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The role of insulin resistance in the association between body fat and autonomic function

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ABSTRACT

Background and Aim
Excess body fat is associated with altered autonomic function. We investigated whether this association is mediated by insulin resistance.

Methods and Results
Cross-sectional analysis of a subgroup of the Netherlands Epidemiology of Obesity study with measurements of autonomic function (heart rate variability calculated as mean interbeat interval, standard deviation of all normal intervals (SDNN), low frequency (LF) power and high frequency (HF) power). We measured BMI (kg/m²), total body fat (%) and waist circumference (cm), and calculated the HOMA-index of insulin resistance (HOMA-IR). We examined the association between body fat and heart rate variability with multivariate linear regression analysis. To investigate whether the association was mediated by insulin resistance, we additionally adjusted for HOMA-IR.

After exclusion of participants with glucose lowering medication (n=19), 466 participants were included. Per SD of BMI, the difference in SDNN was −2.7% (95% CI: −5.5, 0.1) in the multivariate model. Additional adjustment for HOMA-IR attenuated this association to −1.2% (95% CI: −4.2, 1.7), suggesting that 55% of the association between BMI and SDNN was mediated by HOMA-IR. All measures of body fat were associated with mean interbeat interval, SDNN and LF power. Depending on the parameter of body fat or heart rate variability, 29-81% of the association was mediated by HOMA-IR.

Conclusion
In this cross-sectional study, body fat was associated with heart rate variability. This association may at least partially be mediated by insulin resistance. Future studies should investigate whether a reduction in obesity and insulin resistance may prevent the adverse cardiovascular consequences of altered autonomic function.
INTRODUCTION

Excess body fat is associated with altered function of the autonomic nervous system and sympathetic activation.\(^1,2\) Altered autonomic function is an independent risk factor for cardiovascular events in populations with prevalent cardiovascular disease,\(^3,4\) but also for a first event in the general population.\(^5\) The mechanism underlying the association between body fat and altered autonomic function is not elucidated. We hypothesize that insulin resistance may be the underlying factor (Figure 1).

Obesity, especially abdominal obesity, is a risk factor for insulin resistance.\(^6,7\) Insulin resistance has also been associated with altered autonomic function in several cross-sectional studies.\(^8-12\) Based on the results of these cross-sectional results, it remained unclear whether insulin resistance alters autonomic function, or vice versa. We however recently showed that autonomic function was not prospectively associated with the incidence of type 2 diabetes mellitus during a median follow up of 9.2 years.\(^13\) This suggests that the autonomic nervous system may not be involved in the development of insulin resistance, and supports a pathophysiological mechanism in which insulin resistance is the first abnormality in obesity, resulting in sympathetic activation. This mechanism is further supported by the physiological reactions of the autonomic nervous system to infusions of blood glucose and insulin in humans.\(^14,15\)

Therefore, our aim was to study the role of insulin resistance as a mechanism underlying the association between body fat and autonomic function (Figure 1). To that extent, we investigated the association between measures of body fat and parameters of autonomic function and whether this association was mediated by insulin resistance.

METHODS

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study comprising 6673 persons with an oversampling of individuals with a BMI ≥ 27 kg/m\(^2\). The study design and data collection of the NEO study have been described previously.\(^16\) In short, persons aged between 45 and 65 years with a self-reported BMI of 27 kg/m\(^2\) or higher were included between September 2008 and October 2012. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI.
Chapter 4

The baseline visit was performed in the NEO study centre in the Leiden University Medical Centre, during which an extensive physical examination was performed. The present study is a cross-sectional analysis of baseline measurements of the participants with a measurement of heart rate variability. We additionally excluded participants who were using oral glucose lowering medication or insulin. The NEO study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and all participants gave their informed consent.

**Data collection**

Participants reported their highest level of education in ten categories according to the Dutch educational system. We grouped these data into low education (defined as no education, primary education or lower vocational training) versus high education (used as the reference category). Tobacco smoking was categorized into current, former or never (reference) smokers. Self-reported pre-existing cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. Participants reported the frequency and duration of their physical activity using the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH) questionnaire. We calculated the total energy expended during physical activity in hours per week of metabolic equivalents (MET-h/week).

**Body fat**

We measured height and weight without shoes and one kilogram was subtracted to correct for the weight of clothing. Body Mass Index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. Total body fat was measured using a bio-impedance device (TBF-310, Tanita International Division, United Kingdom). We measured the circumference of the waist with a flexible tape in the middle of the distance between the lowest rib and the crista iliaca and used this as a measure of abdominal fat mass.

**Insulin resistance**

Venous blood was sampled after an overnight fast for at least 10 hours. Plasma glucose concentrations were measured with the enzymatic and colorimetric method (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany) and serum insulin concentrations were determined by an immunometric method (Siemens Immulite 2500, Siemens Healthcare Diagnostics, Breda, the Netherlands). We calculated the updated Homeostasis Model Assessment Insulin Resistance (HOMA-IR), a measure of insulin resistance which corresponds well to estimates of insulin resistance derived from the hyperinsulinemic euglycemic clamp, by entering fasting glucose and fasting insulin in a Microsoft Excel spreadsheet available on the internet.\(^7\)
Heart rate variability
Ten Actiheart devices, accelerometers combined with heart rate monitors, were randomly distributed to NEO participants. We used the data from the heart rate monitor to calculate heart rate variability (HRV) parameters as indices of autonomic function. We selected only the Actiheart recordings with the least noise (<1%) for manual reparation and a valid estimation of heart rate variability. Two standard ECG electrodes (H98SG, Tyco Healthcare, Germany) were placed at the level of the second intercostal space, one electrode on the sternum and one ten centimetres to the left of the first electrode. The Actiheart was set up for a recording of four days. During this period, all interbeat intervals (IBIs) were stored in milliseconds. Participants were asked to perform their normal daily activities. For each participant with an Actiheart measurement, we excluded the first 12 hours of the recording because participants were not performing their normal activities during that period due to their visit to the NEO study centre. Then, we determined whether the quality of the IBI data was sufficient for heart rate variability analysis, by the detection of artefacts and non-sinus beats in the data. We calculated the percentage noise by dividing the number of artefacts and non-sinus beats by the total number of IBIs for each 24-h period using a moving average with a 1-h window. We selected the 24-h period with the least artefacts and non-sinus beats for reparation and subsequent HRV analysis, with a maximum of 1% artefacts/non-sinus beats. The raw IBI data were cleaned by removing artefacts and non-sinus beats using a semi-automatic Matlab program (designed by ACM). An overview of this procedure is shown in Figure 2. The Actiheart stores timings of all QRS complexes. Based on these timings, three types of non-sinus beats could be detected: missed QRS complexes, extra QRS complexes and QRS complexes that were detected too early (premature complexes) or too late (post-mature complexes). We repaired non-sinus beats if they either added up to or could be divided in interpolated intervals that differed <5% from the mean of the ten preceding intervals. Artefacts and non-sinus beats that could not be repaired by the algorithm were deselected from the recordings by hand.

Heart rate variability parameters were calculated from the repaired IBI data according to a procedure that was described and applied previously.\textsuperscript{18,19} In short, HRV was calculated using a 5-min moving window and all valid 5-min values were averaged. We calculated the mean interbeat interval and standard deviation of all normal intervals (SDNN). Prior to spectral analysis of the recordings, the tachogram was processed with adjustment for linear trends, tachogram tapering and zero padding. A test of stationarity was performed, and we excluded non-stationary episodes from the analysis. For calculation of low frequency power (LF power) and high frequency power (HF power), a fast Fourier transformation was employed.
Statistical analysis

In the NEO study individuals with a BMI of 27 kg/m² or higher were oversampled. To correctly represent associations in the general population, adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m² were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population. All results were based on weighted analyses.

Baseline characteristics were calculated as mean (standard deviation, SD), median (25th-75th percentile) or as percentage and were stratified for sex-specific tertiles of BMI. Differences in baseline characteristics between NEO participants with and without measurement of heart rate variability were tested with Student t-test for continuous variables and Chi-square for categorical variables. The HOMA-IR and heart rate variability parameters were ln-transformed. Serum insulin concentrations below the detection limit of the assay (2.0 mU/L) were imputed using multiple imputation methods for left censored data, with ten imputation datasets. We standardized the measures of body fat using sex-specific standard deviations. We used linear regression analysis to calculate regression coefficients of the associations between measures of body fat and HRV parameters (the total association, A+B in Figure 1). We adjusted for age, sex, tobacco smoking, ethnicity, education, physical activity, prevalent cardiovascular disease and cardiac medication. Regression coefficients were expressed as percentage difference in heart rate variability with 95% confidence intervals per weighted standard deviation of measures of body fat.

As a last step in our analyses, we included HOMA-IR into our model to investigate whether the total association between body fat and heart rate variability was mediated by insulin resistance (the indirect association, A in Figure 1). To that end, we calculated what percentage of the total association between body fat and heart rate variability was

![Figure 1](image-url)
mediated by HOMA-IR. The percentage mediated association was calculated as \((1 - (\text{direct association} / \text{total association}) \times 100)\). The remaining coefficient after adjustment for HOMA-IR represents the direct association between body fat and heart rate variability, B in Figure 1.

Before adding HOMA-IR as mediator to the model, we tested for interaction between determinant and mediator by adding an interaction term of body fat and HOMA-IR to our linear regression model.

**RESULTS**

**Baseline characteristics**

From the 6673 participants included in the NEO study, an Actiheart device was carried by 955 participants to estimate physical activity. In 50 of this 955, the recording failed due to technical problems (e.g. broken battery, detached electrodes) or incorrect use by the participant (e.g. early removal). From the 905 remaining recordings, we selected 485 recordings with maximum quality of IBI data (<1% artefacts) to use for heart rate variability analysis. After exclusion of participants with oral glucose lowering medication or insulin (n=19), the present analysis included 466 participants. Participants included in our study were more often men (56%) than excluded NEO participants (43%).

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**Figure 2.** Data processing of interbeat intervals for HRV analysis
baseline characteristics did not differ between included and excluded participants (mean age was 56 (SD: 6) years in both groups, mean BMI was 27 (4) kg/m² in included participants and 26 (4) kg/m² in excluded participants, median HOMA-IR was 0.8 (25th-75th percentile: 0.4-1.2) in included and 0.6 (0.4-1.1) in excluded participants). Table 1 shows the baseline characteristics of the study population stratified by sex-specific tertiles of BMI.

Table 1: Baseline characteristics of NEO study participants with data on autonomic function, stratified by sex-specific tertiles of BMI (n=466)

<table>
<thead>
<tr>
<th>Variable</th>
<th>BMI cut-off (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% men)</td>
<td>57</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 (6)</td>
</tr>
<tr>
<td>Smoking</td>
<td>56</td>
</tr>
<tr>
<td>- Former (%)</td>
<td>15</td>
</tr>
<tr>
<td>- Current (%)</td>
<td></td>
</tr>
<tr>
<td>Education (% low)</td>
<td>16</td>
</tr>
<tr>
<td>Physical activity (MET-hours/week)</td>
<td>121 (73-166)</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease (%)</td>
<td>9</td>
</tr>
<tr>
<td>Use of antihypertensive medication (%)</td>
<td>19</td>
</tr>
<tr>
<td>Use of lipid lowering medication (%)</td>
<td>9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 (2)</td>
</tr>
<tr>
<td>- Men</td>
<td>25 (2)</td>
</tr>
<tr>
<td>- Women</td>
<td>25 (2)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93 (8)</td>
</tr>
<tr>
<td>- Men</td>
<td>85 (9)</td>
</tr>
<tr>
<td>- Women</td>
<td>85 (9)</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>23 (4)</td>
</tr>
<tr>
<td>- Men</td>
<td>37 (5)</td>
</tr>
<tr>
<td>- Women</td>
<td>37 (5)</td>
</tr>
<tr>
<td>Mean IBI (ms)</td>
<td>844 (788-901)</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>48 (41-56)</td>
</tr>
<tr>
<td>LF power (ms²)</td>
<td>928 (655-1300)</td>
</tr>
<tr>
<td>HF power (ms²)</td>
<td>218 (142-323)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.5 (0.3-1.1)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD), median (25th-75th percentile) or percentage. Results were based on weighted analyses.

NEO, Netherlands Epidemiology of Obesity; BMI, Body mass index; MET, metabolic equivalent of task; IBI, interbeat interval; SDNN, standard deviation of all normal intervals; LF, low frequency; HF, high frequency; HOMA-IR, HOMA-index of insulin resistance
Body mass index, insulin resistance and heart rate variability

The associations of BMI (per 4 kg/m² for men and 5 kg/m² for women) and parameters of heart rate variability are shown in Table 2. BMI was associated with mean IBI and LF power, with and without adjustment for confounding variables. There was also an association with SDNN, although not significant. We found no association between BMI and HF power. After additional adjustment for HOMA-IR as mediating factor, the association between BMI and parameters of heart rate variability attenuated. For mean IBI, 40% of the association was mediated by HOMA-IR, for SDNN 55% and for LF power 37% of the total association was mediated by HOMA-IR.

An interaction term of BMI and HOMA-IR was added to all models to test for interaction. For mean IBI the regression coefficient was 0.00025 (95% CI: −0.00243, 0.00292, P-value 0.856), for SDNN the coefficient was −0.00014 (95% CI: −0.00775, 0.00749, P-value 0.972), for LF power 0.00166 (95% CI: −0.01555, 0.01887, P-value 0.849) and for HF power 0.003379 (−0.01342, 0.02100, P-value 0.665).

A sensitivity analysis excluding all participants with self-reported cardiovascular disease showed similar results. In the multivariate analyses and per standard deviation of BMI, the difference in mean IBI was −1.8 (95% CI: −2.9, −0.8), the difference in SDNN was −3.4 (95% CI: −6.4, −0.6), in LF power was −8.0 (95% CI: −15.0, −1.4), and in HF power was 1.7 (95% CI: −5.0, 8.0).

Total body fat and heart rate variability

Table 2 shows the associations between total body fat (per 6%) and parameters of heart rate variability. Total body fat was associated with mean IBI and non-significantly associated with SDNN and LF power. Total body fat did not associate with HF power. Of the total association between body fat and heart rate variability, 34% (mean IBI), 67% (SDNN) and 81% (LF power) was mediated by HOMA-IR.

An interaction term of total body fat and HOMA-IR was added to all models to test for interaction, showing regression coefficients of 0.0003192 (95% CI: −0.0014396, 0.0020781, P-value 0.360) for mean IBI, −0.0013493 (95% CI: −0.005757, 0.0030584, P-value 0.547) for SDNN, −0.0052708 (95% CI: −0.0145081, 0.0039666, P-value 0.262) for LF power and −0.0033135 (95% CI: −0.0137051, 0.0070782, P-value 0.651) for HF power.

Repeating the analyses without participants with self-reported cardiovascular disease yielded similar results: per standard deviation of total body fat, the difference in mean IBI was −1.9 (95% CI: −3.0, −0.8), the difference in SDNN was −3.0 (95% CI: −6.2, 0.1), in LF power was −5.8 (95% CI: −11.7, 1.7), and in HF power was 0.8 (95% CI: −7.5, 8.5).
Waist circumference and heart rate variability

The regression coefficients for the associations between waist circumference (per 11 cm for men and 12 cm for women) and parameters of heart rate variability are shown in Table 2. Waist circumference was associated with mean IBI, SDNN and LF power. No association was found for HF power. The total association between waist circumference and heart rate variability was for 29% (mean IBI), 29% (SDNN) and 34% (LF power) mediated by HOMA-IR.

The interaction term of waist circumference and HOMA-IR was non-significant in all models, mean IBI: −0.00019 (95% CI: −0.00119; 0.00081, P-value 0.713), SDNN: −0.00027 (95% CI: −0.00309; 0.00256, P-value 0.854), LF power: 0.00045 (95% CI: −0.00598; 0.00688, P-value 0.890), HF power: 0.00414 (95% CI: −0.00311; 0.01138, P-value 0.262).

Table 2: Percentage difference (95% CI) in autonomic function per standard deviation of body fat measure (n=466)

<table>
<thead>
<tr>
<th>Body fat measure (SD)</th>
<th>Model</th>
<th>Mean IBI</th>
<th>SDNN</th>
<th>LF power</th>
<th>HF power</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (m: 4, w: 5 kg/m²)</td>
<td>Age and sex adjusted</td>
<td>−1.9 (−3.1, −0.8)</td>
<td>−3.3 (−6.3, −0.4)</td>
<td>−7.9 (−14.3, −1.8)</td>
<td>−0.1 (−6.8, 6.2)</td>
</tr>
<tr>
<td></td>
<td>Multivariate¹</td>
<td>−1.6 (−2.7, −0.6)</td>
<td>−2.7 (−5.5, 0.1)</td>
<td>−7.0 (−13.5, −0.9)</td>
<td>0.3 (−5.9, 7.0)</td>
</tr>
<tr>
<td></td>
<td>+ HOMA-IR as mediator²</td>
<td>−1.0 (−2.2, 0.3)</td>
<td>−1.2 (−4.2, 1.7)</td>
<td>−4.4 (−11.0, 1.8)</td>
<td>3.9 (−3.1, 11.5)</td>
</tr>
<tr>
<td>Total body fat (6%)</td>
<td>Age and sex adjusted</td>
<td>−2.1 (−3.4, −0.8)</td>
<td>−2.9 (−6.1, 0.1)</td>
<td>−4.9 (−11.4, 1.3)</td>
<td>0.1 (−7.3, 8.2)</td>
</tr>
<tr>
<td></td>
<td>Multivariate¹</td>
<td>−1.9 (−3.0, −0.8)</td>
<td>−2.4 (−5.4, 0.4)</td>
<td>−4.3 (−10.8, 1.9)</td>
<td>0.8 (−6.7, 9.0)</td>
</tr>
<tr>
<td></td>
<td>+ HOMA-IR as mediator²</td>
<td>−1.3 (−2.5, 0.0)</td>
<td>−0.8 (−3.9, 2.2)</td>
<td>−0.8 (−7.4, 5.4)</td>
<td>4.9 (−4.3, 15.0)</td>
</tr>
<tr>
<td>Waist circumference (m: 11, w: 12 cm)</td>
<td>Age and sex adjusted</td>
<td>−2.3 (−3.6, −1.0)</td>
<td>−4.5 (−7.8, −1.4)</td>
<td>−8.5 (−15.9, −1.6)</td>
<td>−1.2 (−9.1, 6.2)</td>
</tr>
<tr>
<td></td>
<td>Multivariate¹</td>
<td>−2.1 (−3.3, −0.9)</td>
<td>−4.0 (−7.1, −0.9)</td>
<td>−7.5 (−14.7, −0.8)</td>
<td>−1.1 (−9.1, 6.3)</td>
</tr>
<tr>
<td></td>
<td>+ HOMA-IR as mediator²</td>
<td>−1.5 (−2.9, −0.1)</td>
<td>−2.8 (−6.1, 0.4)</td>
<td>−5.0 (−12.1, 1.7)</td>
<td>2.3 (−5.9, 11.1)</td>
</tr>
</tbody>
</table>

Results were based on weighted analyses. CI, confidence interval; SD, standard deviation; BMI, body mass index; IBI, interbeat interval; m, men; w, women; +, additionally adjusted for; SDNN, standard deviation of all normal intervals; LF, low frequency; HF, high frequency, HOMA-IR, Homeostatic model assessment of insulin resistance.

¹Multivariate model adjusted for: age, sex, smoking, ethnicity, education, physical activity, prevalent cardiovascular disease and cardiac medication

²Regression coefficients reflect the direct associations between body fat measures and measures of autonomic function, after adjustment for HOMA-IR as mediating variable
Repeating the analyses without participants with self-reported cardiovascular disease yielded similar results: per standard deviation of waist circumference, the difference in mean IBI was $-2.4$ (95% CI: $-3.6, -1.2$), the difference in SDNN was $-4.8$ (95% CI: $-8.1, -1.6$), in LF power was $-8.3$ (95% CI: $-15.9, -1.1$), and in HF power was $-2.7$ (95% CI: $-11.3, 5.2$).

**DISCUSSION**

In our middle-aged population, measures of body fat (BMI, waist circumference, total body fat) were associated with autonomic function measured as long term heart rate variability (mean NN interval, SDNN, LF power). These associations were most pronounced for waist circumference. Depending on the parameter of body fat or heart rate variability, 29 to 81% of the total association seemed to be mediated by insulin resistance as assessed by HOMA-IR. There was no association between body fat and autonomic function, as measured with HF power.

The results on mean NN interval, SDNN and LF power are in line with previous studies on the association between body fat and parameters of sympathetic activation. Our findings may add to those studies that insulin resistance is a possible mechanism underlying the association between body fat and autonomic function. An explanation for the lack of association with HF power may be that this spectral component is more indicative of the parasympathetic and not directly of the sympathetic outflow.

A strength of the present study is the extensive phenotyping of the NEO participants including the measurement of total body fat and waist circumference, which more accurately quantify body fat and abdominal fat accumulation than BMI does. However, our study also has limitations that need to be considered. The cross-sectional observational nature of our study is a limitation. We cannot exclude the possibility that our outcome (sympathetic activation) may have influenced our determinant (body fat), and so there may be reverse causation in our study. Another limitation is the use of the Homeostasis Model Assessment Insulin Resistance as a measure of insulin resistance instead of the hyperinsulinaemic euglycaemic clamp, but the correlation with the clamp is very high. Also the use of the Actiheart for measurement of heart rate variability may be a limitation, but the agreement of Actiheart with the often used Holter electrocardiogram is high. With regard to the statistical analysis, we used parametric mediation analysis assuming linear models for the outcome and the mediator. This is a valid method to evaluate mediation in absence of mediator-outcome confounding and interaction between the exposure and the mediator under study. It needs to be taken into account that add-
ing a possible mediating variable into a regression model could introduce bias in the estimation of the direct and indirect association if there is residual mediator-outcome confounding or interaction between determinant and mediator.\textsuperscript{27} In our study, we could only identify low-grade systemic inflammation as a potential residual confounder between insulin resistance (mediator) and autonomic function (outcome). Not adjusting for inflammation might have resulted in an overestimation of both the direct association of body fat with sympathetic activation and the association mediated by insulin resistance. However, it may well be that inflammation is not a common cause of insulin resistance and sympathetic activation, but rather an intermediate factor between insulin resistance and sympathetic activation. In that case, adjusting for inflammation would result in an underestimation of the true association in our mediation analyses. To examine the presence of interaction between determinant and mediator, we tested for interaction by adding an interaction term of body fat measures and HOMA-IR to our linear regression models. All were non-significant, meaning that it is unlikely that adding HOMA-IR as a mediator to our models has resulted in bias in the estimation of the direct and indirect association. In addition, estimating direct and indirect associations is affected if the variability in the measurement of determinant and mediator is of different magnitude. In the NEO study, 100 participants were examined for the second time approximately three months after their baseline visit. From this repeated measurements, we calculated intraclass correlation coefficients (ICCs). The ICCs of body fat measures and HOMA-IR were both high (e.g. 0.940 (0.921-0.959) for total body fat and 0.749 (0.675-0.822) for HOMA-IR). This suggests that it is unlikely that a difference the variability in the measurement of determinant and mediator has influenced our results.

Although cross-sectional, our results indicate that insulin resistance may be a mechanism underlying in the association between body fat and autonomic function (Figure 1). This finding is supported by evidence on three associations. First, excess body fat is a well-established risk factor for insulin resistance.\textsuperscript{6,7} Second, the association between body fat and autonomic function has been shown in several previous studies.\textsuperscript{8-12} Adipose tissue secretes a number of hormones (e.g. leptin, adiponectin, resistin, visfatin) and inflammatory markers (e.g. TNF-\alpha, IL-6). Thereby, the adipose tissue is able to directly stimulate the central sympathetic nervous system in the hypothalamus, or to induce a state of low-grade inflammation that can also stimulate the sympathetic nervous system.\textsuperscript{28} Third, insulin resistance has been associated with autonomic function in several cross-sectional studies.\textsuperscript{8-12} We know from experimental studies that higher levels of circulating insulin elevate sympathetic outflow both in the euglycaemic or hyperglycaemic state.\textsuperscript{14,15} The stimulation of the sympathetic nervous system seems at least in part attributable to the central action and peripheral action of insulin.\textsuperscript{29} However, also after the diagnosis of diabetes (and the onset of beta-cell failure), autonomic function
deteriorates. Therefore, it seems unlikely that the impairment of autonomic function is due to hyperinsulinaemia alone. Likely, also the hyperglycaemia and an abundance of advanced glycation end-products in insulin resistant individuals play a role in inducing autonomic dysfunction by damaging the nerves or interfering with chemical signalling.

In conclusion, in this cross-sectional study body fat was associated with autonomic function measured as heart rate variability. This association may at least partially be mediated by insulin resistance. Future studies should investigate whether a reduction in obesity and insulin resistance may prevent the adverse cardiovascular consequences of altered autonomic function.
REFERENCE LIST


