Percentage PWV per SD in time-domain and frequency-domain composite z-score by diabetes status* ***B:*** *Percentage CD per SD in time-domain and frequency-domain composite z-score by diabetes status. Estimates are adjusted for sex, age, educational status, mean arterial pressure, physical activity, smoking behaviour, alcohol use, body mass index, HbA1c, triglycerides, total-to-high density lipoprotein cholesterol ratio, lipid-modifying- and antihypertensive medication. Normal glucose metabolism was defined as reference group.*

**Interaction term p-value < 0.05*

†Interaction term p-value < 0.10