Eur J Nutr (2014) 53:243–249
DOI 10.1007/s00394-013-0522-x

ORIGINAL CONTRIBUTION

Short bouts of anaerobic exercise increase non-esterified fatty acids release in obesity

Alberto Salvadori · Paolo Fanari · Paolo Marzullo · Franco Codecasa · Ilaria Tovaglieri · Mauro Cornacchia · Amelia Brunani · Livio Luzi · Erminio Longhini

Received: 24 May 2012 / Accepted: 2 April 2013 / Published online: 26 April 2013

Abstract
Purpose It is demonstrated that aerobic exercise plays an important role in weight loss programs for obesity by increasing 24 h metabolic rate. While aerobic exercise can result in health and fitness benefits in obese subjects, also independently of weight loss, not completely clear are the effects of bouts of hard exercise on metabolic outcomes. The aim of this study was to test the hypothesis that short-term aerobic activity with anaerobic bouts might result in a greater improvement in the management of obesity than aerobic activity alone.
Methods We studied 16 obese subjects (eight men) during a progressive cycloergometric test up to exhaustion, before and after 4 weeks of two different training schedules (6 days/week). Insulin and glycaemia, non-esterified fatty acids (NEFA) and lactic acid were sampled. Group A (eight subjects, four men) performed an aerobic cycle workout; Group B (eight subjects, four men) performed a 25 min aerobic workout followed by 5 min of anaerobic workout. All the subjects maintained their individual eating habits.
Results The post-training test showed a decrease in AUCs NEFA in Group A ($p < 0.05$) and an increase in Group B ($p < 0.05$), together with an increase in lactic acid in Group A and a decrease in Group B ($p < 0.01$). β-cell function (HOMA2-B) revealed a reduction only in Group A ($p < 0.05$). Group B achieved a greatest reduction in body fat mass than Group A ($p < 0.05$).
Conclusions Aerobic plus anaerobic training seem to produce a greater response in lipid metabolism and not significant modifications in glucose indexes; then, in training prescription for obesity, we might suggest at starting weight loss program aerobic with short bouts of anaerobic training to reduce fat mass and subsequently a prolonged aerobic training alone to ameliorate the metabolic profile.

Keywords Obesity · Exercise · Anaerobic threshold · Non-esterified fatty acids

A. Salvadori · P. Fanari (✉) · F. Codecasa · I. Tovaglieri · M. Cornacchia · E. Longhini
Division of Respiratory Rehabilitation, H. San Giuseppe, Istituto Auxologico Italiano, P. O. Box N. 1, 28921 Verbania, Italy
e-mail: rehab.med@auxologico.it

P. Marzullo
Department of Clinical and Experimental Medicine, University of Piemonte Orientale 'A. Avogadro', Novara, Italy

P. Marzullo
Division of General Medicine, H. San Giuseppe, Istituto Auxologico Italiano, Verbania, Italy

A. Brunani
Division of Rehabilitation Medicine, H. San Giuseppe, Istituto Auxologico Italiano, Verbania, Italy

L. Luzi
Faculty of Exercise Sciences, University of Milan, Milan, Italy

Background

Regular exercise associated with diet is an important strategy in the management of obesity. Exercise increases lipolysis by increased plasma catecholamines, lowers plasma concentrations of insulin which has an antilipolytic activity and reduces insulin resistance [1].

It has been previously shown that endurance training by aerobic exercise without diet intervention in overweight men increases lipolysis and plasma non-esterified fatty acids (NEFA) concentrations greatly lower after exercise

aerobic training which had been performed for 4 months [2].

All forms of physical activities are generally located on an energy continuum composed of two major energy systems that are aerobic and anaerobic systems [3]. When high-intensity training is directed toward the latter system, this system responds with biochemical, neural and anatomic adaptations. Unlike the aerobic system, this response tends to be a local phenomenon rather than a systemic adaptation [3]. The American College of Sport Medicine recommends the addition of resistance exercise to a program of regular aerobic training [4], and up till now, to our knowledge only few studies have analyzed the metabolic effect of low- or high-intensity training in obese subjects [5], in particular in fat oxidation and glucose metabolism indexes.

In the present study we aimed to examine the effects on weight, body composition and metabolism after a brief period of aerobic and aerobic plus anaerobic training in adult obese subjects, being each subject sedentary and maintaining his individual eating habits during the period of reconditioning.

Methods

Subjects

Patients admitted between January and June 2008 to Auxologic Italian Institute (Italy) for weight loss program were evaluated to participate to the study. In a large group initially screened ($n = 40$), 16 subjects (8 male and 8 female, age 38.8 years, range 22–59 years, body mass index (BMI) 35.7 kg/m^2, range 30–43) were accepted to participate and were randomly assigned to aerobic or aerobic plus anaerobic training (respectively, Group A and Group B). Exclusion criteria were smoking, physical inability, hypertension, diabetes or any other cardiovascular or metabolic disorder. Women were analyzed during early follicular phase of the menstrual cycle. A preliminary physical examination, together with anthropometric measurements after voiding, ECG, routine blood and urine analysis, was carried out to exclude medical illnesses. The protocol included bioimpedance analysis (BIA 101/S Akern, Florence, Italy) for the measurements of fat (FM, kg) and fat-free mass (FFM, kg). Patients with fluid overload according to vectorial analysis were excluded to minimize errors in estimating FM in severe obesity. BMI was calculated as weight/height^2 (kg/m^2).

Before exercise testing, subjects were asked to restrain from strenuous activity for at least 24 h. A preliminary nocturnal O_2 saturation monitoring did not demonstrate the presence of significant periods of pathological low values in each subject.

Study design

The protocol was approved by the Ethics Review Committee of the Institute and written informed consent was given by all the subjects before participation, in accordance with the Helsinki Declaration of the World Health Organization (1964, amended in 1975 and 1983) and with the Updated Ethical Standards in Sport and Exercise Science Research [6].

Following an overnight fast, in the morning the subjects performed a continuous incremental test on a Gould bicycle ergometer with power output increased by 20 W (at 60 rpm) every 4 min until the subjects could no longer maintain the pedaling frequency despite verbal encouragement. At the beginning of subsequent recovery, subjects were invited to cycle for 2 more min effortlessly, and then followed for up to 40 min in a sitting position.

A cardiopulmonary exercise test station (Vmax 229; Sensor Medics, Yorba Linda, CA, USA) was used for continuous analysis of oxygen consumption (VO_2), CO_2 production (VCO_2) and minute ventilation (VE). Exercise capacity was assessed by determining the maximum work rate together with ventilatory anaerobic threshold (AT), and VO_2 max defined as the VO_2 averaged over the last minute of exercise and related to theoretical VO_2 max [7]. AT was detected by the V-slope method [8]. Mean values were obtained at baseline and during the last minute of rest and at each power increase. Calibrations were performed prior to each test. Heart rate (HR) and ECG signals were recorded by a CASE 6.5 (GE Medical System Milwaukee, WI, USA), and oxyhemoglobin saturation was determined by a Radiometer percutaneous oximeter every 20 s. Blood hemoglobin was determined in all subjects at baseline.

Sampling was performed from an indwelling Teflon catheter previously placed in the antecubital vein of the right or left arm. Blood samples were analyzed at rest for determination of insulin and glycaemia; at rest, peak exercise, first recovery (5 min) and late recovery (30 min) for determination of NEFA, triglycerides; and at rest, 40, 80, 120 W, and at peak exercise, and at late recovery for lactic acid (Accusport, Boehringer Mannheim, Monza, Italy).

Plasma glucose and lactate concentrations were assessed by amperometric method (Gem Premier 3000; Instrumentation Laboratory) and triglycerides by enzymatic colorimetric method (Trinder). Serum insulin concentrations were measured by chemiluminescence (Immulite 2000 Analyzer; diagnostic Products Corp., Los Angeles, CA). The insulin assay sensitivity was 2 μlU/mL; the inter- and intra-assay coefficients of variation (CV) were 4.0 and 5.1 %, respectively. Serum NEFA concentrations were analyzed spectrophotometrically using enzymatic and colorimetric commercial kit (Randox Laboratories, USA). The NEFA assay sensitivity was 0.072 mmol/l; the inter- and intra-assay CV were 4.51 and 4.74 %, respectively. The modified HOMA model was used to

yield an estimate of insulin sensitivity and β-cell function from fasting plasma insulin and glucose concentrations [9].

Exercise training

Following the baseline evaluation, inpatient obese subjects were randomly assigned to two subgroups each comprising eight subjects matched by sex, age and BMI (Table 1).

Each subgroups underwent a 4-week training protocol consisting of 2 daily 30-min cycloergometric exercise session for 6 days/week. In Group A, the power output of each training session was exclusively aerobic, constantly attaining 70 % of the heart rate registered at AT. In Group B, the power output of each training session was aerobic (25 min) and anaerobic (5 min), attaining 70 % of the HR registered at AT for the aerobic part, and 85 % of the maximal HR for the anaerobic part. Increments of power outputs were imposed in view of the actual improvement by reconditioning, maintaining HR 70 % of AT (Group A and B) and 85 % (Group B) as the reference standards. Oxygen saturation and HR were continuously monitored during each training session. At the end of each training period, each of the participants in Group A and Group B underwent a conclusive ergospirometric test according to the same protocol setting as the entry test.

Eating habits

During the study the subjects performed a balanced diet corresponding to their basal metabolic rate (57.1 % carbohydrate, 25.0 % fat, 18.1 %, protein), but no strict dietary restriction was imposed.

Statistics

ANOVA was employed to determine differences between groups and between pre- and post-training values [10] with a statistical power at least higher than 70 %. Integrated concentrations of NEFA and lactic acid (area under the curve) were calculated during exercise and recovery by using the trapezoidal rule [11]. Variable interactions were evaluated with multiple regression analysis.

Results

Effects of 4-week aerobic training (Group A)

At study entry, the performances of the two groups were comparable, reaching similar VO_2 max expressed in ml/min/kg (18.1 ml/min/kg in Group A vs. 18.7 ml/min/kg in Group B; p = NS).

Peak activity, AT, VO_2 max and exercise VO_2 before and after training in Group A are expressed in Table 2. After training, mean body weight was not significantly decreased from 92.3 to 89.4 kg, FM from 38.9 to 36.3 kg and FFM from 53.2 to 52.1 kg.

At the baseline evaluation, NEFA showed a reduction during exercise followed by an increase from exercise peak to late recovery. After training, NEFA values were significantly lower at each considered point (Fig. 1a). The mean area under the curve AUCs was 37,214 ± 4,005 μEq min/l before training and 24,280 ± 3,614 μEq min/l after training ($p < 0.05$) (Fig. 1b).

Lactic acid increased to its maximal value in correspondence with peak activity and decreased during recovery. After training, at peak activity its values were significantly higher than before training (respectively, 6.96 mmol/l vs 5.01, $p < 0.05$) (Fig. 2a). The mean area under the curve (AUCs) was 177.9 mmol min/l before training and 232.1 mmol min/l after training (p = NS), with a delta value after versus before + 54.2 ± 20.5 mmol min/l (Fig. 2 C).

Triglycerides were constant from rest to peak activity, as well as first and late recovery. After the training period, they did not significantly modify (data not shown).

Table 1 Anthropometric data of the samples

Variable[a]	Group A	Group B	p value*
No. of subjects	8	8	
Sex, M/F	4/4	4/4	NS
Age (years)	40.7 ± 4.5	39.2 ± 4.3	NS
Weight (kg)	92.3 ± 8.2	114.7 ± 7.6	NS
Height (cm)	161 ± 3.9	175 ± 4.4	<0.05
BMI (kg/m^2)	35.2 ± 2.3	37.3 ± 1.1	NS
Fat-free mass (FFM) (kg)	53.2 ± 4.5	68.7 ± 6.0	<0.05
Fat mass (FM) (kg)	38.9 ± 3.8	46.1 ± 2.7	NS

BMI body mass index

* By two-tailed analysis of variance

[a] Values are mean ± SEM

Table 2 Functional data Group A

Variable[a]	Before training	After training	p value*
Mean exercise peak (W)	94.3 ± 8	109.1 ± 7	NS
Mean A.T. (W)	66.2 ± 6	76.4 ± 3	NS
Mean VO_2 max (ml/min/kg)	18.1 ± 1.4 (78 % theoretical VO_2 max)	19.7 ± 1.5 (80 % theoretical VO_2 max)	NS
Exercise VO_2 (ml/min)	770 ± 58 (48 % VO_2 max)	795 ± 64 (47 % VO_2 max)	NS

AT anaerobic threshold, VO_2 = oxygen consumption

* By two-tailed analysis of variance

[a] Values are mean ± SEM

Post-training HOMA 2-B index resulted in a slight, not significant increase in insulin sensitivity and a significant reduction in β-cell function ($p < 0.05$) (Table 3).

Respiratory exchange ratio (RER) increased from starting 0.755 ± 0.02 at rest to 0.983 ± 0.02 at peak of exercise ($p < 0.001$). After the training period, it increased from 0.765 ± 0.01 to 0.967 ± 0.03 ($p < 0.001$).

Finally, looking for possible correlations among FM, NEFA AUCs and HOMA2-B, only the degree of post-training lowering FM was linearly positive correlated to delta post–pre-training NEFA AUCs ($p = 0.034$; $R^2 = 0.55$), and there was a strong correlation among the post-training decreases in FM, delta post–pre-NEFA AUCs and delta post–pre-training HOMA2-B ($p < 0.009$; $R^2 = 0.78$) at a multivariate analysis.

Effects of 4-week aerobic plus anaerobic training (Group B)

Peak activity, AT, VO_2 max and exercise VO_2 before and after the training periods in Group B are expressed in Table 4.

After training, mean body weight was not significantly decreased from 114.7 to 107.1 kg, FM from 46.1 to 38.9 kg and FFM from 68.7 to 68.4 kg. Nevertheless, the decrease in FM resulted significantly higher in Group B compared to Group A (respectively, −7.2 ± 1.0 vs. −2.7 ± 0.5 kg, $p < 0.001$), even when corrected for difference in starting FM (respectively, −16.5 % ± 3.4 vs. −7.2 % ± 1.4, $p < 0.05$).

Differently from Group A, aerobic plus anaerobic training increased NEFA values at rest, peak, first and late recovery, though not significantly (Fig. 1c). The mean area under the curve AUCs was 30,810 ± 3,102 μEq min/l before training and 42,382 ± 4,232 μEq min/l after training ($p < 0.05$)(Fig. 1d).

Lactic acid increased to its maximal value at peak activity and decreased during recovery. Differently from Group A, after training values were constantly, though not significantly, lower than before training (at peak activity, respectively, 6.06 mmol/l vs 6.79, p = NS) (Fig. 2b). The mean area under the curve AUCs was 250.3 before training and 229.3 after training (p = NS), with a delta value after versus before −21.0 ± 11.9 (Fig. 2c) and with a significant difference between the two groups ($p < 0.01$).

When lactic acid at peak exercise before and after training was normalized according to power output achieved at peak exercise before and after training, we

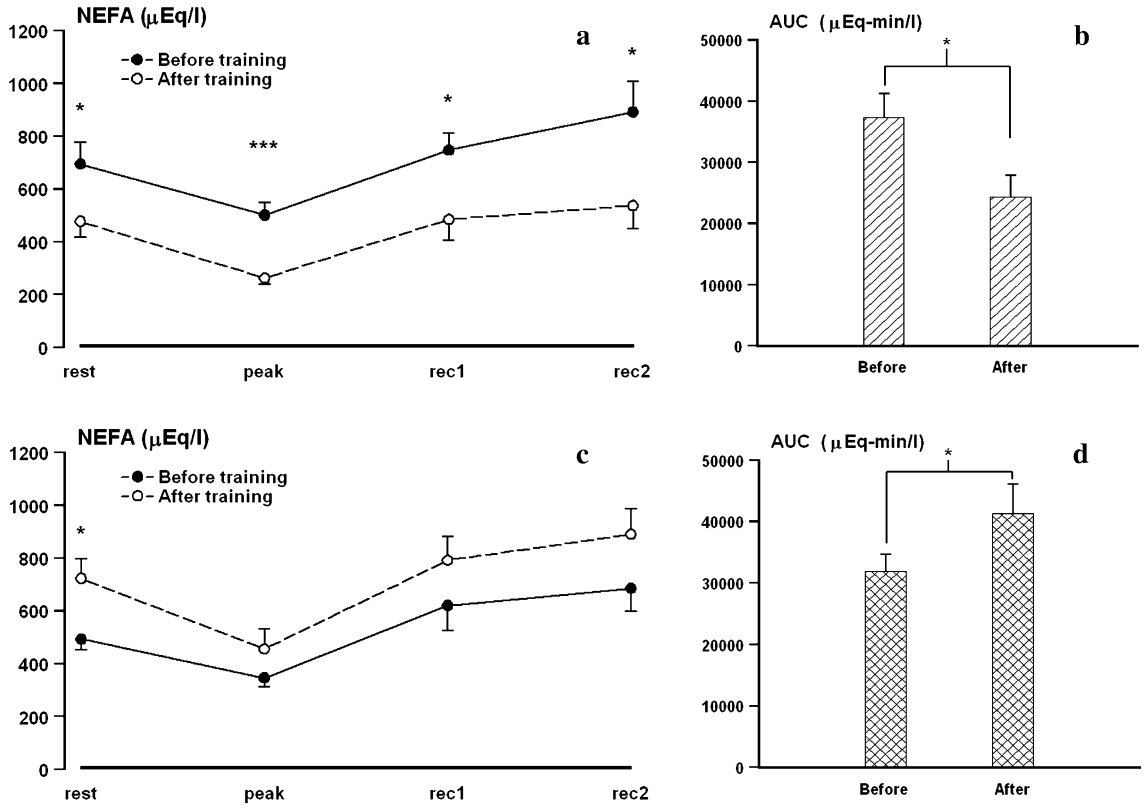

Fig. 1 NEFA at rest, peak activity, first and late recovery before and after the aerobic training period (**a**). AUCs NEFA before and after the training period (**b**) in Group A. NEFA at rest, peak activity, first and late recovery before and after the aerobic and anaerobic training period (**c**). AUCs NEFA before and after the training period (**d**) in Group B (see the text). *Asterisks* indicate a significant difference before and after the training period (see the text) (*$p < 0.05$, ***$p < 0.001$)

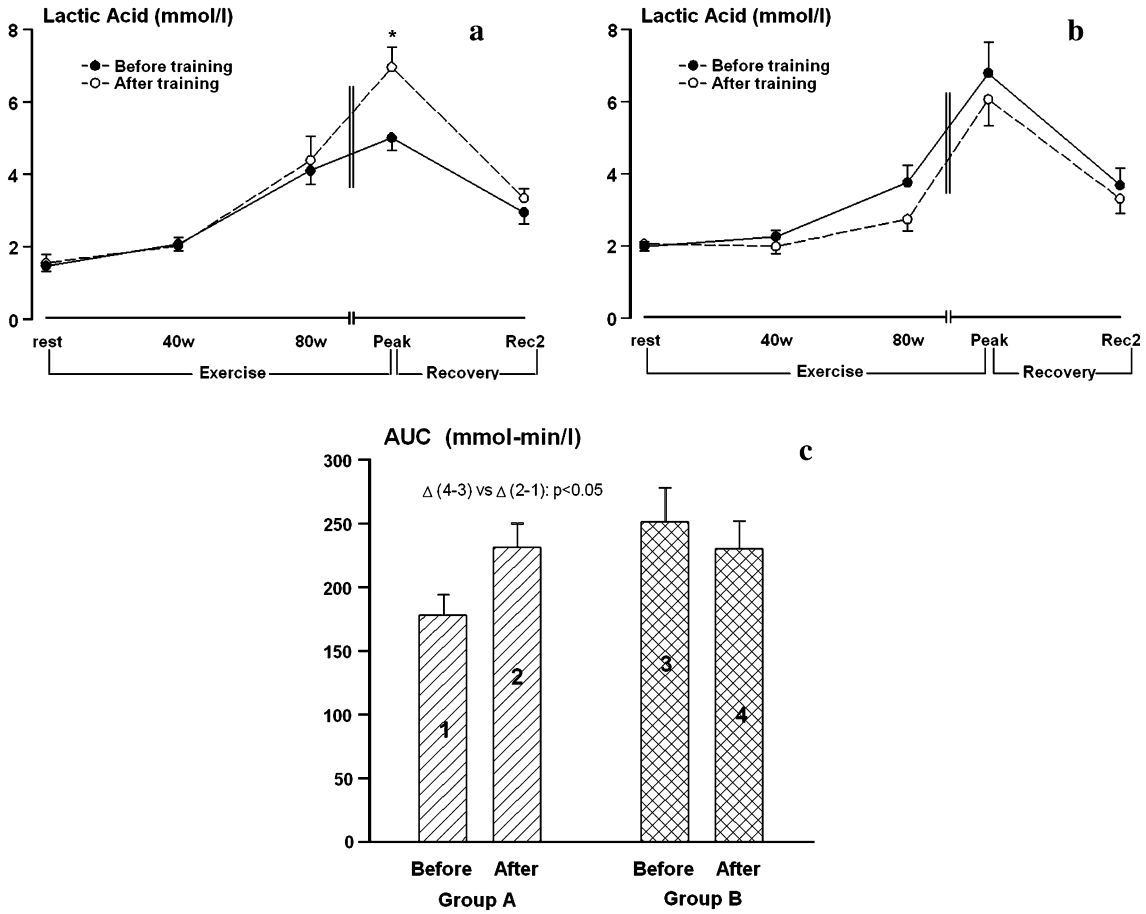

Fig. 2 Lactic acid during and after exercise testing before and after the training period in Group A (aerobic training) (**a**). Lactic acid during and after exercise testing before and after the training period in Group B (aerobic plus anaerobic training) (**b**). AUCs Lactic acid before and after the training periods in Group A and Group B (**c**) (see the text). *Asterisk* indicates a significant difference before and after the training period (see the text) (*$p < 0.05$)

Table 3 Glucose homeostatic model assessment (HOMA), Glucose and Insulin before and after training in Group A and Group B

Variable[a]	Training	Group A			Group B		
		Mean	SE	*p* value	Mean	SE	*p* value*
HOMA2-B	Before	111.18	4.5		118.78	11.3	
	After	90.88	5.2	$p < 0.05$	135.52	14.8	NS
HOMA2-S	Before	87.48	6.6		82.19	7.3	
	After	103.19	14.8	NS	78.18	3.9	NS
Glucose (mmol/l)	Before	4.95	0.1		4.92	0.1	
	After	5.18	0.2	NS	4.74	0.2	NS
Insulin (pmol/l)	Before	64.93	4.9		72.75	11.0	
	After	57.21	6.6	NS	73.53	6.1	NS

* By two-tailed analysis of variance

[a] Values are mean ± SEM

obtained in Group A an increase from 0.055 mmol/l/watt (±0.017 SE) to 0.066 mmol/l (±0.014 SE); in Group B a decrease from 0.056 mmol/l/watt (±0.019 SE) to 0.046 mmol/l (±0.017 SE). Positive delta of Group A (+0.011 ± 0.004) was significantly different from negative delta of Group B (−0.010 ± 0.004) ($p < 0.05$) (Fig. 3).

After training, HOMA2-B index did not change significantly, but with an increasing trend in β-cell function (Table 3).

Respiratory exchange ratio (RER) increased from starting 0.772 ± 0.03 at rest to 0.972 ± 0.03 at peak exercise before training ($p < 0.001$) and from 0.780 ± 0.03 to 0.980 ± 0.01 after training ($p < 0.001$).

Contrary to aerobic exercise, after aerobic plus anaerobic training no correlation among FM, NEFA AUCs and HOMA2-B was detectable.

Discussion

Our study sought to evaluate in simple obese subjects if a brief period of aerobic or aerobic plus anaerobic training could produce different effects in metabolic responses and

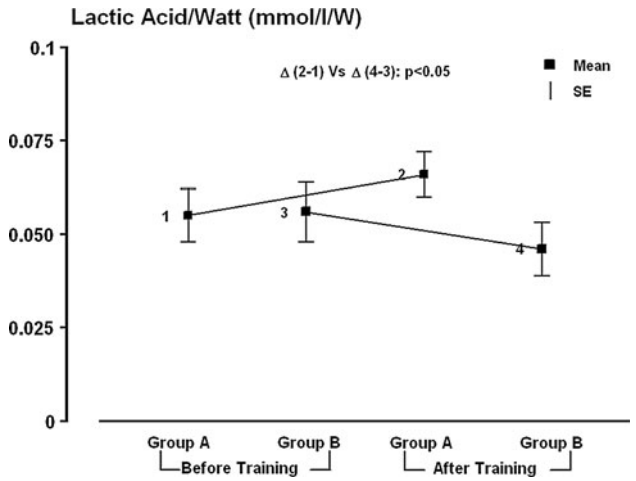

Fig. 3 Lactic acid/watt at peak activity before and after the training periods in Group A and Group B (mean ± SE) (see the text)

Table 4 Functional data Group B

Variable[a]	Before training	After training	*p* value*
Mean exercise peak (W)	125.4 ± 8	138.7 ± 9	NS
Mean A.T. (W)	87.3 ± 7	94.1 ± 7	NS
Mean VO_2 max (ml/min/Kg)	18.7 ± 1.8 (76 % theoretical VO_2 max)	20.2 ± 1.7 (78 % theoretical VO_2 max)	NS
Aerobic Exercise VO_2 (ml/min)	967 ± 75 (47 % VO_2 max)	1,048 ± 82 (48 % VO_2 max)	NS
Anaerobic Exercise VO_2 (ml/min)	1,752 ± 96 (85 % VO_2 max)	1,826 ± 108 (84 % VO_2 max)	NS

AT anaerobic threshold, VO_2 = oxygen consumption

* By two-tailed analysis of variance

[a] Values are mean ± SEM

differences in body weight loss, body composition and performance improvement.

We found that the present study seems to point out the following observations in obesity under physical stress before and after a period of training at different workouts. Differently from other studies [5], we have imposed, as high intensity, a work beyond anaerobic threshold.

Aerobic training did not significantly improve physical performance and did not significantly modify body weight and body composition. In accordance with de Glisezinski [2], aerobic training may act to reduce circulating NEFA in view of increasing the amount of lipids oxidized at rest and during physical activity. The degree of post-training lowering FM was linearly correlated to delta post–pre-training NEFA AUCs. The drop in circulating levels of NEFA may be caused by an imbalance between slow mobilization of fatty acids from adipose tissue and their rapidly increased extraction by skeletal muscle [12]. This hypothesis might explain the increase in RER at peak activity, that is less pronounced (though not significantly) after aerobic training than after aerobic plus anaerobic training.

At the same time, aerobic training ameliorates glucose utilization, as shown by the higher increase in lactic acid beyond anaerobic threshold and HOMA2-B. Its reduction after the training period may probably be linked to a lowering in insulin serum levels with a better utilization of glucose as suggested by enhancement of HOMA2-S, even if not significant. These observations are in accordance with the study of Oshida et al. [13] and, at the same time, suggest that mitochondrial oxidative capacity has improved [14].

The addition of brief periods of work at an intensity beyond AT to a short aerobic training did not appear to improve the physical performance significantly, nor the change in body weight and body composition. Nevertheless, FM loss was greater with aerobic plus anaerobic work than with aerobic work, in accordance with literature data [15]. After aerobic and anaerobic training, we documented an increase in circulating NEFA levels, which may be caused by an excessive mobilization of lipids without an equally concomitant utilization of them. This mobilization may be due to an increased flow of some substances with lipolytic activity after anaerobic stress; among these, an increased flow of GH, as recently observed [16]. At the same time, RER was not changed and lactic acid decreased after aerobic and anaerobic training. In particular, lactic acid at peak activity normalized with external work increased after aerobic activity and lowered after aerobic and anaerobic activity. On the whole, the total secretion of lactic acid after training results significantly lower after aerobic plus anaerobic work than that after aerobic work alone. This may be the result of metabolic effects, considering that Group B had higher FFM and, probably, a higher muscular mass. No modification in insulin sensitivity occurred while function of β-cell showed only a trend to increase. This may imply that the antilipolytic activity of insulin was unchanged by the training scheme.

Strenuous training has been shown to cause large intracellular lipid deposits [17] and a higher reliance of trained muscle on lipids as the substrate for mitochondrial respiration [2, 18]. Thus, the added anaerobic exercise in our obese subjects linked to unchanged insulin sensitivity may be in line with our observations about the observed lowering of lactic acid and the increasing of NEFA, and would suggest a share of lipid utilization that has increased more than that of glucose as fuel for energy.

Our data substantially confirm those of Houmard and Johnson [19, 20], but in our study each subject of both

groups performed training of equal length of time, differently from the other studies where exercise at a lower intensity required more total minutes per week than that at a strenuous intensity.

Some caveats should be considered in the interpretation of our results. First of all, the relative brief period of training. As it was our aim to look for differences between aerobic and anaerobic work, it was necessary to be sure that the appropriate aerobic and anaerobic workloads were carried out. For this reason we enrolled exclusively obese inpatients, and the training period could not be longer than 4 weeks because of organizational questions. Moreover, the two groups had not wide sizes and there were some differences in body measures and composition (see height and FFM). Anyway, we think that the differences between "before training" and "after training" inside each group could be the most relevant outcomes.

Conclusions

Aerobic short-term training seems to confirm its ability to ameliorate NEFA utilization and insulin sensitivity in obesity with an improvement proportional to the degree of FM loss, yet without a sensible FM reduction. The addition of anaerobic training seems to be more efficient in decreasing FM, without a clear improvement in metabolic profile. The higher stress beyond AT does not ameliorate insulin sensitivity, and it further promotes lipids mobilization which probably exceeds their dynamic utilization by increasing some circulating substances (like catecholamines and GH). The results of this study might influence weight loss programs, in particular the choice of physical activity.

More extended periods of aerobic plus anaerobic training and larger numbers of subjects might confirm or not the increased circulating levels of NEFA here observed, meaning possible modifications in membrane fatty acid transporters as time goes by and fitness improves.

Taken together, these observations may prompt to speculate on the opportunity to initially prescribe a period of aerobic with bouts of anaerobic training to decrease fat mass in obese individuals without metabolic alterations, and subsequently to stimulate a prolonged aerobic training alone, to maintain the weight loss and ameliorate the metabolic profile. However, the exercise scheme "aerobic plus anaerobic" might be suitable in patients with simple obesity, but probably inappropriate in obese patients with metabolic syndrome in view of the increase in serum NEFA and the lack of improvement in glucose metabolism.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Horowitz JF, Mora-Rodriguez R, Byerley LO, Coyle EF (1997) Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. Am J Physiol Endocrinol Metab 273:E768–E775
2. De Glisezinski I, Moro C, Pillard F, Marion-Latard F, Harant I, Meste M, Berlan M, Crampes F, Riviere D (2003) Aerobic training improves exercise-induced lipolysis in SCAT and lipid utilization in overweight men. Am J Physiol Endocrinol Metab 285:E984–E990
3. Cahill BR, Misner JE, Boileau RA (1997) The clinical importance of the anaerobic energy system and its assessment in human performance. Am J Sport Med 25:863–872
4. American Medicine College of Sport Medicine (2000) ACSM's guidelines for exercise testing and prescription, 6th edn. Lippincott Williams & Wilkins, Baltimore (MD), pp 141–145
5. Van Aggel-Leijssen DPC, Saris WHM, Wagenmakers AJM, Senden JM, Van Baak MA (2002) Effect of exercise training at different intensities on fat metabolism of obese men. J Appl Physiol 92:1300–1309
6. Harriss DJ, Atkinson G (2011) Update—ethical standards in sport and exercise science research. Int J Sports Med 32:819–821
7. Wasserman K, Hansen JE, Sue DY, Casaburi R, Whipp B (1999) Principles of exercise testing and interpretation, 3rd edn. Lippincot, Williams & Wilkins, Philadelphia (PH), pp 65–67
8. Beaver WL, Wasserman K, Whipp BJ (1986) A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol 60:2020–2027
9. Wallace TM, Levy JC, Mattews DR (2004) Use and abuse of HOMA modelling. Diabetes Care 27:1487–1495
10. Steel RGD, Torrie JH (1980) Principles and procedures of statistics, a biometrical approach. McGraw-Hill, New York (NY), pp 188–190
11. Rowland M, Tozer TN (1995) Clinical pharmacokinetics: concepts and clinical applications, 3rd edn. Media, Williams & Wilkins, Philadelphia (PH), pp 469–472
12. Kiens B (2006) Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev 86:205–243
13. Oshida Y, Yamanouchi K, Hayamizu S, Sato Y (1989) Long-term mild jogging increases insulin action despite no influence on body mass index or $VO_{2\ max}$. J Appl Physiol 66:2206–2210
14. Simoneau JA, Kelley DE (1997) Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NDDM. J Appl Physiol 83:166–171
15. Irving BA, Davis CK, Brock DW, Weltman JY, Swift D, Barrett EJ, Gaesser GA, Weltman A (2008) Effect of exercise training intensity on abdominal visceral fat and body composition. Med Sci Sports Exerc 40:1863–1872
16. Salvadori A, Fanari P, Marzullo P, Codecasa F, Tovaglieri I, Cornacchia M, Walker G, Brunani A, Longhini E (2010) Dynamics of GH secretion during incremental exercise in obesity, before and after a short period of training at different workloads. Clin Endocrinol 73:491–496
17. Hoppeler H (1986) Exercise-induced ultrastructural changes in skeletal muscle. Int J Sports Med 7:187–204
18. Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56:831–838
19. Houmard J, Tanner C, Slentz C, Duscha B, Mc Cartney J, Kraus W (2004) Effect of the volume and intensity of exercise training on insulin sensitivity. J Appl Physiol 96:101–106
20. Johnson JL, Slentz CA, Houmard JA, Samsa GP, Duscha BD, Aiken LB, McCartney JS, Tanner CJ, Kraus WE (2007) Exercise training amount and intensity effects on metabolic syndrome (from studies of a targeted risk reduction intervention through defined exercise). Am J Cardiol 100:1759–1766