Exercise Reduces Resistin and Inflammatory Cytokines in Patients With Type 2 Diabetes

NIKOLAOS P. KADOGLOU, MD[1,2]
DESPINA PERREA, PHD[3]
FOTIOS ILIADIS, MD[1]
NIKOLETA ANGELOPOULOU, MD[4]
CHRISTOS LIAPIS, FACS, FRCS[2]
MILTIADIS ALEVIZOS, MD[1]

Type 2 diabetes is associated with an excessive risk of cardiovascular events (1). On the other hand, physical activity reduces cardiovascular morbidity in diabetic patients (2). Resistin and numerous inflammatory markers (e.g., high-sensitivity C-reactive protein [hsCRP], interleukin [IL]-6, and IL-18) have emerged as novel predictors of cardiovascular diseases (3–5). We hypothesized that exercise could afford pleiotropic cardioprotective actions by modifying these factors in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

The study included 60 overweight/obese patients (BMI >25 kg/m^2) with type 2 diabetes who consented to participate. All patients were on a stable antidiabetes regimen (sulfonylureas and/or metfromin) but with inadequate glycemic control (A1C >6.5%). Smokers and patients receiving lipid-lowering medications, insulin, or thiazolidinediones were rejected. Those with diabetic vascular complications, life-threatening diseases, orthopedic problems, or liver and renal impairment were also excluded. Participants retained their eating patterns, and they were randomly assigned to either the exercise group (n = 30) or control group (n = 30).

Exercise training

All subjects were inactive, and none reported engaging in systemic (more than one time per week) sport activities before the study. Patients in the exercise group underwent a 16-week aerobic exercise training program consisting of four 45–60 min sessions per week (50–85% maximum oxygen consumption [$V\text{O}_{2\text{max}}$]). Exercise modality was based on the recent recommendations of the American Diabetes Association (6). The workload was individualized according to the initial physical fitness assessment and gradually increased with continuous electocardiographic measurement. Aerobic exercise consisted mainly of walking or running on a treadmill, cycling, and calisthenics involving upper and lower limbs. Moreover, subjects in the exercise group were encouraged to increase daily physical activities (brisk walking, etc.). Control subjects were instructed to maintain their habitual activities. Anthropometric parameters (BMI, body weight, and waist-to-hip ratios), fasting plasma glucose, A1C, lipid profile, resistin, hsCRP, IL-18, IL-6, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), cardiorespiratory capacity, and blood pressure were measured at baseline and at the end of the study.

Laboratory and clinical measurements

All participants refrained from any severe physical activity 48 h before measurements. Plasma resistin (BioVendor Laboratory Medicine, Brno, Czech Republic), insulin (DRG Diagnostics, Marburg, Germany), and IL-18 (IBL Immuno-Biological Laboratories, Hamburg, Germany) were quantified using commercially available enzyme-linked immunosorbent assay kits. The inter- and intra-assay coefficients of variance were 6.0 and 3.1% for resistin, 3.0 and 3.4% for insulin, 12.9 and 3.3% for IL-18, and 12.6 and 5.7% for IL-6, respectively. Insulin resistance was estimated by HOMA-IR. Samples were frozen and stored (−80°C) until analysis in the same assay.

All patients performed a graded symptom-limited exercise test on electrically braked ergocycle (7). Oxygen uptake and carbon dioxide output were measured continuously using a Cosmed K4b^2 metabolic system (Cosmed, Rome, Italy). $V\text{O}_{2\text{max}}$ was obtained according to a previous study (8). The heart rate response to the aforementioned test was used to prescribe exercise intensity during exercise intervention.

Statistical analysis

Comparisons within groups were performed by paired Student's t test and between groups by Student's independent t test and Mann-Whitney U test. Normality of distribution was assessed by Kolmogorov-Smirnov test. Correlations were performed using Pearson's correlation coefficient for univariate analysis and multiple regression analysis for all variables. A P value of <0.05 was considered statistically significant.

RESULTS

Of the 54 patients who completed the study, there were 28 in the exercise group and 26 in the control group. Due to time constraints, six patients discontinued the study. Concerning all variables, no significant differences were observed between groups at baseline ($P > 0.05$).

From the [1]First Propedeutic Department of Internal Medicine, AHEPA University Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece; the [2]Department of Vascular Surgery, University Medical School, University of Athens, Athens, Greece; the [3]Laboratory of Experimental Surgery and Surgical Research, University of Athens, Athens, Greece; and the [4]Department of Physical Education and Sports Science, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Address correspondence and reprint requests to Nikolaos P. Kadoglou, 124 Vosporou St., 54454 Thessaloniki, Greece. E-mail: nikoskad@yahoo.com.

Received for publication 4 June 2006 and accepted 27 November 2006.

HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL, interleukin.

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

DOI: 10.2337/dc06-1149. Clinical trial reg. no. NCT00306176, clinicaltrials.gov.

Table 1—*Values of all variables at baseline and after 16 weeks of intervention*

	Exercise group			Control group				
Variable	Baseline	After	P_1	Baseline	After	P_1	P_2	P_3
n (male/female subjects)	28 (12/16)	—	—	26 (11/15)	—	—	—	—
Age (years)	56.83 ± 6.7	—	—	62.32 ± 9.28	—	—	—	—
Duration of diabetes (years)	6.50 ± 4.64	—	—	7.78 ± 4.90	—	—	—	—
SULF	3	—	—	3	—	—	—	—
MET	10	—	—	9	—	—	—	—
SULF + MET	15	—	—	14	—	—	—	—
Patients on anti-hypertensive medication (%)	19 (67.9%)	—	—	16 (61.5%)	—	—	—	—
BMI (kg/m^2)	31.79 ± 3.79	31.12 ± 3.46	0.430	29.92 ± 3.41	30.17 ± 3.46	0.670	0.570	0.620
Weight (kg)	81.91 ± 14.48	80.64 ± 16.47	0.570	77.55 ± 15.10	78.16 ± 14.17	0.720	0.290	0.680
Waist-to-hip ratio	0.970 ± 0.070	0.957 ± 0.048	0.075	0.940 ± 0.100	0.943 ± 0.130	0.390	0.170	0.720
Cholesterol (mg/dl)	225.22 ± 38.28	206.69 ± 40.85	0.016	234.95 ± 39.04	242.37 ± 42.51	0.580	0.012	0.048
HDL (mg/dl)	50.26 ± 15.25	53.48 ± 15.92	<0.001	54.60 ± 11.31	52.16 ± 12.5	0.340	0.007	0.840
LDL (mg/dl)	148.30 ± 32.90	129.45 ± 30.86	0.016	150.74 ± 36.68	151.65 ± 2.40	0.940	0.011	0.036
Triglycerides (mg/dl)	145.57 ± 54.33	130.44 ± 51.94	0.033	145.37 ± 63.7	165.32 ± 85.82	0.075	0.009	0.014
A1C (%)	7.70 ± 1.11	6.99 ± 1.08	<0.001	7.65 ± 1.01	7.96 ± 0.97	0.013	<0.001	0.005
Fasting plasma glucose (mg/dl)	167.65 ± 33.31	145.55 ± 32.56	0.002	175.94 ± 26.83	184.22 ± 31.00	0.029	<0.001	0.001
Systolic blood pressure (mmHg)	133.95 ± 19.75	126.05 ± 20.03	0.007	141.11 ± 16.05	141.39 ± 17.13	0.980	0.010	0.021
Diastolic blood pressure (mmHg)	81.15 ± 9.99	79.20 ± 9.83	0.059	78.89 ± 9.16	80.83 ± 9.43	0.340	0.084	0.460
Span class = hsCRP (mg/dl)	0.52 ± 0.15	0.33 ± 0.23	0.028	0.39 ± 0.17	0.38 ± 0.11	0.790	0.045	0.130
Insulin (mU/l)	11.00 ± 0.41	9.56 ± 5.15	0.043	8.68 ± 0.53	9.94 ± 5.63	0.480	0.049	0.870
HOMA-IR	4.44 ± 2.49	3.40 ± 2.26	0.027	4.27 ± 2.38	5.01 ± 2.70	0.310	0.035	0.026
Resistin (ng/ml)	17.4 ± 6.59	11.88 ± 4.10	0.031	15.65 ± 2.94	16.73 ± 6.04	0.420	0.033	0.038
IL-6 (pg/ml)	4.51 ± 0.76	2.98 ± 0.60	0.042	4.64 ± 0.55	4.62 ± 1.05	0.860	0.035	0.027
IL-18 (pg/ml)	375.15 ± 112.74	223.7 ± 176.76	0.032	292.98 ± 153.18	342.84 ± 151.26	0.092	0.018	0.013
$V\mathrm{O}_{2max}$ (mg · kg^{-1} · min^{-1})	22.57 ± 4.31	26.26 ± 5.08	<0.001	23.80 ± 5.79	22.71 ± 5.27	0.090	0.001	0.031
Duration test (min)	9.00 ± 1.48	9.77 ± 1.65	<0.001	9.45 ± 1.68	9.06 ± 1.53	0.020	<0.001	0.360

Data are means ± SD unless otherwise indicated. MET, number of patients receiving metformin; P_1, *P* values of levels of variables between baseline versus the end of the study within groups; P_2, *P* values of changes of variables in the exercise versus control group; P_3, *P* values of levels of variables in the exercise versus control group at the end of the study; SULF, number of patients receiving sulfonylurea agent; SULF + MET, number of patients receiving sulfonylurea agent and metformin.

Effects of intervention

Changes of all variables are listed in Table 1. Anthropometric parameters negligibly altered in both groups ($P > 0.05$). As expected, the exercise training induced considerable improvement in mean ± SD A1C (−0.61 ± 0.44 vs. 0.41 ± 0.07%, $P < 0.001$), fasting plasma glucose (−16.58 ± 3.42 vs. 8.28 ± 14.67 mg/dl, $P < 0.001$), HOMA-IR, fasting insulin levels, systolic and diastolic blood pressure, and lipid profile ($P < 0.05$), in comparison with the control group. Exercise capacity ($V\mathrm{O}_{2max}$) increased only in the exercise group (~16.3%).

In contrast to the control group, resistin reduced remarkably in the exercise group (−4.99 ± 0.84 vs. 1.08 ± 0.44 ng/ml, $P = 0.033$). Exercise-treated subjects demonstrated larger reduction of hsCRP (−0.184 ± 0.017 vs. −0.012 ± 0.011 mg/dl, $P = 0.045$) than control subjects. Besides this, exercise training decreased both plasma IL-6 and IL-18 ($P = 0.035$ and $P = 0.018$, respectively).

Correlations

Following training, resistin reduction was associated with alterations in hsCRP ($r = 0.4$, $P = 0.044$), IL-18 ($r = 0.73$, $P = 0.002$), and $V\mathrm{O}_{2max}$ ($r = -0.72$, $P = 0.015$). Changes of IL-18 additionally correlated with body weight ($r = -0.49$), HOMA-IR ($r = 0.31$), $V\mathrm{O}_{2max}$ ($r = -0.49$), and hsCRP ($r = -0.74$, $P < 0.05$). The latter variable was also related to changes in HOMA-IR ($r = 0.74$, $P = 0.009$), glucose ($r = 0.66$, $P = 0.02$), IL-6 ($r = 0.58$, $P < 0.01$), and insulin ($r = 0.65$, $P = 0.046$). Finally, exercise-induced changes of IL-6 were negatively related to $V\mathrm{O}_{2max}$ ($r = -0.54$, $P < 0.01$).

After multiple regression analysis, IL-18, hsCRP, and $V\mathrm{O}_{2max}$ remained independent contributors of resistin changes. These variables explained 77.6% of resistin variance.

CONCLUSIONS — Supervised exercise training reduces resistin, IL-6, hsCRP, and IL-18, while exercise capacity inversely correlates with inflammatory cytokines in diabetic patients, suggesting an alternative explanation for the above anti-inflammatory effects.

As expected, our training program improved all parameters of glucose and insulin regulation (9). Resistin is an emerging cardiovascular risk factor implicated in diabetes (3). Contrary to previous

reports (10,11), our exercise program induced considerable reduction of resistin. This discrepancy may be attributed to different intervention modality (duration, type, and intensity) and selection criteria. Interestingly, subgroup analysis (data not shown) indicated a remarkable decline of resistin in exercise-trained male subjects ($P = 0.01$), whereas this difference became less marked among female subjects ($P = 0.058$). Despite the recent conflicting data (12) on sexual dimorphism of resistin, we cannot exclude a sex-dependent response to exercise.

In agreement with previous studies (12,13), we demonstrated that resistin levels were independently associated with inflammatory markers. In regards to macrophages and adipocytes as the predominant sources of resistin (14), type 2 diabetes (as a low-grade inflammatory condition) seems to induce macrophage expression of resistin. Thus, the inflammation-lowering effects of exercise merely explain resistin decrease.

Nowadays, the key role of IL-6 in diabetes is undisputed (15). Despite previous conflicting data (10,11,16,17), we showed that exercise, per se, lowered IL-6 and hsCRP production, which is a promising finding. IL-18, another novel member of the IL-1 cytokine superfamily, is an independent predictor of cardiovascular events (4,18). Combined intervention of diet and physical activity in postmenopausal obese women has been proven (19) to downregulate IL-18 concentration. This is the first study to provide evidence of decreased IL-18 after long-term supervised exercise, without weight decline. We and others (20) observed that alterations of serum IL-18 correlated with insulin resistance modulation. We postulated that exercise may lower IL-18 concentration via insulin-signaling modification. The anti-inflammatory impact of our exercise regimen is of clinical importance due to the prominent role of inflammation in atherosclerosis (5).

The principal limitation of our study was the small sample. Concerning medications, our study cohort was homogeneous. It is unknown if the same intervention would confer similar results in a group with different characteristics. This study suggests that exercise training, without weight loss, decreases resistin and inflammatory cytokines in patients with type 2 diabetes. Further larger-scale studies would elucidate the underlying mechanisms.

Acknowledgments— This study was financially supported by the Pythagoras I project of the Greek Ministry of National Education and Religion and European Union. N.K. was also awarded a grant by the Propondis Foundation.

References

1. Williams R, Van Gaal L, Lucioni C, the CODE-2 Advisory Board: Assessing the impact of complications on the costs of type II diabetes. *Diabetologia* 45:S13–S17, 2002
2. Tanasescu M, Leitzmann MF, Rimm EB, Hu FB: Physical activity in relation to cardiovascular disease and total mortality among men with type 2 diabetes. *Circulation* 107:2435–2439, 2003
3. Kershaw EE, Flier JS: Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89:2548–2556, 2004
4. Mallat Z, Corbaz A, Scoazec A, Besnard S, Leseche G, Chvatchko Y, Tedgui A: Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation* 104:1598–1603, 2001
5. Ross R: Atherosclerosis: an inflammatory disease. *N Engl J Med* 340:115–126, 1999
6. Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C, White RD: Physical activity/exercise and type 2 diabetes: a consensus statement from the American Diabetes Association. *Diabetes Care* 29: 1433–1438, 2006
7. Lakka TA, Laaksonen DE, Lakka HM, Mannikko N, Niskanen LK, Rauramaa R, Salonen JT: Sedentary lifestyle, poor cardiorespiratory fitness, and the metabolic syndrome. *Med Sci Sports Exerc* 35:1279–1286, 2003
8. McGavock JM, Mandic S, Muhll IV, Lewanczuk RZ, Quinney HA, Taylor DA, Welsh RC, Haykowsky M: Low cardiorespiratory fitness is associated with elevated C-reactive protein levels in women with type 2 diabetes. *Diabetes Care* 27: 320–325, 2004
9. Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C: Physical activity/exercise and type 2 diabetes. *Diabetes Care* 27: 2518–2539, 2004
10. Monzillo LU, Hamdy O, Horton ES, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, Moussa A, Mantzoros CS: Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. *Obes Res* 11:1048–1054, 2003
11. Giannopoulou I, Fernhall B, Carhart R, Weinstock RS, Baynard T, Figueroa A, Kanaley JA: Effects of diet and/or exercise on the adipocytokine and inflammatory cytokine levels of postmenopausal women with type 2 diabetes. *Metabolism* 54:866–875, 2005
12. Steppan CM, Lazar MA: The current biology of resistin. *J Intern Med* 255:439–447, 2004
13. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ: Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 111:932–939, 2005
14. Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA: An inflammatory cascade leading to hyperresistinemia in humans. *PLoS Med* 1:e45, 2004
15. Kristiansen OP, Mandrup-Poulsen T: Interleukin-6 and diabetes: the good, the bad, or the indifferent? *Diabetes* 54 (Suppl. 2):S114–S124, 2005
16. Marcell TJ, McAuley KA, Traustadottir T, Reaven PD: Exercise training is not associated with improved levels of C-reactive protein or adiponectin. *Metabolism* 54: 533–541, 2005
17. Goldhammer E, Tanchilevitch A, Maor I, Beniamini Y, Rosenschein U, Sagiv M: Exercise training modulates cytokines activity in coronary heart disease patients. *Int J Cardiol* 100:93–99, 2005
18. Blankenberg S, Luc G, Ducimetiere P, Arveiler D, Ferrieres J, Amouyel P, Evans, Cambien F, Tiret L, the PRIME Study Group: Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation* 108:2453–2459, 2003
19. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Guglano D: Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 289:1799–1804, 2003
20. Fischer CP, Perstrup LB, Berntsen A, Eskildsen P, Pedersen BK: Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. *Clin Immunol* 117:152–160, 2005