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Two weekly sessions of combined aerobic and resistance exercise are sufficient to provide beneficial effects in subjects with Type 2 diabetes mellitus and metabolic syndrome

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ABSTRACT. This study was performed to establish whether only 2 sessions per week of combined aerobic and resistance exercise are enough to reduce glycated hemoglobin (HbA_{1c}) and to induce changes in skeletal muscle gene expression in Type 2 diabetes mellitus (DM2) subjects with metabolic syndrome. Eight DM2 subjects underwent a 1-yr exercise program consisting of 2 weekly sessions of 140 min that combined aerobic [at 55-70% of maximal oxygen uptake (VO_{2max})] and resistance circuit training [at 60-80% of 1 repetition maximum (RM)]. The training significantly improved VO_{2max} (from 33.5±3.8 ml/kg/min to 38.2±3.5 ml/kg/min, p=0.0085) and muscle strength (p<0.05). Changes over baseline were significant for HbA_{1c}, reduced by 0.45% (p=0.0084), fasting blood glucose (from 8.8±1.5 to 6.9±2.2 mmol/l, p=0.0132), waist circumference (from 98.9±4.8 to 95.9±4.6 cm, p=0.0054), body weight (from 87.5±10.7 to 85.7±10.1 kg, p=0.0375), systolic blood pressure (from 137±15 to 126±8 mmHg, p=0.0455), total cholesterol (from 220±24 to 184±13 mg/dl, p=0.0057), and LDL-cholesterol (from 150±16 to 105±15 mg/dl, p=0.0004). Mitochondrial DNA/nuclear DNA ratio at 6 and 12 months did not change. There was a significant increase of mRNA of peroxisome proliferator-activated receptor (PPAR)-γ after 6 months of training (p=0.024); PPARα mRNA levels were significantly increased at 6 (p=0.035) and 12 months (p=0.044). The mRNA quantification of other genes measured [mitochondrially encoded cytochrome c oxidase subunit II (MTCO2), cytochrome c oxidase subunit Vb (COX5b), PPARγ coactivator 1α (PGC-1α), glucose transporter 4 (GLUT 4), forkhead transcription factor BOX O1 (FOXO-1), carnitine palmitoyltransferase 1 (CPT-1), lipoprotein lipase (LPL), and insulin receptor substrate 1 (IRS-1)] did not show significant changes at 6 and 12 months. This study suggests that a twice-per-week frequency of exercise is sufficient to improve glucose control and the expression of skeletal muscle PPARγ and PPARα in DM2 subjects with metabolic syndrome.
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INTRODUCTION

It is clearly established that adequate amounts of regular physical activity are an effective treatment for subjects with Type 2 diabetes mellitus (DM2) and metabolic syndrome (1-4). For healthy adults, the American College of Sport Medicine and the American Heart Association recommend at least 150 min per week of moderate aerobic physical activity (>10 MET-h/week), or minimum 60 min per week of intense aerobic physical activity, that should be combined with twice per week sessions of resistance exercise to increase muscle strength (5). The American Diabetes Association confirms these recommendations for people with DM2, suggesting that, in addition to aerobic exercise, resistance exercise should be performed at least 3 times per week (1). Recently, a scientific statement from the American Heart Association on Exercise Training for Type 2 Diabetes Mellitus (6), recommends accumulating a minimum of 150 min/week of at least moderate-intensity physical activity and/or 90 min/week of at least vigorous-intensity cardiorespiratory exercise, for reducing cardiovascular risk. Additionally, resistance training 3 times per week should be encouraged and exercise should be completed on at least 3 days per week, with no more than 2 consecutive days without training (6). A *post-hoc* analysis of an intervention study of ours, designed to validate a counselling strategy to increase leisure time physical activity in DM2 subjects (7), has confirmed that the minimal amount of exercise to significantly reduce glycated hemoglobin (HbA_{1c}) must be >10 MET-h/week (8). In this study the great majority of subjects chose walking as a form of exercise with a frequency of 3 or more times per week (7, 8). Three or more exercise sessions per week are not possible for some DM2 subjects because of lack of time, one of the most frequent obstacles reported during physical activity counseling (7, 9). At present, it is not well defined if 2 weekly sessions of physical activity improve metabolic parameters and increase muscle oxidative capacity. Ibañez et al. (10) demonstrated that 2 weekly sessions of a progressive resistance exercise program are adequate to reduce fasting plasma glucose levels and abdominal fat in older DM2 men, but did not significantly decrease HbA_{1c} (10). In contrast, Honkola et al. showed

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that a 5-month individualized progressive resistance training program (moderate intensity, high volume) twice a week, in obese, sedentary DM2 subjects, improved lipid profile and HbA_{1c} (11). Using moderate aerobic endurance training, Brun et al. showed that 1 yr of 2 weekly sessions of exercise at home did not improve blood pressure, lipid profile and HbA_{1c}, but decreased significantly insulin resistance (12).

The aim of the present study was to establish whether only 2 sessions per week of physical activity, achieving the minimal amount of energy expenditure suggested by Scientific Societies, are enough to reduce HbA_{1c} and to induce changes in skeletal muscle gene expression in DM2 subjects with metabolic syndrome.

Thus, we planned to totalize the minimal amount of 10 MET-h/week of energy expenditure and chose an intensity of exercise [~65% of maximal oxygen uptake (VO_{2max})] in the moderate range because this level is better accepted by sedentary DM2 patients. We used a mixed exercise program because there is evidence of additional benefits resulting from combining aerobic and resistance exercise (2, 13). Combined exercise is more effective in reducing HbA_{1c} levels of DM2 subjects in comparison to only aerobic exercise (2, 13).

We report our results for the primary outcome (change in HbA_{1c} after 1 yr of intervention) and for the secondary outcomes: anthropometric modifications and skeletal muscle (vast lateral) mitochondria DNA concentration and gene expression. Molecular changes of skeletal muscle were measured because muscle represents about 90% of insulin sensitive tissues (14). A growing body of evidence points to the role of aerobic exercise capacity and skeletal muscle mitochondrial function, in the development of insulin resistance, DM2, metabolic syndrome, and cardiovascular disease (4). Exercise is extremely effective in modulating muscle gene expression, involving mitochondrial biogenesis, muscular oxidative capacity, and muscular insulin sensitivity (4). This is the first study investigating changes in muscle gene expression after 2 weekly sessions of exercise lasting for 1 yr.

MATERIALS AND METHODS

Subjects

We recruited 8 subjects (2 females and 6 males), aging 38 to 74 yr (55.6±10.9, mean±SD), with DM2 and metabolic syndrome. All gave their informed consent to the study that was approved by the University Research Ethics Board. The main entry criteria were: diagnosis of DM2 for more than 1 yr, waist circumference >94 cm (in men) and >80 cm (in women), plus >1 other metabolic syndrome trait (according to International Diabetes Federation I criteria) (Tables 1 and 2), sedentary lifestyle for at least 6 months, ability to walk without assistance and eligibility after cardiovascular evaluation. Additional requirements were no change in any medication and diet for the past 6 months before starting exercise program. All patients executed fundoscopic evaluation and cardiological examination [resting electrocardiogram (ECG) and, based on clinical judgment, an echocardiogram and/or an ECG treadmill test]. None of patients suffered from conditions that limited or contraindicated exercise (like overt retinopathy or nephropathy).

Protocol

The exercise intervention took place in a local exercise facility (Gym Elisir, Perugia, Italy) where all participants were provided with free admission for 12 months. Exercise was in addition to standard medical care and supervised by personal trainers. The exercise program consisted of 140 min/week divided in 2 sessions that combined aerobic training (at 55-70% of predicted VO_{2max}), using a treadmill and/or cycloergometer, and resistance circuit training [at 60-80% of one repetition maximum (1RM)] (15). Resistance training was performed at 60 or 80% of predicted 1RM and consisted of 4 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 3 stretching positions. The number of exercises ranged from 2 sets of 15 repetitions to 3 sets of 8 repetitions, for each muscle group, for patients working at 60% and 80% of 1RM, respectively. Energy expenditure during supervised sessions was calculated automatically by the machines (Technogym, Gambettola, FC, Italy). Subjects were evaluated at baseline and after 3, 6, 9, and 12 months for cardiorespiratory fitness by VO_{2max} estimation (Fitmate, Cosmed srl, Rome, Italy), and for muscle strength by a 5-8 RM test. Exercise loads were eventually adjusted accordingly to the changes in cardiorespiratory fitness or in muscle strength measured every 3 months.

Standard medical care integrated physical activity counseling (7) with a treatment regimen aimed at achieving the glucose, lipid, blood pressure, and body weight targets, suggested by current guidelines, by means of glucose-, lipid-, and blood pressure-lowering drugs plus 3-monthly nutritional counseling. For promoting weight loss, the dietician did not prescribe a restricted diet, but provided patients with nutritional information and used food log for monitoring dietary habits and their change. In particular, in the nutritional counselling sessions, dietitian suggested reducing high energy density food consumption, in order to reduce daily caloric intake of about 300-400 Kcal and daily caloric intake from fat ≤30% of total caloric intake (ideal composition of diet: CHO 55%, FAT 30%, protein 12-15% of total calories).

Measurements

HbA_{1c} was measured by high-performance liquid chromatography (HPLC) Adams TMA1C HA-8160 (Menarini Diagnostics, Florence, Italy), plasma lipids and blood glucose by VITROS 5,1 FS Chemistry System, Ortho-Clinical Diagnostics Inc, Raritan (NJ, USA). Blood pressure was measured after 10 min at rest; the mean of 2 readings was used in statistical analysis. Cardiorespiratory fitness (VO_{2max}) was predicted as oxygen uptake at maximal heart rate (220 minus age), by extrapolation of the regression line established during the individual calibration for the relationship between oxygen consumption and heart rate during a submaximal graded treadmill exercise test. The test was stopped when patients reached 80% of the predicted maximal heart rate. For all subjects the treadmill speed, predetermined during a practice session, was held constant (4.3 or 5.3 km/h). The initial grade was set at 0% for the 1st and 2nd min, was 2% for the 3rd min and was increased by 1% every 2 min thereafter (16). All tests used gas exchange analyzer FitMate (Cosmed srl, Rome, Italy).

Skeletal muscle mitochondria DNA (mtDNA) content and gene expression were obtained by fine needle aspiration (FNA) from

Table 1 - *Age, sex, diabetes duration, complications, drug treatment (at baseline, T0 and after 12 months, T12), job and boby mass index (BMI) of 8 Type 2 diabetes mellitus (DM2) subjects.*

Subjects	Age	Sex	Diabetes duration (yr)	Complications (T0)	Drugs T0	Drugs T12	no	BMI kg/m^2 T0	BMI kg/m^2 T12
1	58	M	2	None	Rosiglitazone 4 mg Metformin 1000 mg Repaglinide 6 mg Irbesartan 300 mg Amlodipine 10 mg HCT 12.5 mg	Rosiglitazone 4 mg Metformin 1000 mg Repaglinide 6 mg Irbesartan 300 mg Amlodipine 10 mg HCT 12.5 mg	Pensioner	31	28.9
2	56	M	6	None	Glicazide 160 mg Simvastatin 10 g Ramipril 5 mg HCT 25 mg Atenolol 25 mg	Glicazide 160 mg Simvastatin 10 g Ramipril 5 mg HCT 25 mg Atenolol 25 mg	Engineer	30	29.7
3	59	M	6	Coronary heart disease (cardiac infarction and by-pass Ao-C 3 yr before)	Lyspro 21 U Sinvastatin 20 mg ASA 100 mg Atenolol 100 mg	Lyspro 21 U Sinvastatin 20 mg ASA 100 mg Atenolol 100 mg	Pensioner	30	30
4	55	M	6	Nephropathy st. 1 (microalbuminuria)	Metformin 1 g/die	Metformin 1.7 g/die (3rd m) Rosuvastatin 10 g/die (3rd)	Medical Doctor	27.1	26.5
5	44	F	2	Nephropathy st. 1 (microalbuminuria)	Metformin 1000 mg Atorvastatin 20 mg Enalapril 20 mg HCT 6 mg ASA 100 mg	Metformin 1000 mg Atorvastatin 20 mg Enalapril 20 mg HCT 6 mg ASA 100 mg	Factory Worker	27.1	26.5
6	74	M	6	Nephropathy st. 1 (microalbuminuria) hypertensive retinopathy	Metf ormin 2.8 gr die Glibenclamide 2.5 mg/die Terazosin 4 mg/die Ramipril 5 mg ASA 100 mg	Metformin 2.2 gr/die (9th m) Terazosin 4 mg Ramipril 5 mg ASA 100 mg	Pensioner	26.3	26
7	38	F	9	Background retinopathy	45U lyspro + 30U glargine Perindopril 4 mg Amlodipine 5 mg Furosemide 25 mg	Metformin 1.5 gr/die (3rd m) Perindopril 4 mg Amlodipine 5 mg Furosemide 25 mg	Housewife	36.9	36.5
8	61	M	3	None	14 U glargine+12 U lyspro 40 mg simvastatin 1000 mg omega3 10 mg bisoprolol 2 mg alprazolam	20 U glargine+12 U lyspro (3rd m) 40 mg simvastatin 1000 mg omega3 10 mg bisoprolol	Manager	28.1	27.8
Mean	55.6							29.6	29
SD	10.9							3.4	3.4

HCT: ramipril 5 mg + hydrochlorotiazide 25 mg; ASA: acetylsalicytic acid.

the *vastus lateralis* muscle of all subjects at baseline, 6, and 12 months. Muscle FNA were performed more than 24 h after the last exercise session; we used 22 gauge spinal needles (Becton Dickinson, Madrid) under ultrasound guidance as previously described by Guescini et al. (17). Briefly, the needle was kept firm in the middle of the muscle, and samples were collected keeping a constant vacuum for a period of 2 min through a 60 ml plastic syringe maintained at its maximal extension by a firm support. No local anesthetic was required because needle insertion is painless, like common im injections. The muscle sample, trapped in the fine needle following aspiration process, was recovered by repeated needle washing with 1 ml of RLT solution (Qiagen GmbH, Hilden, Germany) diluted 1:3 by diethyl pyrocarbonate treated water. Nuclear (nDNA) and mitochondrial DNA content from FNA sample were measured by real-time PCR (17). The amount of mtDNA was related to nDNA by the method described by Pfaffl (18). Gene expression analysis was achieved by Multiplexed Tandem real-time RT-PCR strategy as reported in Guescini et al. (17) using the specific primers reported in Supplementary Material I. Real-time PCR amplifications were conducted using Light-Cycler® 480 SYBR Green I Master (Roche, Basilea, Switzerland) according to the manufacturer's instructions, with 500 nM primers and a variable amount of DNA standard in 20 µl final reaction volume. Thermocycling was conducted using a LightCycler® 480 (Roche) initiated by a 10-min incubation at 95 C, followed by 40 cycles (95 C for 5 sec; 60 C for 5 sec; 72 C for 10 sec) with a single fluorescent reading taken at the end of each cycle. Each reaction was conducted in triplicate and completed with a melt curve analysis to confirm the specificity of amplification and lack of primer dimers.

Statistical analysis

Data are expressed as mean±SD. Statistical differences were assessed by repeated measures analysis of variance. Dunnett's test was used as *post hoc*. The significance threshold was set to 0.05. We calculated that the primary outcome (change in HbA_{1c} after 1 yr of intervention) required 8 subjects to achieve 87% power and to detect a difference of –0.5 between the null hypothesis mean of 0.0 and the alternative hypothesis mean of 0.5 with an estimated SD of 0.4 and with a significance level (alpha) of 0.05000 using a two-sided one-sample t-test (19).

Table 2 - *Waist circumference (WC, cm), weight (W, kg), fasting plasma glucose (FG, mmol/l), glycated hemoglobin (HbA_{1c}, %), systolic and diastolic blood pressure (SBP and DBP, mmHg), total, HDL, LDL-cholesterol (T-, HDL-, LDL-Chol, mg/dl), plasma triglycerides (TG, mg/dl) in the Type 2 diabetes mellitus subjects at baseline (T0) and after 12 months (T12) of physical exercise intervention (p=T12 vs T0).*

	WC <94 cm M <80 cm W		W		FG <5.55 mmol/l		HbA_{1c} % <6.5%		SBP		DBP		T-Chol <200 mg/dl		HDL-Chol >40 mg/dl		LDL-Chol <100 mg/dl		TG <150 mg/dl	
	T0	T12	T0	T12	T0	T12	T0	T12	T0	T12	T0	T12	T0	T12	T0	T12	T0	T12	T0	T12
n. 1*	107	100	98	91.5	6.9	5.9	6	5.4	130	120	80	80	230	162	47	44	159	95.6	121	112
n. 2*	102	100	91	90	8.3	6.4	5.8	5.5	140	120	90	90	208	186	54	52	135	107.2	96	134
n. 3*	98	98	80	80	9.7	8.5	5.9	6.1	120	120	80	80	201	203	45	57	137	121.6	86	122
n. 4#	94	92	88	85.8	9.5	4.2	5.6	5.1	135	120	80	70	246	185	42	52	157	81.6	235	257
n. 5*	93	88	73	71.2	8.1	6.7	6.9	5.9	130	130	70	70	238	195	43	51	179	125.6	81	92
n. 6#	95	92	76	75	6.6	4.6	6.1	5.8	140	140	80	80	172	169	28	57	131	99.4	64	63
n. 7#	102	100	104	103	10.6	10.9	7.5	7.1	130	125	80	75	235	189	51	49	152	113.6	160	132
n. 8#	100	97	90	89	10.6	8.2	7.2	6.5	170	135	85	75	227	184	45	40	154	95.4	869	243
Mean	98.9	95.9	87.5	85.7	8.8	6.9	6.38	5.93	137	126	81	77	220	184	44	50	150	105	215	144
SD	4.8	4.6	10.7	10.1	1.5	2.2	0.72	0.64	15	8	6	6	24	13	8	6	16	15	270	69
p		0.00540		0.0375		0.0132		0.0084		0.0455		0.095		0.0057		0.1894		0.0004		0.4031

*Patients who did not change therapy. #Patients who changed therapy: 4: increased metformin from 1 to 1.7 g/day and started 10 mg rosuvastatin; 6: reduced metformin from 2.8 to 2.2 g/day and quit glibenclamide; 7: stopped glargine and lyspro insulin (75 total U/day) and started metformin (1.5 g/day).

RESULTS

During the 1-yr period of the study, 4 patients did not modify the therapy (Table 1). The other 4 patients changed drug treatment as follows: 1 patient increased metformin from 1 to 1.7 g/day and started 10 mg rosuvastatin after 3 months; 1 reduced metformin from 2.8 to 2.2 g/day and quit glibenclamide after 9 months; 1 increased glargine insulin dosage from 14 U to 20 U after 3 months and started ramipril (2.5 mg/day) after 6 months; 1 stopped glargine and lyspro insulin (75 total U/day) after 6 months and started metformin (1.5 g/day) after 3 months. Dietary intake (caloric and fat intake) did not change significantly throughout the study, according to food log examination. All subjects during the 1-yr study did not engage in leisure time physical activity and did not change their habitual daily life physical activity.

From baseline to 12 months, the median exercise training attendance was 80±3%. The training significantly improved cardiovascular fitness as documented by increase in VO_{2max} by 15% (from 33.5±3.8 ml/kg/min to 38.2±3.5 ml/kg/min, p=0.0085) and in lower body, chest and upper body muscle strength, tested by 5-8 RM test (p<0.05, Table 3).

Changes over baseline were significant for the major outcome of the study: HbA_{1c} was reduced by 0.45%, from a baseline value of 6.38±0.72 to 5.93±0.64 (p=0.0084) at 12 months (Table 2). For anthropometric and metabolic secondary end-points, we observed a significant reduction in fasting blood glucose (from 8.8±1.5 to 6.9±2.2, p=0.0132), waist circumference (from 98.9±4.8 to 95.9±4.6, p=0.0054), and body weight (from 87.5±10.7 to 85.7±10.1, p=0.0375). Also, systolic blood pressure (from 137±15 to 126±8, p=0.0455), total cholesterol (from 220±24 to 184±13, p=0.0057), and LDL-cholesterol (from 150±16 to 105±15, p=0.0004) were significantly reduced.

The real-time PCR did not show a significant change of mitochondrial DNA/nuclear DNA ratio (baseline 203±34) after 6 (275±100) and 12 months (236±53, p=ns). The Multiplexed Tandem real-time RT-PCR strategy used to

Table 3 - *Aerobic capacity [maximal oxygen uptake (VO_{2max}) (ml/kg/min)] and strength (kg) of lower body, chest, dorsal, and upper body muscles in 8 Type 2 diabetes mellitus subjects at baseline (T0) and after 12 months (T12) of physical exercise intervention (p=T12 vs T0).*

	VO_{2max} ml/kg/min	VO_{2max} T12	Lower body T0	Lower body T12	Chest T0	Chest T12	Dorsal T0	Dorsal T12	Upper body T0	Upper body T12
n. 1	35	37	286	354	45	68	58	84	52	76
n. 2	39.4	42.6	310	324	68	81	73	81	71	81
n. 3	29.2	39.9	186	348	62	67	68	68	65	68
n. 4	37.9	38.1	248	225	62	79	73	62	68	71
n. 5	30	37.6	236	314	39	59	48	59	44	59
n. 6	32.3	37.6	111	161	27	34	42	50	35	42
n. 7	30	31.2	216	278	58	68	64	72	61	70
n. 8	33.9	41.4	186	354	45	99	51	96	48	98
Mean	33.5	38.2	222	295	51	69	60	72	55	70
SD	3.8	3.5	63	70	14	19	12	15	13	16
p		0.0085		0.017		0.011		0.087		0.027

evaluate gene expression showed a significant increase of mRNA of peroxisome proliferator-activated receptor (PPAR)-γ after 6 months of training (p=0.024), that was no longer significant after 12 months (p=0.061); also, PPARα mRNA levels were significantly increased at 6 (p=0.035) and 12 months (p=0.044). The mRNA quantification of key genes involved in oxidative phosphorylation [mitochondrially encoded cytochrome c oxidase subunit II (MT-CO2) and cytochrome c oxidase subunit Vb (COX5b)], in the control of mitochondrial biogenesis [PPAR-γ coactivator 1α (PGC-1α)], in glucose and lipid metabolism and insulin sensitivity [glucose transporter 4 (GLUT 4), forkhead transcription factor BOX O1 (FOXO-1), carnitine palmitoyltransferase 1 (CPT-1), lipoprotein lipase (LPL), and insulin receptor substrate 1 (IRS-1)] did not show significant changes at 6 and 12 months (p=ns) (Fig. 1).

DISCUSSION

Our study suggests that a twice per week frequency of exercise at moderate intensity is sufficient to improve glucose control in DM2 patients with metabolic syndrome, as shown by a 0.45% decrease of HbA_{1c} at 12 months (p=0.008) and by a 1.9 mM decrease in fasting plasma glucose (p=0.0013).

The twice weekly frequency of exercise is also sufficient to increase the expression of skeletal muscle PPARγ (at 6 months) and PPARα (at 6 and 12 months), to improve cardiovascular fitness (VO_{2max}) and muscle strength and to reduce body weight and waist circumference. We observed a modest decrease in body weight probably because subjects did not change their habitual diet and increased their lean body mass; instead, waist circumference was more significantly reduced. This is in agreement with the known effects of exercise on visceral fat mass in subjects with metabolic syndrome (4).

Our study has some methodological limitations that need to be discussed. We have studied only 8 patients with very wide range of age, therapy and different sex and we do not have a control group. Thus it is possible that the decrease in HbA_{1c} could be, at least in part, time dependent. However, the mean 0.45% decrement in HbA_{1c} observed in this study is in agreement with previous literature data on the effects of exercise (2). The beneficial effects observed in our study can be likely attributed to the exercise intervention. In fact, during the study, food log examination did not show relevant changes in diet and fat intake; moreover, drug treatment of subjects did not change significantly. In particular, drugs changes observed in 4 subjects were mainly in the direction of a re-

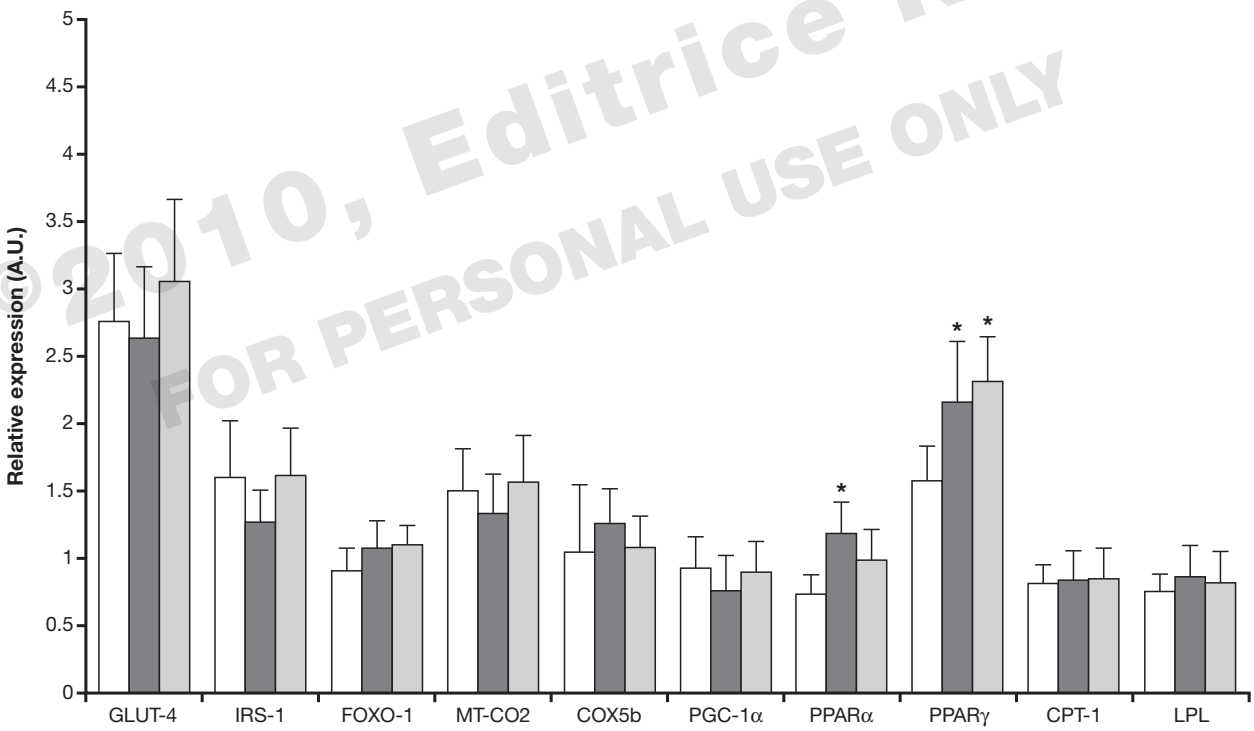

Fig. 1 - *Gene expression analysis from* vastus lateralis *skeletal muscle of Type 2 diabetes mellitus patients in response to physical exercise. Muscle fine needle aspirations were obtained from right lateral vast muscle before exercise intervention (white bars), after 6 months of physical training (black bars) and at the end of the exercise programme (gray bars). Target gene expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase mRNA transcription and expressed as arbitrary units (AU). Values are means±SEM, no.=8. Statistical differences were assessed by analysis of variance followed by Dunnett's test. *(p<0.05) were significantly different vs baseline. GLUT 4: glucose and lipid metabolism and insulin sensitivity [glucose transporter 4; IRS-1: insulin receptor substrate 1; FOXO-1: forkhead transcription factor BOX O1; MTCO2: mitochondrially encoded cytochrome c oxidase subunit II; COX5b: cytochrome c oxidase subunit Vb; PPAR: peroxisome proliferator-activated receptor; PGC-1α: PPAR-γ coactivator 1α; CPT-1: carnitine palmitoyltransferase 1; LPL: lipoprotein lipase.*

duction, than of an increase (Table 1), according also to analysis of medications cost. The cost of drugs for the 4 patients who changed therapy, was 915±423 €/patient/yr at baseline and 789±307 €/patient/yr after intervention. In particular, the mean cost for antidiabetic drugs was 438±261 before and 183±124 €/patient/yr after 1 yr. Thus, drug treatment for diabetes control was less expensive at the end of the intervention, and changes of therapy, made over the year in the 4 subjects, followed a trend of a reduction (a reduced amount of 69 U of daily insulin) in drug intake.

The efficacy of our twice weekly exercise programme on VO_{2max} and muscle strength was expected because the subjects enrolled in the present study were unfit, due to their previous long-term sedentary condition (20). The improvement of VO_{2max} (15%) was associated with a considerable increase of PPARγ (after 6 months) and PPARα (at 6 and 12 months) expression, two skeletal muscle regulatory genes promoting lipid and glucose oxidation and mitochondrial biogenesis (21, 22). However, we could not detect a concomitant significant increment in skeletal muscle mtDNA content. Recently, Toledo et al. were able to demonstrate a significant ($p<0.05$) 18% increment in mtDNA content, following a 4 to 6 days per week exercise and weight loss programme; they showed also a reduction of HbA_{1c} (–1.38%) and body weight (–7.1 kg), greater than those achieved in our study (–0.45% and –1.8 kg, respectively) (23). Thus, it is plausible that a twice per week exercise programme is not sufficient to induce a detectable augment of mtDNA content, but is enough to increase expression of key genes, like PPARγ and PPARα, able to enhance glucose and lipid oxidation in mitochondria of skeletal muscle in DM2 subjects. We analyzed also genes involved in different aspects of muscle metabolism (24, 25): PGC-1α, mainly related to mitochondrial biogenesis (26, 27), COXVb and COXII, part of the mitochondrial respiratory chain and major point of regulation of muscle oxidative capacity (28, 29), GLUT-4, responsible for muscle glucose uptake (30), IRS1, factor of insulin signaling pathway (31) and FOXO-1, factor that positively regulates fatty acid metabolism and negatively regulates glucose metabolism (32, 33). The expression of these skeletal muscle genes did not significantly change at the end of the study. However, since fine needle aspiration was obtained more than 24 h after the last exercise session, it is likely that we did not detect transient increments of gene trascripts (their mRNA). Indeed, several study showed that increase of PGC-1α and GLUT-4 mRNA expression (34, 35) (in contrast to that of GLUT-4 protein) is transient and not detectable 24 h after an exercise session.

In our study, the intensity of exercise program was set at 40-70% of VO_{2max} because it corresponds to a range of intensity that can be accepted without difficulty by sedentary people (20). It is not possible to rule out that 2 weekly sessions at greater exercise intensities might result in further significant beneficial effects. However, it is unrealistic to start with a high intensity physical activity programme with untrained people (20).

In conclusion, our study confirms the recommendations of American Diabetes Association (ADA), American College of Sports Medicine (ACSM) and American Heart Association (AHA) guidelines, suggesting a minimum amount of 150 min/week of exercise. In addition, it suggests an affermative answer to the frequent question of DM2 subjects with reduced time to exercise: "Is it enough to practice moderate exercise only twice per week?". Our data, obtained with a small group of DM2 subjects, show beneficial effects on glucose control and skeletal muscle gene expression with a 2-weekly exercise program. However, it must be underlined that our positive results were obtained by means of supervised aerobic and resistance exercise sessions, structured to maximize the time spent in physical activity. Patients must keep in mind that a strong and positive relationship exists between the amount of energy expenditure due to physical exercise and improvements of several metabolic parameters (5, 4).

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