Low Cardiorespiratory Fitness Is Associated With Elevated C-Reactive Protein Levels in Women With Type 2 Diabetes

JONATHAN M. MCGAVOCK, MSC[1,2]
SANDRA MANDIC, MSC[1,2]
ISABELLE VONDER MUHLL, MD[3]
RICHARD Z. LEWANCZUK, MD[4]
H. ARTHUR QUINNEY, PHD[2]
DYLAN A. TAYLOR, MD[3]
ROBERT C. WELSH, MD[3]
MARK HAYKOWSKY, PHD[1,3]

OBJECTIVE — The purpose of this study was to examine differences in novel markers of cardiovascular disease (CVD) in women with type 2 diabetes stratified according to cardiorespiratory fitness.

RESEARCH DESIGN AND METHODS — A total of 28 women (mean age 57 ± 6 years) with type 2 diabetes who were free from overt CVD were placed into low cardiorespiratory fitness (LCF) or average cardiorespiratory fitness (ACF) groups based on a graded exercise test to exhaustion. A group of eight women without type 2 diabetes were also examined and served as healthy control subjects. The median $\dot{V}O_{2peak}$ value was used as a cutoff for group determination. We assessed both conventional CVD risk factors, including blood pressure, BMI, and lipid profile, as well as novel CVD risk factors, such as left ventricular filling dynamics, arterial stiffness, fasting insulin, and C-reactive protein (CRP).

RESULTS — $\dot{V}O_{2peak}$ values were 69 ± 14 and 91 ± 24% of predicted values for sedentary age-matched healthy individuals in the LCF and ACF groups, respectively. BMI was significantly greater in the LCF group ($P < 0.05$); however, no differences were observed in age, lipid profile, or resting hemodynamics. CRP was 3.3-fold higher in the LCF group (6.3 ± 41. vs. 1.9 ± 1.7 mg/l, $P < 0.05$), whereas other novel markers of CVD were not significantly different between the groups. Significant negative relationships were observed between $\dot{V}O_{2peak}$ and both CRP ($r = -0.49$) and the homeostasis model assessment index ($r = -0.48$) ($P < 0.05$).

CONCLUSIONS — The novel finding of this investigation is that low cardiorespiratory fitness is associated with elevated CRP and reduced fasting glucose control in women with type 2 diabetes.

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Type 2 diabetes is associated with an increased risk for the development of cardiovascular disease (CVD) (1) that may be explained, at least in part, by synergistic comorbidities such as hypertension, dyslipidemia, and obesity. In addition to conventional risk factors for CVD, growing evidence suggests that novel markers may play a role in determining CVD risk in patients with type 2 diabetes. These include measures of subacute inflammation, such as C-reactive protein (CRP) (2); vascular effects of excessive insulin (3); and cardiac (4) and vascular (5) maladaptations specific to type 2 diabetes.

Low cardiorespiratory fitness is associated with increased CVD morbidity and mortality in men with and without type 2 diabetes (6). In men with type 2 diabetes, this association remained significant after adjusting for conventional CVD risk factors such as hypertension, dyslipidemia, smoking status, and family history of CVD (6). It is possible, however, that the adverse effects of low cardiorespiratory fitness in patients with type 2 diabetes contribute to or exacerbate the cardiovascular (7,8) and metabolic derangements (9) that increase the risk for CVD in this population. The cardiovascular consequences of type 2 diabetes are significantly more deleterious in women than in men (10), possibly due to sex-related differences in the cholesterol profile (11) and/or significant reductions in cardiorespiratory fitness (12). Therefore, the purpose of this investigation was to examine conventional and novel CVD risk factors in women with type 2 diabetes stratified according to their cardiorespiratory fitness.

RESEARCH DESIGN AND METHODS

— A total of 32 women with type 2 diabetes, free from diabetes-related complications, were screened for participation in this investigation. Presence of type 2 diabetes was confirmed by a chart review and with fasting HbA_{1c}. Pharmacological treatment strategies for metabolic control, hypertension, dyslipidemia, and preventative therapy (i.e., acetylsalicylic acid) were evenly distributed between both groups of women with type 2 diabetes (Table 1). Groups were evenly matched for the number of participants

From the [1]Faculty of Rehabilitation Medicine, University of Alberta, Edmonton, Alberta; the [2]Faculty of Physical Education, University of Alberta, Edmonton, Alberta; the [3]Faculty of Medicine, Division of Cardiology, University of Alberta, Edmonton, Alberta; and the [4]Division of Endocrinology, University of Alberta, Edmonton, Alberta.

Address correspondence and reprint requests to Jon McGavock, 2-50 Corbett Hall, Faculty of Rehabilitation Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G4. E-mail: mcgavock@ualberta.ca.

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Abbreviations: ACF, average cardiorespiratory fitness; CRP, C-reactive protein; CVD, cardiovascular disease; ECG, electrocardiogram; HOMA, homeostasis model assessment; LCF, low cardiorespiratory fitness.

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

Table 1—*Subject characteristics*

	LCF	ACF	Control subjects
n	14	14	8
Demographic characteristics			
Age (years)	59 ± 7	56 ± 5	69 ± 4*†
Weight (kg)	94.6 ± 13.1	84.4 ± 17.4	70.5 ± 10.1*†
BMI (kg/m^2)	37.5 ± 4.1	32.2 ± 5.6*	28.5 ± 3.7*
Years since diagnosis	5 ± 2	4 ± 3	—
$\dot{V}O_{2peak}$ (ml · kg^{-1} · min^{-1})	15.7 ± 2.0	23.2 ± 2.7*	21.9 ± 3.0*
$\dot{V}O_{2peak}$ (% predicted)	69 ± 14	91 ± 24*	111 ± 14*
Diabetes therapy (D/O/I)	2/12/0	4/10/0	—
Hypertension therapy	8	7	—
Dyslipidemia therapy	4	4	—
Resting hemodynamics			
Heart rate (beats/min)	76 ± 10	74 ± 11	74 ± 8
Systolic blood pressure (mmHg)	146 ± 17	132 ± 13	143 ± 13
Diastolic blood pressure (mmHg)	78 ± 9	72 ± 12	78 ± 9
Large artery compliance (ml/mmHg)	0.82 ± 0.34	1.0 ± 0.38	0.77 ± 0.18
Oscillatory compliance (ml/mmHg)	0.037 ± 0.036	0.040 ± 0.033	0.022 ± 0.014
Left ventricular morphology (n = 8 LCF group, n = 11 ACF group)			
LVIDd (cm)	5.4 ± 1.1	4.8 ± 0.6	—
LVMI (g/m^2)	134 ± 62	96 ± 13	—
RWT	0.40 ± 0.05	0.40 ± 0.14	—
Estimates of left ventricular function			
E/A ratio	0.95 ± 0.21	1.04 ± 0.29	—
Deceleration time (ms)	0.24 ± 0.05	0.25 ± 0.04	—
PVs:PVd	1.58 ± 0.28	1.48 ± 0.25	—
Pad-Mad (s)	−27 ± 35	−33 ± 48	—
Ejection fraction (%)	69 ± 10	71 ± 8	—

Data are means ± SD. *$P < 0.05$ vs. LCF, †$P < 0.05$ vs. ACF. D, diet; E/A ratio, ratio of early to late transmitral filling velocities; LVIDd, left ventricular internal diameter in diastole; LVMI, left ventricular mass index; I, insulin; O, oral antidiabetic therapy; Pad-Mad, difference between pulmonary venous and transmitral flow duration during atrial contraction; PVs and PVd, pulmonary venous flow in systole and diastole.

on hormone replacement therapy. Exclusion criteria for this investigation included evidence of ischemic heart disease documented by a history of CVD or by resting or exercise electrocardiogram (ECG) abnormalities (>1.5 mm flat or down-sloping ST segment depression), angina or any other cardiac symptoms potentially limiting exercise capacity, and the presence of musculoskeletal or peripheral vascular abnormalities that would limit exercise capacity. Informed consent was obtained from all subjects before the investigation, and the Research Ethics Review Board within the Faculty of Medicine at the University of Alberta approved the study protocol.

Study protocol

Patients reported to the Division of Cardiology, University of Alberta Hospital, for a clinical and physical examination as well as a resting and exercise ECG. A graded exercise test was performed on an electronically braked cycle ergometer to determine peak rate of consumption ($\dot{V}O_{2peak}$). On a second visit, subjects reported to the Metabolic Unit after an overnight fast for blood sampling, an assessment of arterial compliance, and a resting echocardiogram. All subjects were asked to refrain from medications with vascular or cardiac effects for 48 h before testing. A period of at least 48 h separated the two visits. Subjects were then stratified into two groups according to relative $\dot{V}O_{2peak}$ values obtained from the graded maximal exercise test. The group median was used to separate participants into groups of low cardiorespiratory fitness (LCF) and average cardiorespiratory fitness (ACF). Eight healthy older women without type 2 diabetes were also assessed and served as a control group.

Graded maximal exercise test

Oxygen consumption, carbon dioxide production, and minute ventilation were sampled breath by breath at rest and during exercise, and values were averaged every 15 s using a MedGraphics Metabolic Cart (Medical Graphics, Minneapolis, MN). At least two of the following criteria needed to be achieved for the determination of $\dot{V}O_{2peak}$: respiratory exchange ratio >1.10; <100 ml/min increase in $\dot{V}O_2$ for >30 s, despite an increase in workload; and heart rate within five beats of age-predicted and volitional exhaustion. Arm blood pressure (auscultation) was performed according to American Heart Association guidelines, and heart rate was determined from 12-lead ECG recordings.

Assessment of arterial compliance and resting hemodynamics

Arterial compliance was assessed using computerized arterial pulse waveform

analysis. This technique involves 30-s recordings of signal-averaged arterial pulse waves by applanation tonometry using a surface residing pressure transducer on the radial artery (Hypertension Diagnostics, Eagan, MN). Blood pressure was measured oscillometrically on the opposite arm. The diastolic decay of the waveform was analyzed mathematically, and the two components of arterial compliance were calculated based on a modified Windkessel model of circulation: capacitive compliance (large artery) and oscillatory (or reflective) compliance reflecting smaller more peripherally located arteries and arterioles. This technique has been validated previously with invasive measurements of arterial waveforms (13).

Table 2—*Hematological markers of CVD women with type 2 diabetes stratified according to cardiorespiratory fitness*

	LCF	ACF	Control subjects
n	14	14	8
HbA_{1c} (%)	7.4 ± 1.7	6.9 ± 1.0	5.7 ± 0.2*
Glucose (mmol/l)	8.2 ± 4.0	7.5 ± 1.2	5.3 ± 0.5*
Insulin (mU/l)	14.7 ± 6.8	11.0 ± 6.0	6.4 ± 2.7*
HOMA index	5.3 ± 2.6	3.8 ± 2.2	1.53 ± 0.63*
Total cholesterol (mmol/l)	5.3 ± 1.4	5.1 ± 0.78	5.4 ± 0.6
HDL (mmol/l)	1.4 ± 0.36	1.3 ± 0.21	1.7 ± 0.3
LDL (mmol/l)	3.1 ± 0.65	2.8 ± 0.85	3.0 ± 0.5
Triglyceride-to-HDL ratio‡	1.9 ± 1.5	1.6 ± 0.8	0.9 ± 0.8
CRP (mg/l)	6.3 ± 4.3	1.9 ± 1.7*	4.1 ± 4.0*†

Data are means ± SD. *$P < 0.05$ vs. LCF; †$P < 0.05$ vs. ACF; ‡estimate of particle size.

Echocardiographic measurements

Left ventricular imaging was performed with a commercially available ultrasound instrument (Sonos 5500; Hewlett Packard) with a 3.5-MHz transducer. Left ventricular two-dimensional transthoracic images were obtained from the parasternal short-axis view at the level of the midpapillary muscles according to American Society of Echocardiography guidelines (14). Left ventricular filling dynamics were assessed using pulsed-wave Doppler analysis of transmitral and pulmonary venous flow patterns recorded in the apical four-chamber view (15). All echocardiographic images were averaged over three cardiac cycles.

Blood collection and analysis

Blood was drawn in the fasted state before ultrasound imaging. Fasting hematological measurements included glucose, insulin, HbA_{1c}, total cholesterol, HDL, triglycerides, and LDL. Insulin was measured using a Roche Diagnostics Elecsys 2010 System using the sandwich principle (16). Glucose was measured using a hexokinase method. Total cholesterol values were obtained using a cholesterol oxidase method (17), whereas triglyceride values were obtained using a fully enzymatic colorimetric assay reaction for glycerol after the removal of all serum-free glycerol (18). HDL values were determined from a photometric method after the addition of polyethylene glycol–modified enzymes (19). Plasma lipids, glucose, and HbA_{1c} were determined on a Synchron LX20 analyzer (Beckman Coulter, Fullerton, CA). Plasma CRP was analyzed using a fully automated Behring Nephelometer Analyzer System (Behring Diagnostics, Mannheim, Germany) using anti-CRP mouse monoclonal antibodies coupled with latex microparticles. This is not an assay for high-sensitivity CRP. The homeostasis model assessment (HOMA) index was used as an estimate of insulin sensitivity (20).

Statistical analysis

The data are presented as means ± SD. Baseline characteristics between the groups were compared using a Student's *t* test. Stepwise multiple linear regression analyses were performed to determine the relationship between $\dot{V}O_{2peak}$ and CVD risk factors. A *P* value of ≤0.05 was considered statistically significant.

RESULTS

Demographics and hemodynamics

Subject characteristics are provided in Table 1. Of the initial 32 women screened, 4 women were excluded from the investigation because of either ECG abnormalities, suggestive of underlying coronary artery disease ($n = 2$), or failure to achieve adequate criteria for determination of $\dot{V}O_{2peak}$ ($n = 2$). Therefore, 28 women were distributed evenly into LCF and ACF groups. Two women in each of the ACF and LCF groups and all the women in the control group were taking hormone replacement therapy. BMI was higher in the LCF group because of a difference of ~10 kg ($P = 0.09$) in body weight. No differences were observed in age or duration of diabetes between the ACF and LCF groups. The control group was significantly older than the ACF and LCF groups ($P < 0.05$). $\dot{V}O_{2peak}$ values were significantly lower in the LCF groups than in the ACF group as per the study design. $\dot{V}O_{2peak}$ was significantly lower in the LCF groups than in the control group. Few subjects were able to attain a respiratory exchange ratio >1.10 ($n = 2$ LCF, $n = 4$ ACF); however, average values were identical between the two groups (LCF 1.06 ± 0.04 vs. ACF 1.06 ± 0.04). Heart rate at peak exercise was 93 ± 14 and 99 ± 9% of age-predicted values in the LCF and ACF groups, respectively. Taken together, these data suggest similar maximal efforts between the two groups.

There were no significant differences in resting heart rate, diastolic blood pressure, and mean arterial pressure between the groups. A substantial difference in systolic blood pressure (146 ± 17 vs. 132 ± 13 mmHg in the LCF and ACF groups, respectively) was observed between the two type 2 diabetes groups; however, it did not achieve statistical significance ($P = 0.056$). No differences were observed in large or oscillatory compliance, or systemic vascular resistance, among all three groups.

Measures of left ventricular structure and function were similar in both type 2 diabetes groups. Technically adequate measures of left ventricular morphology could only be obtained in 19 subjects (8 LCF and 11 ACF). Although left ventricular mass indexed to body surface area tended to be higher in the LCF group (134 ± 62 vs. 96 ± 13 g/cm^2), this difference did not reach statistical significance ($P = 0.061$). Echocardiographic

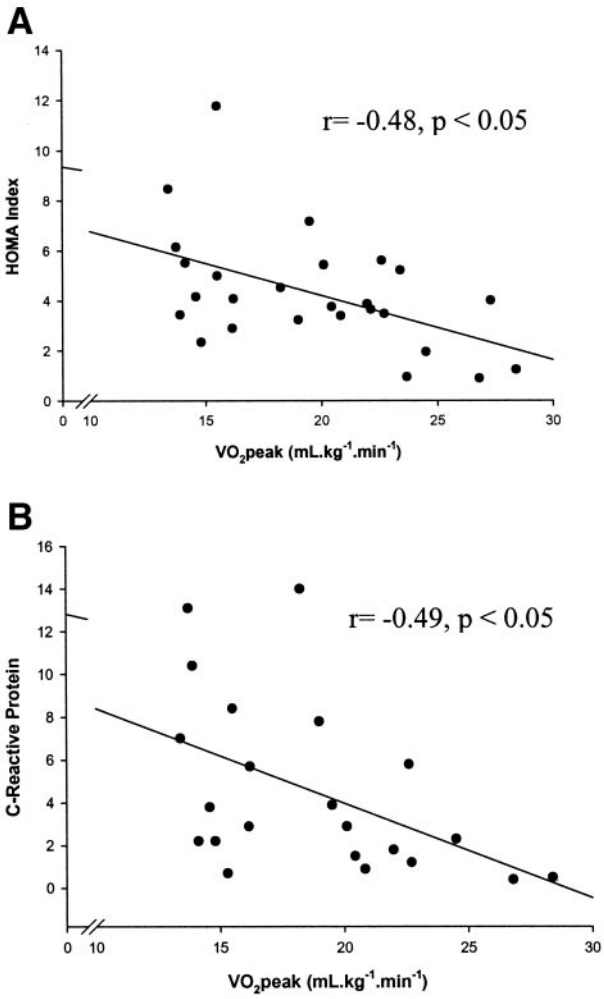

Figure 1—*Relationship of $\dot{V}O_{2peak}$ to CRP and the HOMA index in women with type 2 diabetes.*

analyses were not performed in healthy control subjects.

Hematological variables

Hematological variables are reported in Table 2. There were no differences observed in classic markers of CVD, such as total cholesterol, LDL, HDL, or triglycerides, among all three groups. HOMA index and HbA_{1c} were significantly higher in the LCF group than in the control group ($P < 0.05$). CRP levels were ~1.5- and 3-fold higher in the LCF group than in the control and ACF groups, respectively ($P \leq 0.05$). CRP values were not different between the ACF and control groups ($P \leq 0.05$). Significant negative correlations were observed between $\dot{V}O_{2peak}$ and both CRP and the HOMA index in the women with type 2 diabetes (Fig. 1). A stepwise multiple linear regression analysis between CRP and $\dot{V}O_{2peak}$, BMI, and age revealed β values of −0.651 ($P < 0.05$), −0.216 ($P = 0.46$), and −0.203 ($P = 0.32$), respectively. Finally, we observed a significant positive correlation between the HOMA index and CRP in women with type 2 diabetes ($r = 0.49$, $P < 0.05$, Fig. 2).

CONCLUSIONS — This study is the first to assess the interaction between cardiovascular fitness with conventional and novel markers of CVD risk in women with type 2 diabetes. The primary findings of this investigation are the association of low cardiorespiratory fitness with increased HOMA index and CRP in women with type 2 diabetes. Furthermore, no relationships were observed between low cardiorespiratory fitness and classic risk factors for CVD, such as age, duration of diabetes, and lipid profile, or novel risk factors, such as left ventricular filling dynamics or arterial compliance.

CRP has been shown to be prospectively associated with an increased risk for the development of CVD (2) and type 2 diabetes (21). Because physical inactivity is a primary risk factor in the development of both these diseases, it is possible that this trend could be, in part, a function of reduced physical activity patterns. In support of this hypothesis, inverse associations between CRP and self-reported physical activity levels have recently been reported in multiethnic populations of healthy men and women (22,23). Additionally, reports from the National Health and Nutrition Examination Survey III demonstrated that individuals who participate in vigorous exercise (>6 metabolic equivalent levels) have a 60% reduction in CRP when compared with individuals who reported no leisure-time physical activity (24). These data suggest

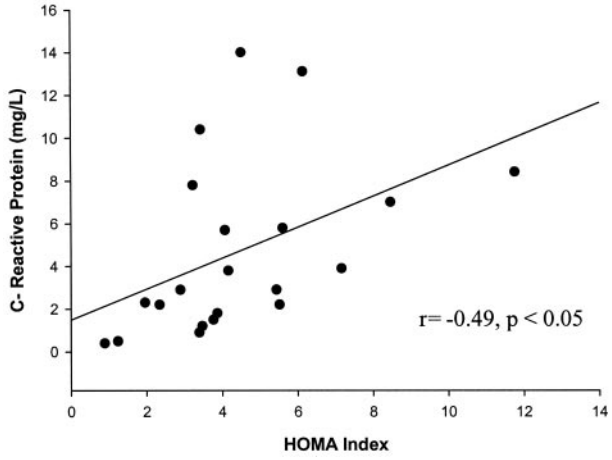

Figure 2—*Relationship between the HOMA index and CRP in women with type 2 diabetes.*

that increased levels of physical activity or caloric expenditure may reduce or attenuate the expression of markers of subclinical vascular inflammation in healthy older individuals. Our data support these findings and extend them to a population at significant risk for CVD, because we observed a negative correlation between cardiorespiratory fitness and CRP in our sample of women with type 2 diabetes.

The mechanisms underlying the negative correlation between cardiorespiratory fitness and markers of subclinical vascular inflammation have yet to be determined; however, some evidence suggests that poor metabolic control may play a role. First, prospective population-based studies have demonstrated that CRP is a strong predictor of the development of type 2 diabetes in both sexes (21). Second, a recent analysis of the Women's Health Study data demonstrated that fasting insulin levels are strongly and independently associated with CRP expression (25). Insulin resistance precedes the development of type 2 diabetes, and these observations suggest that elevated CRP expression may occur secondary to reductions in insulin's metabolic actions. In line with these investigations, we observed a significant relationship between the HOMA index and CRP (Fig. 2).

Wei et al. (6) reported a 2.9-fold increased risk for all-cause mortality associated with "low fitness" (defined as the lowest quintile of ~1,250 individuals) in men with type 2 diabetes, even after adjustment for conventional risk factors such as hypertension, dyslipidemia, and smoking habits. Our data provide a possible explanation for this finding because we observed elevated markers of subclinical vascular inflammation in women with type 2 diabetes who had low cardiorespiratory fitness. Our data suggest that the increased CRP with low cardiorespiratory fitness may be mediated through a reduction in fasting glucose control (i.e., the HOMA index). Furthermore, we demonstrate that in a small sample of women with type 2 diabetes, low cardiorespiratory fitness is not associated with changes in left ventricular filling dynamics or arterial compliance.

Several limitations to our study need to be addressed. Because our sample size was less robust than larger epidemiological studies, we chose the median value of cardiorespiratory fitness as criteria for group stratification. We believe that had we tested a larger sample of women with type 2 diabetes and used the lowest quintile to classify "low cardiorespiratory fitness," as others have done (6,26), we would have likely observed much greater differences in fitness and subsequently greater differences in conventional and novel markers of CVD.

It is also possible that our observations were a consequence of differences in body mass rather than cardiorespiratory fitness (27,28). Inasmuch, similar to others (28), we did observe a significant relationship between the HOMA index and BMI (data not shown). However, we did not observe a relationship between BMI and CRP levels ($r = 0.28$, $P = 0.3$). Because increased body mass has a negative impact on cardiorespiratory fitness, it is difficult to distinguish the independent effects of cardiorespiratory fitness or body mass on subclinical vascular inflammation in this investigation. To address this issue, we performed a second analysis of the data with the groups stratified according to time to exhaustion, as others have done (6,26). When the groups were stratified according to time to exhaustion, the differences in body mass were negated (91 ± 16 vs. 89 ± 17 kg in the LCF and ACF groups, respectively); however, the differences in CRP remained significantly different (5.9 ± 4.5 vs. 2.2 ± 1.7 g/l).

Another limitation to this investigation is that the HOMA index is a crude estimate of insulin sensitivity and not a direct measure of insulin-mediated glucose disposal. Despite this limitation, the relationship between the $\dot{V}O_{2peak}$ and HOMA index observed in this investigation was remarkably similar to a recent investigation that measured insulin sensitivity using the euglycemic-hyperinsulinemic clamp technique ($r = 0.42$, $P < 0.001$) (29). Furthermore, HOMA index estimates of insulin sensitivity are closely related to measured values obtained using hyperinsulinemic-euglycemic clamp techniques (-0.796, $P < 0.0001$) in women with type 2 diabetes (30). Although we cannot state that low cardiorespiratory fitness is associated with a reduction in insulin sensitivity in women with type 2 diabetes, our data suggest that this is likely.

Our findings have several important clinical implications. It has been suggested that the increased incidence of type 2 diabetes and CVD in technologically advanced societies is a function of a caloric surplus due to reduced levels of physical activity (31). Furthermore, recent evidence suggests that reduced cardiorespiratory fitness is an independent risk factor for mortality in healthy and diseased populations (6,26). It is possible that a sedentary lifestyle may lead to reduced insulin sensitivity and increased subclinical vascular inflammation, both of which are considered novel risk factors for CVD (3,14) and type 2 diabetes (21). Our finding of elevated CRP and reduced glucose control in women with type 2 diabetes with low cardiorespiratory fitness demonstrates the negative impact of physical inactivity on CVD risk in this population. Taken together, these data support the role of regular exercise in the prevention and treatment of metabolic and cardiovascular disorders associated with type 2 diabetes.

In conclusion, the results of the present study demonstrate that low cardiorespiratory fitness is associated with elevated CRP levels and reduced fasting glucose control in women with type 2 diabetes. Furthermore, our results suggest a link between fasting glucose control and CRP in women with type 2 diabetes.

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