Influence of Autonomic Nervous System Dysfunction on the Development of Type 2 Diabetes

The CARDIA study

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OBJECTIVE — We investigated whether autonomic nervous system dysfunction, estimated by slow heart rate recovery (HRR) following cessation of an exercise treadmill test, was associated with increases in insulin and glucose over time and the development of type 2 diabetes.

RESEARCH DESIGN AND METHODS — Maximal exercise tests were performed by 3,295 healthy adults aged 18–30 years in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Repeat measurements of insulin and glucose collected at 7-, 10-, and 15-year examinations were compared by quartiles of HRR (maximum heart rate minus heart rate 2 min after cessation of the test). Incident diabetes was identified at any follow-up examination as glucose ≥7 mmol/l or the use of diabetes control medication.

RESULTS — Among participants who did not develop diabetes, fasting insulin concentrations increased from baseline to year 15. Following adjustment (for age, race, sex, smoking status, and BMI), participants with the slowest HRR (quartile 1) had higher fasting insulin at each examination than participants with faster HRR (e.g., year 15 examination: 88.1 vs. 81.3 pmol/l for quartile 1 vs. quartile 4, respectively, P = 0.05). Glucose did not differ by quartile of HRR at any examination. Among participants with poor fitness, the risk of developing diabetes (n = 98) was 3.4-fold greater (95% CI 1.5–8.0) when HRR was <42 vs. >42 bpm. This persisted following adjustment for baseline insulin.

CONCLUSIONS — Autonomic dysfunction, in combination with poor physical fitness, may be a mechanism associated with early glucose dysmetabolism and the development of diabetes.

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Abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults; HRR, heart rate recovery.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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HRR, the rapid decrease in heart rate to preexercise levels following cessation of exercise, has been inversely associated with mortality (14,15), fasting glucose, and diabetes (4,6) in previous studies of middle-aged adults. Vagal reactivation is the principal determinant of the decrease in heart rate in the first 30 s of recovery; low values of HRR (slow HRR) may reflect decreased parasympathetic activity (16,17). In a sample from the longitudinal Coronary Artery Risk Development in Young Adults (CARDIA) study, we tested the hypothesis that participants with slow HRR, indicative of decreased parasympathetic tone, had larger increases in insulin and glucose over time and an increased incidence of type 2 diabetes over 15 years of follow-up.

RESEARCH DESIGN AND METHODS — The CARDIA study is a longitudinal cohort study designed to investigate factors that influence the evolution of coronary heart disease risk factors.
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in black and white young adults. Adults aged 18–30 years (n = 5,115) from four geographic areas, Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California, were recruited and examined in 1985–1986 (18). Roughly half of the participants were women (54.5%), and roughly half were African American (51.6%). Participants were reexamined at years 2, 5, 7, 10, and 15; 74% (n = 3,672) of the original cohort returned for examination at year 15.

For this analysis, we excluded participants for the following reasons: 1) prevalent diabetes or impaired fasting glucose at baseline (glucose >6.1 mmol/l) (n = 75), 2) no completion of exercise treadmill testing at baseline (n = 217), 3) non-fasting (n = 141), 4) pregnancy (n = 32), 5) heart rate reserve ([maximum heart rate – resting heart rate]/[220 – age – resting heart rate] × 100) <80% (n = 1,275) (19), and 6) use of medication for hypertension or cardiovascular disease (n = 80). Following these exclusions, 3,295 participants remained. However, for analyses of changes in insulin and glucose, we required that participants have at least two consecutive measurements (insulin n = 2,534; glucose n = 2,762).

Data collection
All measurements in CARDIA were collected according to standardized protocols across study sites (18). Subjects were asked to fast for at least 12 h before examination and to avoid smoking or engaging in heavy physical activity for at least 2 h before the examination.

Exercise testing
At baseline, all medically eligible participants underwent a graded symptom-limited maximal exercise test according to the Balke protocol (20). Participants were determined to be ineligible for the test for the following reasons: history of ischemic heart disease, use of cardiovascular medications (except high blood pressure medication), elevated resting blood pressure (systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg), or acute illness with a fever (test was rescheduled for a later date whenever possible). Additionally, we excluded participants who did not achieve an estimated heart rate reserve of at least 80% for two reasons: 1) these participants likely did not exercise to their maximum capacity, a requirement for determining HRR; and, 2) heart rate reserve <80% is a marker of chronotropic incompetence (19), an independent marker for mortality (21).

The exercise test procedure has been previously described (20). In brief, the test included up to nine 2-min stages of progressively increasing difficulty, and participants were instructed to exercise to maximal exertion. At the end of each stage, and at 1, 2, and 3 min after cessation of the test, heart rate and blood pressure were measured. Metabolic equivalent energy expenditure at peak exercise (equal to a multiple of oxygen consumption of 3.5 ml · kg⁻¹ · min⁻¹) was estimated. Participants in the uppermost quartile of sex- and age-specific (18–24 years and ≥25 years) heart rate at the end of stage 2 (4 min) of the exercise test were classified as being in poor physical fitness (22). In secondary analyses, fitness was evaluated according to treadmill test duration. Participants in the lowest quintile of sex-specific test duration (men <10.7 min; women <7 min) were classified as being in low fitness.

Heart rate recovery. HRR was defined as the difference between the maximum heart rate and the heart rate 2 min following the cessation of exercise. A smaller value of HRR indicates a slower (less favorable) recovery, which is indicative of impaired vagal reactivation. We categorized HRR into sex-specific quintiles for analysis and dichotomized HRR at 42 bpm based on previously published reports that have indicated an increased risk of mortality below that value (15).

Clinical evaluation
Blood was drawn into vacuum tubes from participants in the seated position and was centrifuged at 4°C within 60 min. Serum and plasma were stored in cryovials (containing fluoride for the glucose assay) and then frozen at −70°C for shipment to a central laboratory. Glucose was measured by the hexokinase method. Impaired fasting glucose was defined as glucose ≥6.1 mmol/l (110 mg/dl) and <7.0 mmol/l (126 mg/dl). Diabetes was defined as a fasting glucose ≥7.0 mmol/l (126 mg/dl) or the use of oral hypoglycemic medications or insulin. Incident events were identified at years 7, 10, and 15. Fasting insulin was analyzed using a radioimmunoassay technique that used overnight equilibrium incubation (23,24).

With the participant in light examination clothes and no shoes, height, weight, and waist circumference were measured. BMI was calculated as the ratio of weight (kilograms) to standing height (meters) squared. Waist circumference was measured to the nearest 0.5 cm at the minimum abdominal girth with participants standing upright. The average of two measurements was used. After a 5-min rest, pulse was recorded for a 30-s interval.

Age, race, education, cigarette smoking status, and medication use were ascertained by interview at all examinations. First-degree family history of diabetes was collected by self-report at baseline, 5-, and 10-year examinations. Self-reported physical activity was assessed by a validated interview-administered questionnaire in which participants were asked about the frequency of participation in 13 different categories of recreational sports and exercise in the past 12 months. Physical activity scores were computed by multiplying the frequency of participation by the intensity of the activity; details of the instrument have been published (25).

Statistical methods
Demographic and clinical characteristics were computed for the total population and stratified by sex-specific quintiles of 2-min HRR. Tests of linear trend were performed using continuously measured HRR in linear (continuous) and logistic (categorical) regression models. Before modeling, we evaluated homogeneity of effect across race and sex by using stratified modeling and including a multiplicative interaction term in regression models. Because there was no qualitative or quantitative difference of effect between race and sex, the results were pooled and adjusted for race and sex. Next, we used generalized estimating equations to examine the association between HRR and average annual change in insulin and glucose over follow-up. The results were comparable whether or not values were log-transformed, so values are presented without transformation. We tested for statistical interaction between characteristics hypothesized to modify the association between autonomic nervous system function and insulin and glucose using maximum-likelihood χ² tests. Poor physical fitness [≥25th percentile of heart rate at 4 min of treadmill test (22)] emerged as a signifi-
cant effect modifier. Multivariable logistic regression modeling was used to evaluate the association between HRR and incident diabetes. Effects are presented specific to each stratum. Statistical significance was denoted at $P < 0.05$. All analyses were conducted using SAS software version 8.1 (SAS, Cary, NC).

**RESULTS** — The distribution of demographic, anthropometric, and clinical characteristics and health behaviors in this sample is consistent with previous CARDIA reports (Table 1). Sex-specific quartile cutpoints of HRR were 34, 41, and 49 bpm for women and 37, 44, and 52 bpm for men. Participants with slower HRR were slightly older, reported their race as black less frequently, were more highly educated, and were less frequently current smokers. BMI and waist circumference were inversely associated with HRR.

On average, participants spent 10.5 min (SD 2.7) on the treadmill, and the length of the test was directly associated with HRR. Resting heart rate and the prevalence of poor physical fitness were inversely associated with HRR quartiles, although there was no difference in maximum heart rate. The increase in heart rate from resting to maximum and the self-reported physical activity participation were directly associated with HRR.

At baseline, mean insulin was 75.8 pmol/l (95% CI 74.5–77.1) and was highest among participants in the lowest quartile (80.9 pmol/l, 77.7–84.0), compared with the uppermost quartile (70.7 pmol/l, 68.5–73.0), of HRR. Following the exclusion of participants who developed diabetes during follow-up, insulin increased over time and remained inversely associated with HRR (Fig. 1A). When models were adjusted additionally for baseline smoking status and BMI change, insulin no longer increased with time (Fig. 1B), but differences in insulin between the upper and lower quartiles persisted.

At baseline, mean glucose in all participants was 4.53 mmol/l (95% CI 4.52–4.55) and did not differ markedly across quartiles of HRR (Q1 = 4.56 mmol/l, 95% CI 4.53–4.59; Q2 = 4.54, 4.51–4.57; Q3 = 4.52, 4.49–4.55; and Q4 = 4.51 mmol/l, 4.48–4.54). In the subset of participants who did not develop diabetes, there was no association between HRR and glucose at any examination and no pattern of glucose change over time (Fig. 2).

During the study, 98 participants developed incident diabetes. The association between HRR and incident diabetes was not linear, but the risk of developing diabetes was significantly elevated in individuals with abnormally slow HRR (<42 bpm) (Table 2). However, following adjustment for BMI, cigarette smoking (model 2), and baseline fasting insulin (model 3), this finding was attenuated.
While HRR was only weakly correlated with physical fitness, estimated as the heart rate at stage 2 of the exercise test ($r = -0.33$), it was a significant ($\chi^2 = 9.77, P = 0.002$) modifier of the association between HRR and incident diabetes. The risk of developing diabetes among participants in low fitness and with abnormally slow HRR was threefold higher than in those with faster HRR. This association persisted with adjustment for fasting insulin.

In secondary analysis, we tested whether this interaction with fitness was present using a different marker of fitness, treadmill test duration. Treadmill test duration was weakly correlated with HRR ($r = 0.23$) but also proved to be a modifier of the association between HRR and incident diabetes ($\chi^2 = 6.19, P = 0.01$). Less fit participants with HRR <42 bpm were 2.2 times (95% CI 1.2–4.2) more likely to develop diabetes over follow-up than less fit participants with higher HRR.

Among participants in good fitness, slow HRR was not associated with the development of diabetes (OR 0.9, 95% CI 0.5–1.4).

**CONCLUSIONS** — Young adults in this population-based sample with impaired vagal reactivation following exercise were hyperinsulinemic compared with their counterparts, and if they were also in poor fitness, they were at an elevated risk of developing incident diabetes.
Despite an inverse association between HRR and insulin at baseline and each follow-up examination, which is consistent with previous research (7,8,10), we did not observe differences in the increase in insulin over time between individuals by HRR once we accounted for changes in BMI. Our findings imply a complex association among insulin, glucose, and autonomic dysfunction. Previous animal (26), clinical (27), and therapeutic (10,28) studies postulate that persistently elevated levels of glucose damage peripheral nerve fibers, thus stimulating sympathetic activity and decreasing parasympathetic control. This is plausible because the pancreas is heavily innervated by parasympathetic and sympathetic nerve fibers. Parasympathetic fibers stimulate the β-cells to release insulin in response to circulating glucose levels, whereas sympathetic activation inhibits insulin secretion from the β-cells, resulting in impaired transport of blood glucose to the muscle cells. Gradual glucose increases in response to declining insulin secretion and decreased liver and muscle metabolism are one mechanism for the development of frank diabetes. Findings from this study confirm those of

Figure 2—Adjusted changes in fasting glucose over time by HRR. A: Model adjusted for age, race, and sex. B: Model adjusted for age, race, sex, cigarette smoking, and BMI change. HRR quartiles: ■, quartile 1 (slow); □, quartile 2; ●, quartile 3; ○, quartile 4 (fast).
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Table 2—Multivariable-adjusted ORs (95% CIs) for the 15-year incidence of diabetes by abnormal (<42 bpm) HRR

<table>
<thead>
<tr>
<th>Total population OR (95% CI)</th>
<th>Low fitness OR (95% CI)</th>
<th>High fitness OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1. Adjusted for age, race, and sex</td>
<td>1.53 (1.01–2.31)</td>
<td>3.54 (1.56–8.03)</td>
</tr>
<tr>
<td>Model 2. Adjusted for model 1 + smoking status and BMI</td>
<td>1.29 (0.84–1.99)</td>
<td>3.43 (1.47–8.00)</td>
</tr>
<tr>
<td>Model 3. Adjusted for model 2 + baseline fasting insulin</td>
<td>1.15 (0.72–1.85)</td>
<td>3.27 (1.34–7.94)</td>
</tr>
</tbody>
</table>

*Low fitness is the highest quartile of age- and sex-specific heart rate at stage 2 (4 min) of the exercise test.

References


10. Van De Borne P, Hausberg M, Hoffman RP, Mark AL, Anderson EA: Hyperin-