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Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss

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Abstract *Background and aims:* We investigated the effect of different exercise modalities on high sensitivity-C reactive protein (hs-CRP) and other inflammatory markers in patients with type 2 diabetes and the metabolic syndrome.
Methods and results: Eighty-two patients were randomized into 4 groups: sedentary control (A); receiving counseling to perform low-intensity physical activity (B); performing prescribed and supervised high-intensity aerobic (C) or aerobic + resistance (D) exercise (with the same caloric expenditure) for 12 months. Evaluation of leisure-time physical activity and assessment of physical fitness, cardiovascular risk factors and inflammatory biomarkers was performed at baseline and every 3 months. Volume of physical activity increased and HbA_{1c} decreased in Groups B–D. VO_{2max}, HOMA-IR index, HDL-cholesterol, waist circumference and albuminuria

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improved in Groups C and D, whereas strength and flexibility improved only in Group D. Levels of hs-CRP decreased in all three exercising groups, but the reduction was significant only in Groups C and D, and particularly in Group D. Changes in VO_{2max} and the exercise modalities were strong predictors of hs-CRP reduction, independent of body weight. Leptin, resistin and interleukin-6 decreased, whereas adiponectin increased in Groups C and D. Interleukin-1β, tumor necrosis factor-α and interferon-γ decreased, whereas anti-inflammatory interleukin-4 and 10 increased only in Group D.

Conclusion: Physical exercise in type 2 diabetic patients with the metabolic syndrome is associated with a significant reduction of hs-CRP and other inflammatory and insulin resistance biomarkers, independent of weight loss. Long-term high-intensity (preferably mixed) training, in addition to daytime physical activity, is required to obtain a significant anti-inflammatory effect.

Introduction

Cardiovascular disease (CVD) represents the main cause of morbidity and mortality in patients with type 2 diabetes. The 2—4-fold increase in CVD risk in diabetic vs. nondiabetic subjects [1] has been attributed primarily to traditional CVD risk factors, including chronic hyperglycemia as well as central obesity, dyslipidemia and hypertension and clustering with insulin resistance in the setting of the metabolic syndrome (MS) [2], the prevalence of which is >80% in type 2 diabetic patients.

Chronic low-grade inflammation has recently emerged as the common denominator linking type 2 diabetes, MS, insulin resistance, endothelial dysfunction and CVD [3]. In particular, a growing body of evidence has indicated a fundamental role for inflammation in mediating all stages of atherosclerosis [4] and several pro-inflammatory mediators have been associated with CVD, independent of traditional CVD risk factors.

In particular, the acute phase reactant C-reactive protein (CRP) was shown to be an independent predictor of CVD [5,6] and of the outcome of acute coronary syndromes [7]. The most recent American Heart Association (AHA) consensus statement recommended the use of high sensitivity (hs)-CRP to further stratify patients at intermediate (10—20%) 10-year risk according to the Framingham score [8]. Recently, CRP, known to be produced primarily by the liver in response to inflammatory cytokines such as IL-6, was also shown to be generated in adipose tissue [9] and atherosclerotic plaques [10] and to actively participate in the pathogenesis of atherosclerosis by promoting endothelial cell activation, macrophage recruitment, and foam cell generation within the arterial wall [11]. Other pro-inflammatory cytokines have also been implicated in CVD, including interleukin (IL)-6 [5] and tumor necrosis factor (TNF)-α [6].

In the general population, several studies have shown that levels of physical activity and cardiorespiratory fitness are inversely correlated to CRP [12] and that regular exercise significantly reduces circulating levels of CRP and other inflammatory mediators [13,14]. From a review of cross-sectional and longitudinal studies, chronic training produces an anti-inflammatory effect [15], though a number of studies, including a meta-analysis [16], have shown that exercise programs do not significantly influence inflammatory markers. Moreover, it is uncertain whether exercise has a direct effect on CRP levels independent of weight loss, which has consistently been shown to reduce CRP levels [17].

A previous large trial in subjects with impaired glucose tolerance (IGT) demonstrated that physical activity is effective in reducing CRP [18], whereas few studies have prospectively examined the effect of exercise on elevated levels of inflammatory biomarkers in diabetic subjects and found contrasting results in terms of efficacy and dependence on weight loss [19,20]. Moreover, the type, dose and intensity of physical activity needed to obtain a significant anti-inflammatory effect in this high-risk population are largely unknown.

This study was aimed at investigating the effect of different exercise modalities on circulating levels of several inflammatory markers, including high sensitivity (hs)-CRP considered as the primary endpoint, in type 2 diabetic patients with the MS and no history of CVD.

Methods

Subjects and design

Eighty-two subjects with type 2 diabetes and the MS, defined according to the IDF criteria [2], and without any known CVD were recruited. Additional requirements were age 40—75 years, diabetes duration >1 year, BMI 27—40 kg/m^2, ability to walk without assistance and eligibility after cardiovascular evaluation.

Patients were randomized into 4 groups: control subjects who remained sedentary throughout the study period (Group A; $n = 20$); subjects who received a structured exercise counseling [21] to perform aerobic physical activity of low-intensity (Group B; $n = 20$); subjects who performed a sustainable program of prescribed and supervised aerobic activity only (Group C; $n = 20$) or combined aerobic and resistance (Group D; $n = 22$) exercise of high-intensity for 12 months. All patients received standard diabetes care including pharmacological treatment and dietary prescriptions. Physicians were blinded to the assignment of groups.

Gender, age, diabetes duration and smoking habits were registered at enrolment. Blood pressure (BP), body weight and height, and waist circumference were measured at baseline and every 3 months throughout the study using standard methods. At the same timepoints, fat and fat-free mass were assessed, fasting blood and morning urine samples were obtained for the measurement of

biochemical markers of CVD risk and leisure-time physical activity (LTPA) was estimated as indicated below.

Exercise program

The training program consisted of twice a week supervised sessions of 60 min of aerobic exercise at 70—80% VO_{2max} for Group C patients and 40 min aerobic exercise at 70—80% VO_{2max} + 20 min resistance exercise at 80% 1 repetition maximum (RM) for Group D subjects. Aerobic exercise was performed using a treadmill and/or cycloergometer. The strength training consisted of four resistance exercises, i.e. thrust movement on the transverse plane (chest press or equivalent), traction movement on the frontal plane (lateral pull down or equivalent), squat movement (leg press or equivalent), trunk flexion for the abdominals, and three stretching positions. Volume was progressively increased by 0.1 kcal/kg body weight/month and the intensity level was adjusted according to improvements in VO_{2max} and 1RM, as recorded every 3 months (see below). To allow comparisons, the caloric expenditure was the same in subjects from Groups C and D.

LTPA

The Minnesota LTPA questionnaire [22] was used to retrospectively estimate the volume of physical activity at baseline. LTPA during the study period was evaluated prospectively by asking patients to fill in a daily diary based on the range of physical activities considered in the Minnesota LTPA questionnaire. Volume was calculated by multiplying the metabolic equivalent (MET) scores (one MET expresses oxygen consumption, taken by convention to be 3.5 ml) corresponding to each Minnesota code (according to the Compendium of Physical Activities Tracking Guide [23]) by the hours per week spent in each activity, and expressed as METs h^{-1} $week^{-1}$. Energy expenditure during supervised sessions was calculated automatically by the machines from workload (i.e. the combination of velocity and slope for treadmill and power for cycloergometer) and time, using standard equations [24], for aerobic exercise, and conventionally fixed at 3 METs h^{-1}, for resistance.

Physical fitness

Estimation of VO_{2max}, a 5—8-RM test and a standard bending test were performed to evaluate cardiorespiratory fitness, strength and flexibility and results were expressed as ml/kg/min, kg and cm of distance from the ground, respectively [25]. Groups C and D subjects were evaluated every 3 months, in order to adjust the intensity level, whereas Groups A and B subjects only at timepoints 0 and 12 months.

Body composition

Fat and fat-free mass were measured by a BF664 bioimpedance monitor (Tanita, Vernon Hills, IL).

Biochemical parameters

HbA_{1c} was measured by HPLC (Adams TMA1C HA-8160, Menarini Diagnostics, Florence, Italy), blood glucose, serum triglycerides, total and HDL-cholesterol, high sensitivity (hs)-CRP, BUN, uric acid, and serum and urinary creatinine by VITROS 5,1 FS Chemistry System (Ortho-Clinical Diagnostics, Raritan, NJ), serum C-peptide and insulin by chemiluminescent immunometric assays (Immulite 2000 Thes, Diagnostic Products Co., Los Angeles, CA), urinary albumin by mAlb VITROS (Ortho-Clinical Diagnostics), serum IL-1β, IL-4, IL-6, IL-10, TNF-α, and interferon (IFN)-γ by Biochip Array Technology (Evidence®, Randox Laboratories, Crumlin, UK), and serum leptin, resistin and adiponectin by ELISA kits (from DRG Instruments, Germany, for leptin, and Biovendor, Czech Republic, for resistin and adiponectin). Intra- and inter-assay coefficients of variation for cytokine assays were 5—10%. LDL-cholesterol, the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index and the albumin/creatinine ratio (A/C) were calculated using standard formulas.

Power calculation

This sample size allowed us to detect a minimum difference between groups in hs-CRP levels of 2 mg/L, assuming a SD of 2.2 mg/L ($\alpha = 0.05$, $1-\beta = 0.80$), derived from baseline values of a large trial using the same assay [25].

Statistical analysis

Values are reported as mean ± SD (SEM in graphs), except for categorical variables (frequencies and percentages) and albuminuria (median and interquartile range). Patient characteristics at baseline according to study group were compared using ANOVA and Kruskall Wallis one-way ANOVA for normally and non-normally distributed continuous variables, respectively; categorical variables were compared using the Pearson χ^2 test. Within-group comparisons were performed by ANOVA repeated measures and Friedman test, followed by Student—Neuman—Keuls test and Dunn test, for parametric and nonparametric data, respectively, for variables assessed every 3 months, and using paired *t* test and Wilcoxon matched-pairs signed-ranks test, for parametric and nonparametric data, respectively, for variables assessed at baseline and 12 months. All endpoints were further analyzed with a hierarchical linear model for repeated measurements to assess differences between groups over time [26]. Post hoc comparisons between groups were assessed and *p*-values were adjusted for multiple testing according to Hochberg's method [27]. *p*-Values < 0.05 were considered statistically significant. To verify the independent effect of exercise modality on hs-CRP, multiple regression analysis with backward variable selection was applied, with hs-CRP changes from baseline as the dependent variable and mean value of METs, exercise study groups and changes in VO_{2max}, BMI, waist circumference, HbA1c, HDL-cholesterol and triglycerides as covariates. All analyses were done according to an intention-to-treat protocol and were performed using SAS Statistical Package Release 9.1 (SAS Institute, Cary, NC).

Results

No significant difference among groups was detected at baseline for any clinical characteristics, including gender,

age, diabetes duration, smoking habits and current drug therapy, including statin treatment, which is known to affect CRP [28] (Table 1). Fitness parameters, traditional CVD risk factors and inflammatory biomarkers were also similar among groups (Tables 2 and 3 and Figs. 1 and 2).

Minor musculo-skeletal injury/discomfort requiring temporary modification of the exercise program occurred in 3 of the 20 Group B subjects (15%) and 16 of the 42 subjects (38%) randomized to the supervised and prescribed intervention ($p < 0.01$ vs. Group B). Nevertheless, attendance to supervised sessions was as high as 80% (interquartile range 71–87%) in Group C and 81% (interquartile range 72–89%) in Group D. Five (6.1%) subjects withdrew between randomization and the end of the study: 1 (5%) in Group B, 2 (10%) in Group C and 2 (9.1%) in Group D but they were included in the analysis. No episode of severe hypoglycemia requiring assistance occurred.

LTPA increased significantly from baseline in Groups B, C and D although overall volume of activity was slightly, but not significantly higher in Groups C and D than in Group B. LTPA values from Groups C and D included activity performed during the sessions of prescribed and supervised exercise, which was similar in the two groups according to the study design (8.2 ± 1.2 and 8.1 ± 1.7 METs h^{-1} $week^{-1}$, respectively). Also VO_{2max} increased significantly with time in Groups C and D, whereas strength and flexibility improved only in Group D (Table 2). Increases in VO_{2max} were not due to weight loss since data in absolute values were similar to those normalized per body weight (not shown).

Glucose, triglycerides, total and LDL-cholesterol, systolic and diastolic BP, body weight, BMI, fat and fat-free mass, BUN, uric acid and creatinine did not change significantly during the 12-month study period in all groups, whereas HbA_{1c} decreased in the three exercising groups, and HOMA-IR index (together with insulin and C-peptide), HDL-cholesterol, waist circumference and A/C ratio improved in C and D Groups (Table 2). Pharmacological treatment did not change significantly from baseline in all study groups for any of the drug classes considered (not shown). Beta estimate and *p*-value adjusted for multiple comparisons showed improvements of HbA_{1c}, waist circumference, fat and fat-free mass in Group D and HDL-cholesterol and insulin levels in Groups C and D vs. Group A (Table 3).

Levels of hs-CRP decreased in all three exercising groups, with a 12%, 28% and 54% reduction from baseline observed at 12 months in Groups B, C and D, respectively, which was significant in Group C and to a much higher extent in Group D subjects (Fig. 1A); Group D subjects also showed significantly higher changes than Group A over 1 year (Table 3). Moreover, approximately 50% of Group C and D patients passed from a higher to a lower hs-CRP risk class according to the AHA consensus statement [8] (vs. 30% in

Table 1 Baseline clinical characteristics of patients.

Characteristics	Group A	Group B	Group C	Group D	*p*-Value
Gender, F/M	9/11	9/11	8/12	8/14	
Age, years (mean ± SD)	61.1 ± 7.1	62.5 ± 7.1	64.3 ± 8.1	60.6 ± 9.3	ns
Duration of diabetes, years (mean ± SD)	7.8 ± 5.2	10.1 ± 7.3	9.4 ± 6.0	8.5 ± 5.7	ns
Smoking, *n* (%)					
Never	14 (70)	14 (70)	15 (75)	17 (77)	ns
Former	3 (15)	4 (20)	3 (15)	2 (9)	ns
Current	3 (15)	2 (10)	2 (10)	3 (14)	ns
Medications, *n* (%)					
Oral hypoglycemic agents	14 (70)	18 (90)	15 (75)	19 (86)	ns
Sulfonylurea	3 (15)	8 (40)	5 (25)	5 (23)	ns
Glinide	6 (30)	6 (30)	7 (35)	4 (18)	ns
Metformin	9 (45)	13 (65)	11 (55)	18 (82)	ns
Thiazolidinedione	4 (20)	4 (20)	4(20)	5 (23)	ns
Insulin use	4 (20)	2(10)	3 (15)	3 (14)	ns
Antihypertensive agents	13 (65)	15 (75)	13 (65)	13 (59)	ns
Angiotensin-converting enzyme inhibitor	5 (25)	5 (25)	3 (15)	6 (27)	ns
Angiotensin-receptor blocker	12 (60)	9 (45)	8 (40)	8 (36)	ns
Diuretic	6 (30)	13 (65)	7 (35)	5 (23)	ns
Calcium-channel blocker	2 (10)	2 (10)	2 (10)	2 (9.1)	ns
Beta-blocker	2 (10)	0 (0)	1 (5)	3 (14)	ns
Alpha1-adrenergic blocker	0 (0)	0 (0)	1 (5)	2 (9.1)	ns
Lipid-lowering agents	9 (45)	9 (45)	10 (50)	11 (50)	ns
Statin	8 (40)	8 (40)	10 (50)	9 (41)	ns
Fibrate	0 (0)	0 (0)	0 (0)	1 (4.5)	ns
ω-3	2 (10)	2 (10)	0 (0)	0 (0)	ns
Antiplatelet agents	10 (50)	9 (45)	7 (35)	6 (27)	ns

Table 2 Values of physical fitness and selected traditional cardiovascular risk factors at baseline and at 3, 6, 9 and 12 months (mean ± SD, *p*-values for ANOVA repeated measures, except for A/C ratio, median and interquartile range, *p*-values for Friedman test).

	Baseline	3 Months	6 Months	9 Months	12 Months	*p*-Value
LTPA,[a] METs h^{-1} $week^{-1}$						
Group A	12.9 ± 2.7	13.0 ± 5.4	11.6 ± 5.0	12.7 ± 5.6	12.31 ± 3.6	ns
Group B	13.9 ± 2.8	19.9 ± 5.3	21.0 ± 6.0	19.9 ± 6.0	19.9 ± 6.3	0.0001
Group C[a]	13.4 ± 5.3	22.9 ± 4.2	22.7 ± 4.1	22.8 ± 4.0	22.3 ± 4.0	0.0001
Group D[a]	13.2 ± 5.3	23.4 ± 7.2	22.6 ± 7.3	25.4 ± 7.7	23.7 ± 7.2	0.0001
VO_{2max}, ml/kg/min						
Group A	23.8 ± 6.5	NA	NA	NA	23.6 ± 7.1	ns
Group B	25.1 ± 7.4	NA	NA	NA	26.3 ± 6.4	ns
Group C	25.0 ± 6.1	28.8 ± 4.9	29.8 ± 4.6	29.3 ± 4.6	31.5 ± 4.7	0.0001
Group D	25.5 ± 6.6	29.7 ± 5.7	30.7 ± 5.5	30.8 ± 5.7	32.0 ± 6.3	0.0001
Muscle strength, kg						
Upper body						
Group A	36.2 ± 17.7	NA	NA	NA	37.2 ± 19.6	ns
Group B	41.8 ± 23.2	NA	NA	NA	43.2 ± 22.7	ns
Group C	42.5 ± 21.7	44.7 ± 21.6	44.3 ± 19.4	43.1 ± 20.4	43.3 ± 21.3	ns
Group D	47.3 ± 20.9	59.8 ± 23.6	64.0 ± 24.2	65.5 ± 22.0	68.4 ± 23.4	0.0266
Lower body						
Group A	68.7 ± 43.9	NA	NA	NA	68.1 ± 45.6	ns
Group B	84.5 ± 61.4	NA	NA	NA	86.1 ± 57.5	ns
Group C	73.1 ± 56.4	77.5 ± 56.5	81.1 ± 47.3	82.7 ± 52.1	84.9 ± 55.9	ns
Group D	85.9 ± 54.1	114.6 ± 64.2	114.8 ± 59.8	119.4 ± 62.1	134.2 ± 68.8	0.0001
HbA1c, %						
Group A	7.08 ± 1.28	7.09 ± 1.30	7.07 ± 1.29	7.21 ± 1.50	6.93 ± 1.29	ns
Group B	7.50 ± 1.26	7.50 ± 1.15	7.42 ± 1.19	7.11 ± 1.06	7.05 ± 1.22	0.0019
Group C	7.29 ± 1.35	6.94 ± 0.92	7.14 ± 1.05	6.72 ± 1.01	6.34 ± 0.96	0.0001
Group D	7.74 ± 1.72	7.37 ± 1.28	7.25 ± 1.08	6.92 ± 1.32	6.65 ± 1.10	0.0001
HOMA-IR, index						
Group A	4.33 ± 2.87	4.68 ± 2.89	4.95 ± 2.29	4.59 ± 2.77	4.89 ± 1.90	ns
Group B	4.15 ± 3.47	4.13 ± 2.93	4.77 ± 2.41	4.53 ± 2.56	4.22 ± 2.07	ns
Group C	4.13 ± 1.66	3.46 ± 1.66	3.39 ± 1.69	2.84 ± 1.31	3.28 ± 1.77	0.0084
Group D	4.56 ± 1.73	3.40 ± 1.87	3.12 ± 1.39	3.24 ± 1.81	2.73 ± 1.26	0.0001
Triglycerides, mg/dl						
Group A	181.3 ± 33.0	181.4 ± 29.0	172.7 ± 30.9	175.2 ± 31.6	159.3 ± 26.6	ns
Group B	150.1 ± 29.6	176.6 ± 22.2	187.3 ± 24.9	157.4 ± 16.6	199.9 ± 27.0	ns
Group C	139.4 ± 14.7	158.6 ± 14.7	144.9 ± 10.5	171.4 ± 11.9	156.6 ± 18.9	ns
Group D	191.8 ± 39.2	179.5 ± 38.1	178.0 ± 23.9	145.0 ± 16.5	164.9 ± 20.5	ns
Total cholesterol, mg/dl						
Group A	225.4 ± 7.8	194.9 ± 9.0	218.2 ± 11.5	227.5 ± 11.5	221.2 ± 7.0	0.0062
Group B	188.5 ± 9.8	188.6 ± 9.2	199.6 ± 8.3	193.9 ± 8.2	201.3 ± 8.3	ns
Group C	199.3 ± 6.2	193.1 ± 9.9	197.8 ± 8.8	207.4 ± 8.8	199.1 ± 6.8	ns
Group D	205.9 ± 7.8	186.6 ± 8.3	201.0 ± 5.0	192.9 ± 4.2	190.3 ± 4.8	ns
HDL-cholesterol, mg/dl						
Group A	45.7 ± 2.3	46.4 ± 2.3	45.8 ± 2.4	45.5 ± 2.4	44.9 ± 2.0	ns
Group B	45.5 ± 2.0	46.1 ± 2.0	46.6 ± 2.1	46.8 ± 1.9	45.3 ± 1.5	ns
Group C	44.1 ± 2.0	46.6 ± 2.2	46.9 ± 2.0	47.2 ± 1.6	47.6 ± 1.8	0.0010
Group D	44.0 ± 2.3	46.0 ± 2.1	49.0 ± 1.9	49.0 ± 1.7	48.2 ± 1.6	0.0001
LDL-cholesterol, mg/dl						
Group A	125.6 ± 7.2	110.8 ± 6.5	136.4 ± 9.0	145.6 ± 8.0	144.4 ± 9.1	0.0057
Group B	112.4 ± 10.0	106.7 ± 8.9	115.0 ± 7.0	115.2 ± 6.9	115.9 ± 6.4	ns
Group C	127.1 ± 6.4	114.8 ± 8.7	121.6 ± 8.9	125.3 ± 8.2	119.8 ± 7.0	ns
Group D	127.2 ± 8.3	112.9 ± 8.1	117.2 ± 5.3	117.4 ± 4.9	113.2 ± 5.2	ns

Table 2 (*continued*)

	Baseline	3 Months	6 Months	9 Months	12 Months	*p*-Value
Systolic BP, mmHg						
Group A	145.0 ± 4.0	146.0 ± 4.0	144.5 ± 4.3	141.0 ± 3.9	142.3 ± 4.5	ns
Group B	138.5 ± 3.2	142.5 ± 3.7	141.4 ± 3.5	144.0 ± 3.0	140.8 ± 3.2	ns
Group C	139.8 ± 3.1	136.0 ± 3.3	138.0 ± 2.6	136.0 ± 2.4	134.8 ± 1.9	ns
Group D	142.1 ± 3.3	137.3 ± 3.3	137.3 ± 2.7	138.0 ± 3.2	136.8 ± 2.2	ns
Diastolic BP, mmHg						
Group A	87.4 ± 2.2	85.3 ± 2.4	85.0 ± 2.0	83.8 ± 2.3	85.5 ± 1.9	ns
Group B	84.8 ± 1.2	84.0 ± 1.2	82.2 ± 1.6	85.5 ± 1.7	82.8 ± 1.8	ns
Group C	82.5 ± 1.5	81.3 ± 1.7	83.0 ± 1.4	81.8 ± 1.2	80.0 ± 1.5	ns
Group D	83.4 ± 1.8	82.6 ± 1.6	82.7 ± 0.9	82.7 ± 1.1	80.2 ± 0.8	ns
Body weight, kg						
Group A	82.2 ± 2.7	80.5 ± 2.6	81.6 ± 2.6	81.9 ± 2.5	82.6 ± 2.7	0.0495
Group B	80.3 ± 3.2	80.0 ± 3.2	80.2 ± 3.2	80.2 ± 3.3	81.5 ± 2.6	ns
Group C	79.5 ± 3.6	78.9 ± 3.6	78.0 ± 3.6	79.2 ± 3.5	79.0 ± 3.5	ns
Group D	84.6 ± 2.9	83.8 ± 2.8	84.0 ± 2.8	84.0 ± 2.8	85.2 ± 2.9	0.0196
BMI, kg/m^2						
Group A	30.9 ± 1.1	30.3 ± 1.0	30.6 ± 1.1	30.6 ± 1.1	31.0 ± 1.1	ns
Group B	30.0 ± 1.0	29.9 ± 1.0	30.6 ± 1.2	30.0 ± 0.9	30.0 ± 1.0	ns
Group C	29.4 ± 1.1	29.14 ± 1.2	29.0 ± 1.1	29.2 ± 1.2	29.1 ± 1.1	ns
Group D	30.5 ± 0.9	30.0 ± 0.9	30.0 ± 0.8	30.1 ± 0.9	30.2 ± 0.8	ns
Waist circumference, cm						
Group A	101.3 ± 3.1	100.2 ± 2.6	101.3 ± 2.6	101.3 ± 2.6	101.3 ± 2.6	ns
Group B	100.0 ± 2.4	98.9 ± 2.1	99.2 ± 2.2	98.9 ± 1.9	99.7 ± 2.1	ns
Group C	99.8 ± 2.7	98.2 ± 2.8	97.9 ± 2.5	97.7 ± 2.6	97.6 ± 2.6	0.0033
Group D	102.0 ± 2.8	99.6 ± 2.6	98.6 ± 2.4	97.5 ± 2.4	97.0 ± 2.4	0.0001
Fat mass, %						
Group A	33.2 ± 2.3	33.7 ± 2.3	33.7 ± 2.3	34.5 ± 2.4	33.9 ± 2.3	ns
Group B	32.9 ± 2.5	33.5 ± 2.6	33.2 ± 2.5	33.9 ± 2.6	33.9 ± 2.4	ns
Group C	32.2 ± 2.4	31.3 ± 2.6	31.4 ± 2.6	33.6 ± 2.9	32.4 ± 2.4	ns
Group D	32.2 ± 1.8	31.0 ± 1.8	31.5 ± 1.8	31.7 ± 1.8	31.6 ± 1.7	ns
Fat-free mass, %						
vGroup A	66.8 ± 2.3	66.3 ± 2.3	66.3 ± 2.3	65.5 ± 2.4	66.1 ± 2.3	ns
Group B	67.1 ± 2.5	66.5 ± 2.6	66.8 ± 2.5	66.1 ± 2.6	66.1 ± 2.4	ns
Group C	67.8 ± 2.4	68.7 ± 2.6	68.6 ± 2.6	66.4 ± 2.9	67.64 ± 2.4	ns
Group D	67.8 ± 1.8	69.0 ± 1.8	68.5 ± 1.8	68.3 ± 1.8	68.46 ± 1.7	ns
A/C ratio, mg/g						
Group A	10.3 (6.1—16.4)	8.6 (6.3—22.8)	11.0 (7.7—15.6)	11.5 (8.7—18.14)	9.9 (5.2—13.7)	ns
Group B	11.1 (7.2—21.9)	8.7 (5.1—22.8)	14.5 (7.5—19.5)	10.0 (6.9—18.2)	11.3 (7.4—22.1)	ns
Group C	11.9 (8.5—18.5)	6.1 (4.9—15.0)	7.5 (5.9—11.4)	8.5 (5.8—13.6)	11.1 (7.3—17.3)	0.0011
Group D	11.7 (7.3—39.5)	8.1 (5.8—15.0)	11.6 (7.0—17.8)	9.7 (6.9—13.2)	7.7 (5.6—18.6)	0.0446

[a] LTPA values for Group C and D patients include the high-intensity physical activity performed during the supervised sessions, as measured by the machines.

Group B), whereas none in Group D and only 2 subjects in Group C increased their hs-CRP risk class. Multiple regression analysis showed that VO_{2max} ($\beta = -2.68$; $p = 0.009$), exercise volume (i.e. the mean value of METs: $\beta = -2.08$; $p = 0.04$) and type (Group D only: $\beta - 4.52$; $p = 0.000$) and waist variation ($\beta = -5.66$; $p = 0.000$) were independent predictors of changes in hs-CRP levels. BMI change became an independent predictor when waist circumference was excluded from the model, whereas fat mass, though significantly correlated to waist circumference ($R = 0.64$; $p < 0.0001$), was not associated with hs-CRP changes even when both waist circumference and BMI were excluded. All together, these variables explained 73% of the variance in hs-CRP changes ($R^2 = 0.73$).

Significant reductions from baseline were observed for leptin (by 27% and 47%) and resistin (by 14% and 22%) levels, together with an increase of adiponectin (by 36% and 38%), in Groups C and D respectively (Fig. 1B,D). In addition, the pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and IFN-γ decreased significantly from baseline in Group D (by 41%,

Table 3 Beta estimate and *p*-value adjusted for multiple comparisons for 1-year changes in metabolic parameters and inflammatory biomarkers.

	B vs. A	C vs. A	D vs. A	Overall
HbA1c, %	−0.31 (0.68)	−0.80 (0.06)	−0.94 (0.02)	0.026
Insulin, ng/ml	−1.20 (0.54)	−4.59 (0.01)	−3.70 (0.03)	0.12
HDL-C, mg/dl	0.55 (0.71)	4.25 (0.016)	4.98 (0.004)	0.04
Waist, cm	−0.3 (0.84)	−2.25 (0.25)	−4.95 (0.003)	0.017
Fat mass, %	−1.89 (0.72)	−5.16 (0.04)	−5.12 (0.04)	0.06
Fat-free mass, %	1.89 (0.72)	5.16 (0.04)	5.12 (0.04)	0.06
hs-CRP, mg/dl	−0.5 (0.51)	−1.0 (0.46)	−1.9 (0.04)	0.30
IL-1β, pg/ml	−0.24 (0.95)	−0.03 (0.95)	−0.46 (0.95)	0.79
IL-6, pg/ml	0.09 (0.90)	−1.40 (0.22)	−1.95 (0.047)	0.016
TNF-α, pg/ml	−0.07 (0.98)	−5.48 (0.11)	−5.88 (0.09)	0.024
IFN-γ, pg/ml	0.09 (0.91)	−1.05 (0.60)	−1.40 (0.33)	0.16
IL-4, pg/ml	0.08 (0.80)	0.39 (0.62)	0.68 (0.10)	0.09
IL-10, pg/ml	0.21 (0.38)	0.23 (0.38)	0.53 (0.11)	0.17
Leptin, ng/ml	1.03 (0.75)	−2.06 (0.75)	−5.06 (0.42)	0.22
Resistin, ng/ml	0.34 (0.86)	−0.08 (0.86)	−0.41 (0.86)	0.46
Adiponectin, μg/ml	−0.49 (0.97)	5.66 (0.27)	5.56 (0.27)	0.10

59%, 44%, and 18% respectively) and, in the case of IL-6, also in Group C (by 41%). Instead, the anti-inflammatory cytokines IL-4 and IL-10 increased significantly over baseline values only in Group D subjects (by 47% and 84%, respectively) (Fig. 2). Analysis with the hierarchical linear model for repeated measurements (Table 3) showed significant overall changes for hs-CRP, IL-6 and TNF-α variation and multiple comparisons indicated significant differences in hs-CRP and IL-6 changes between Group D and Group A.

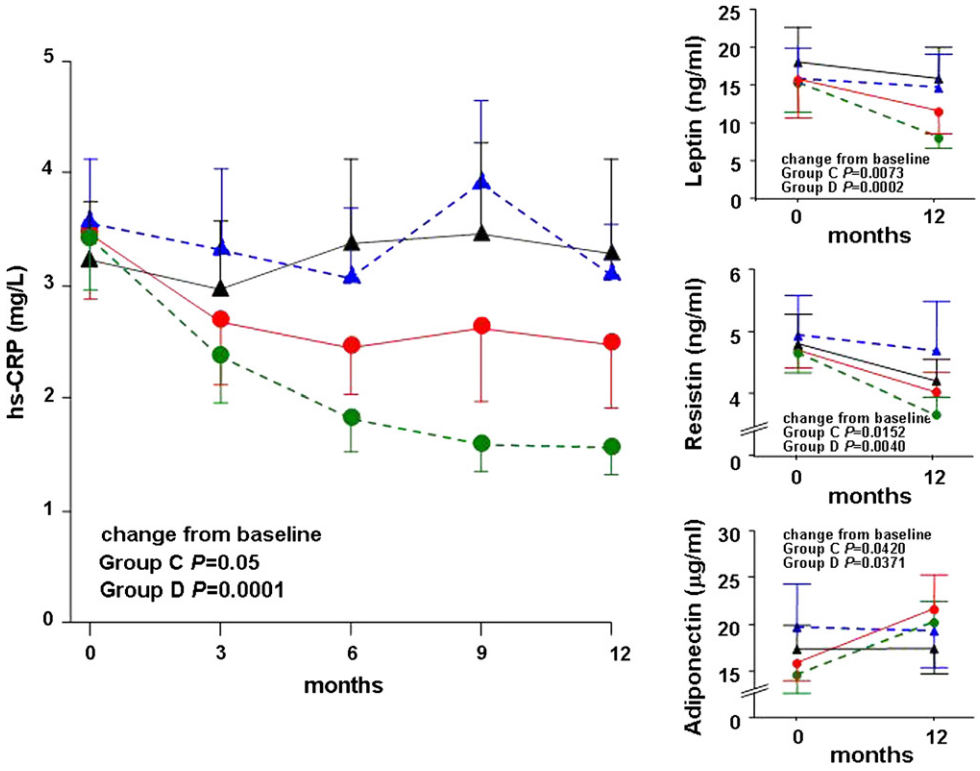

Figure 1 hs-CRP, leptin, resistin and adiponectin levels in Group A (black closed line and triangles), B (blue dashed line and triangles), C (red closed line and circles) and D (green dashed line and circles) patients at time 0 and after 3, 6, 9 (only for hs-CRP) and 12 months of sedentary or active (leasure-time ± prescribed and supervised physical activity) lifestyle (mean ± SEM, *p*-values for Friedman test for hs-CRP, Wilcoxon matched-pairs signed-ranks test for leptin, and paired *t* test for resistin and adiponectin). hs-CRP = high sensitivity-C reactive protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

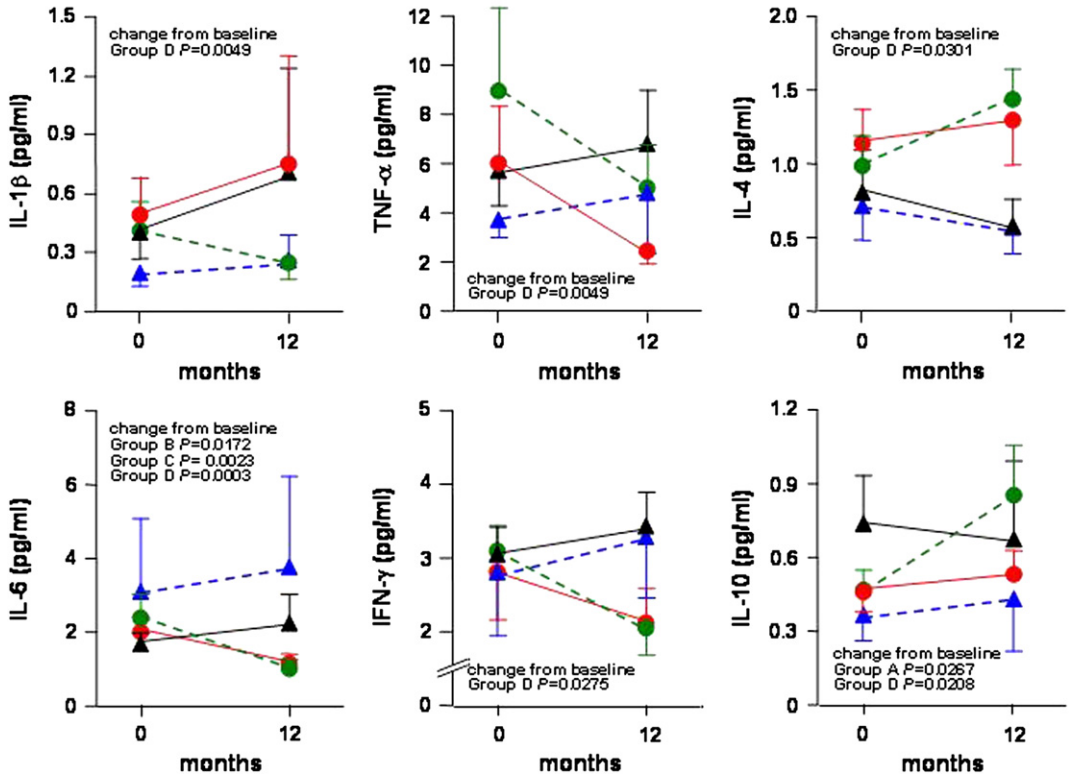

Figure 2 IL-1β, IL-γ, TNF-α, IFN-γ, IL-4 and IL-10 levels in Group A (black closed line and triangles), B (blue dashed line and triangles), C (red closed line and circles) and D (green dashed line and circles) patients at time 0 and after 12 months of sedentary or active (leasure-time ± prescribed and supervised physical activity) lifestyle (mean ± SEM, *p*-values for Wilcoxon matched-pairs signed-ranks test, for IL-1b;, IL-6, TNF-α and IFN-γ, or paired *t* test, for IL-10). IL = interleukin; TNF-α = tumor necrosis factor-α; and IFN-γ = interferon-γ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Discussion

This paper conclusively demonstrates that physical activity is effective in reducing hs-CRP levels in type 2 diabetic patients with the MS and indicates that the anti-inflammatory effect is strictly dependent on the exercise modalities, including volume, intensity and type, consistent with a previous large trial in subjects with IGT [18]. Several lines of evidence support this interpretation. Firstly, only subjects performing high-intensity physical activity, as in the prescribed and supervised exercise programs, showed a significant improvement in hs-CRP levels. The long duration of intervention (12 months) might have contributed to achieve a significant improvement in hs-CRP. This is consistent with the observation that reductions in hs-CRP values observed in the high-intensity Groups D and C (54% and 28%, respectively) were more pronounced than those previously reported in healthy subjects with baseline hs-CRP levels either in the low range [14] or falling into the high-risk class [29] with supervised high-intensity exercise programs of shorter duration. Secondly, mixed (aerobic plus resistance) exercise was more effective than aerobic training in reducing hs-CRP levels, despite similar caloric expenditure. The dependence of hs-CRP reduction on intensity and type of exercise and duration of training program might explain the discordant results of previous studies in the general population [15,16] and in subjects with type 2 diabetes [19,20] with exercise protocols of different modalities and duration. Thirdly, high-intensity exercise accounted only for approximately one-third of total energy expenditure in Group C and D subjects, thus pointing to the importance of reaching a certain volume of physical activity, as also indicated by the significant relation between hs-CRP changes and the mean value of METs. Daytime activity, in addition to the supervised sessions of high-intensity exercise, may have contributed to the higher extent of hs-CRP reductions observed in our study compared with previous reports in healthy subjects who followed only supervised programs of high-intensity exercise [14,29].

In addition to hs-CRP, high-intensity training, particularly the combined exercise program, induced an improvement of other biomarkers of inflammation and insulin resistance, with a reduction of IL-1β, IL-6, TNF-α, IFN-γ, leptin, and resistin (associated with decreased insulin, C-peptide, and HOMA-IR) and an increase of IL-4, IL-10, and adiponectin, thus indicating that exercise has a full anti-inflammatory and insulin-sensitizing effect. These results suggest that the beneficial effect of physical activity on CVD morbidity and mortality may depend, at least partly, on the anti-inflammatory effect of exercise, though it is unproven that reducing hs-CRP and other inflammatory biomarkers is effective in decreasing CVD risk [30].

Changes in inflammatory biomarkers in the high-intensity exercise groups were paralleled by improvements though, to a lesser extent, in HbA_{1c}, HDL-cholesterol, waist circumference, fat and fat-free mass and albuminuria, the latter also considered as an inflammatory marker. However, multiple regression analysis indicated that hs-CRP reduction was largely independent from changes in these traditional CVD risk factors. In particular, among measures of adiposity, changes in waist circumference were significantly associated with hs-CRP reduction, whereas the effect of changes in BMI and fat mass appeared to be driven by waist circumference variation. This finding, which is in keeping with data from IGT subjects [18], is of major clinical and research interest, since it indicates that physical activity has independent anti-inflammatory effects, which could be added to those of weight reducing programs.

However, further studies are needed to identify the molecular mechanisms underlying the anti-inflammatory effect of exercise and the site(s) where this action is predominantly exerted. In fact, circulating inflammatory biomarkers originate from multiple sources, particularly visceral adipose tissue, where excess fat promotes macrophage recruitment and both adipocytes and macrophages secrete numerous cytokines (adipokines), including IL-1β, IL-6, TNF-α, leptin, adiponectin and resistin [4]. Skeletal muscle is another source of cytokines, called myokines, among which IL-6 is considered to play a dual role: anti-inflammatory and pro-inflammatory, the latter by inducing production of CRP and other acute phase reactants by the liver [30]. We may speculate that, while adipokines are likely affected only in part in the absence of weigh loss, high-intensity training of long duration may significantly influence myokine production, thus driving the anti-inflammatory effect of exercise. However, the clinical significance of improvement in inflammatory biomarkers is uncertain because circulating levels may not reflect vascular synthesis and changes possibly induced by exercise in the vessel wall [30].

In conclusion, our findings are consistent with the view that physical exercise is anti-inflammatory per sè, independent of weight loss, by acting through several mechanisms involving inhibition of pro-inflammatory, and stimulation of anti-inflammatory pathways, as well as modulation of adipokines regulating insulin sensitivity. However, high-intensity, preferably combined (aerobic plus resistance) exercise training, in addition to daytime physical activity, is required for achieving a significant anti-inflammatory effect in type 2 diabetic patients with MS.

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