Time-course effects of aerobic interval training and detraining in patients with metabolic syndrome

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Abstract Background and aims: Exercise training can improve health of patients with metabolic syndrome (MetS). However, which MetS factors are most responsive to exercise training remains unclear. We studied the time-course of changes in MetS factors in response to training and detraining. Methods and results: Forty-eight MetS patients (52 ± 8.8 yrs old; 33 ± 4 BMI) underwent 4 months (3 days/week) of supervised aerobic interval training (AIT) program. After 1 month of training, there were progressive increases in high density lipoprotein cholesterol (HDL-c) and reductions in waist circumference and blood pressure (12 ± 3%, −3.9 ± 0.4, and −12 ± 1%, respectively after 4 months; all P < 0.05). However, fasting plasma concentration of triglycerides and glucose were not reduced by training. Insulin sensitivity (HOMA), cardiorespiratory fitness (VO2peak) and exercise maximal fat oxidation (FOmax) also progressively improved with training (−17 ± 4; 21 ± 4 and 31 ± 8%, respectively, after 4 months; all P < 0.05). Vastus lateralis samples from seven subjects revealed that mitochondrial O2 flux was markedly increased with training (71 ± 11%) due to increased mitochondrial content. After 1 month of detraining, the training-induced improvements in waist circumference and blood pressure were maintained. HDL-c and VO2peak returned to the values found after 1–2 months of training while HOMA and FOmax returned to pre-training values. Conclusions: The health related variables most responsive to aerobic interval training in MetS patients were waist circumference, blood pressure and the muscle and systemic adaptations to consume oxygen and fat. However, the latter reverse with detraining while blood pressure and waist circumference are persistent to one month of detraining. © 2014 Published by Elsevier B.V.
Aerobic interval training (AIT) is more efficacious than continuous moderate-intensity training to reverse MetS [29] although this finding is not without discussion [14]. Low level of aerobic fitness has revealed as an independent and strong predictor of mortality compared to classical risk factors [5]. Aerobic fitness level is low in individuals with MetS [9] and it is possible that their low aerobic capacity underlies the metabolic and cardiovascular abnormalities that compose their syndrome. Further, exercise training reverses some of the MetS components in direct association with improved maximal aerobic capacity (i.e., VO2max) in the elderly [13]. The adaptations that lead to increased VO2max with training involve, improved cardiovascular function, oxygen carrying capacity of the blood and mitochondria biogenesis. With exercise training, MetS patients improve diastolic function [4] and oxygen carrying capacity of blood [26] to a similar magnitude than in healthy subjects. However, to our knowledge nobody has reported weather exercise training results in improved mitochondrial respiration in MetS patients.

In this study our aim was to determine the time-course progression towards healthy values of the different components of MetS with aerobic interval training (AIT). The identification of metabolic syndrome factors that do not readily respond to exercise could help to design interventions combining exercise, diet and medication for these less modifiable factors. We also studied which of the exercise modifiable factors relapse towards pre-training values after 1 month of detraining. This information may help to prescribe training-break duration to avoid losing and important health benefits obtained from exercise training in these patients.

Methods

Study population

This study was conducted between January 2012 and September 2012 in accordance with a protocol approved by the local Hospital’s Ethics Committee. Forty-eight obese subjects (22 men and 26 women) between 31 and 68 years old (mean 52.0 ± 8.8 yr old) completed the study. Participants were enrolled based on fulfilling ≥3 MetS criteria as per harmonized definition [1] using population-Europid waist circumference cutoffpoints. Subjects were instructed to continue with their current medication prescriptions during the study. Exclusion criteria included cardiovascular or renal disease, peripheral vascular disease and any disease associated with exercise intolerance. Body weight stability in the last six months was also a requirement. All subjects provided written, witnessed, informed consent.

Exercise training and dietary records

Subjects underwent supervised aerobic interval training (AIT) with a frequency of 3 times per week during 4 months. Training consisted on pedaling for 10-min as warm up at 70% HRmax followed by 4 x 4-min intervals at 90% of HRmax interspersed with 3-min active recovery at 70% HRmax and a 5-min cool-down period for a total of 43 min. Exercise intensity was increased as training adaptations developed to maintain the target heart rate (Accurex coded, Polar, Finland). Participants were required to attend at least 85% of all the exercise sessions. Subjects were instructed to maintain their dietary patterns during the duration of the study. A three-day dietary log was collected monthly and analyzed for caloric intake and macronutrient composition.

Clinical investigation

Before training, monthly during the 4 months of training and after 1 month of detraining we tested all subjects for body composition, anthropometry (weight and waist circumference), resting blood pressure, blood metabolites, exercise maximal fat oxidation (FOmax) and peak oxygen consumption (VO2peak) using a graded exercise test. Blood was drawn in the morning after a 10 h overnight fast. All tests were scheduled at least 72 h after the last exercise training session to avoid measuring the acute effects of the last exercise bout rather than the chronic effects of the exercise training program. In addition, percent body fat, trunk body fat and fat-free mass were determined by dual energy X-ray absorptiometry (DXA Hologic Serie Discovery Wi QDR, Bedford, USA). Supine resting blood pressure was recorded using a hand-held aneroid sphygmomanometer (Gamma GST, Heine, Germany) as the average of 4 measurements.

Cardio-respiratory and metabolic fitness

Peak aerobic capacity (VO2peak) was assessed on an electronically-braked cycle ergometer (Ergoselect 200, Ergoline, Germany) during a graded exercise testing using indirect calorimetry, (Quark b2, Cosmed, Italy) with 12 lead ECG monitoring (Quark T12, Cosmed, Italy). The highest heart rate value obtained during the test was considered HRPEAK. Maximal fat oxidation (FOmax) was assessed in a fasted state using a graded exercise test with 3 min stages until respiratory exchange ratio exceeded 1.0. The last minute of each stage was averaged to calculate non-protein respiratory quotient and fat oxidation rate [10].

Blood analyses

Plasma glucose was analyzed using the glucose oxidase-peroxidase method with intra-inter assay coefficient of variation (iCV) of 0.9–1.2%. Glycated hemoglobin (HbA1c), apolipoprotein B (Apo B) and high sensitive protein C reactive (hsPCR) using immune-turbidimetry tests (iCV; 0.7–2.1%). HDL-c using accelerator selective detergent method (iCV; 1.7–2.9%). Blood triglycerides (TG) with glycerol-3-phosphate oxidize method (iCV; 0.8–1.7%). Total serum cholesterol (T Chol) by an enzymatic method (iCV; 0.9–1.2%) using accelerator selective detergent method (iCV; 2.1%). HDL-c using accelerator selective detergent method (iCV; 1.7–2.9%). Blood triglycerides (TG) with glycerol-3-phosphate oxidize method (iCV; 0.8–1.7%). Total serum cholesterol (T Chol) by an enzymatic method (iCV; 0.9–1.2%). Low-density lipoprotein-cholesterol (LDL-c) was calculated as proposed by Friedewald [11]. All the above analyses were run in an automated Mindray BS 400 Chemistry Analyzer (Mindray Automation, China).
Medical instrumentation, USA). Insulin concentration was measured in duplicate using chemiluminescent micro particle immunoassay (iCV: 2.0–2.8%) in an automated immunoassay analyzer (Architect c4100, Abbott Laboratories, USA). Insulin sensitivity was calculated using the homeostasis model assessment (HOMA\textsuperscript{[20]}).

**Mitochondrial respiration**

Muscle biopsies were obtained in seven subjects (5 men and 2 women) before training and 4 days after the last training session. Biopsies from the vastus lateralis were obtained under local anesthesia (2% lidocaine without epinephrine; Braun, Germany) and rapidly cleaned of blood, fat and connective tissue. Then a 20–40 mg piece was immersed in BIOPS solution for mitochondrial respiration analysis \textsuperscript{[16]}. The remaining of the biopsy sample was immediately frozen in liquid nitrogen and stored at −80°C for latter fluorometric analysis of citrate synthase activity \textsuperscript{[21]}. Mitochondria respiration was assessed in saponin-permeabilized muscle fibers using a high-resolution respirometer (Oxygraph, Hansatech Instruments Ltd, Norfolk, England). Malate (M), octanoylcarinate (O), glutamate (G), succinate (S), NADH, ADP were progressively added to the medium to measure the different mitochondria respiration states. Intactness of the outer mitochondrial membrane was tested by quantifying respiration after addition of cytochrome c (10 μM).

**Statistical analyses**

We calculated a Z score to assess the continuous rather than dichotomous evolution on the MetS risk factors. Z score was calculated in each MetS criteria using the monthly standard deviations. Blood and muscle biochemistry data, body composition, anthropometry and exercise data, were analyzed during training and detraining using one-way ANOVA with repeated measures. Tukey's post-hoc analysis was performed when a significant F value was obtained. Data are presented as the mean ± s.e. except for descriptive data which are presented as mean ± s.d. Statistical significance level was set at $P < 0.05$.

**Results**

**Subjects and diet**

Participants were all Caucasians and their responses to training did not vary between genders (46% males and 54% females). Thus data were analyzed as a group without gender distinctions. Two subjects dropped out after baseline testing, however, they did not differ from the others with regards to age, fitness or blood chemistry (Fig. 1). No significant diet alterations were detected during the 4 month exercise intervention period or during detraining. Subjects ingested an average of 1880 ± 85 kcal · day\(^{-1}\) with a distribution of 41 ± 12%, 38 ± 11% and 21 ± 10% for carbohydrate, fat and protein. Saturated fat ingestion was maintained as 40% of total fat ingested.

**Factors comprising metabolic syndrome**

Evolution of the MetS factors with exercise is depicted in Table 1. After 4 months of training patients had a reduction in waist circumference of 3.9 ± 0.4% a 12 ± 3% increase in HDL-c, a 12 ± 1% reduction in SBP and DBP (all $P < 0.05$). However, plasma glucose and triglycerides concentrations were not significantly reduced after 4 months of training. Z scores revealed that at pre-training patients were 3.2 ± 0.4 standard deviations above the mean of a healthy value for their respective gender. After four months of training, subjects were only 1.9 ± 0.4 standard deviations away from normal values (Table 1; $P < 0.05$). Most of these changes were already significant after 1–2 months of training.

**Additional physiological parameters**

Subjects progressively reduced their body weight by 1.8 ± 0.4 kg, fat mass by 1.1 ± 0.3 kg and trunk body fat by 0.9 ± 0.2 kg after 4 months of training (Table 2; all $P < 0.05$). HbA1c showed a progressive reduction with exercise training reaching the lower values after 4 months of training (4 ± 1% reduction; Table 2; $P < 0.05$). Insulin and HOMA suggested improved insulin sensitivity although the improvements were not progressive with training duration (Table 2). Fasting glucose and blood parameters of lipid metabolism (Apo B, T Chol and LDL-c) were not reduced from pre-training values at any time during the 4 months of training. hsPCR was reduced below pre-training values after 1 month of training and further after 3 and 4 months of training (Table 2; $P < 0.05$).

**Exercise parameters**

FO\textsubscript{MAX} during exercise increased above pre-training values after 3 months of training and increased further with 4
months of training (31 ± 8%; Table 2; *P* < 0.05). VO₂peak increased progressively throughout the 4 months of training reaching a 21 ± 2% improvement. This increase was accompanied by enhanced maximal pedaling workload (W Max; 39 ± 3%, Table 2; *P* < 0.05) and peak heart rate (HR peak; 4 ± 2%, Table 2; *P* < 0.05).

**Mitochondrial respiration**

O₂ flux in *vastus lateralis* per mg of wet tissue increased after 4 months of AIT in state 3 respiration representing electron input from complex I and fatty acid β-oxidation (i.e., M03, Fig. 2; *P* < 0.05). The substrate control ratio remained elevated for complexes I and II and maximal coupled state 3 flux rate was also elevated (i.e., GMOS, Fig. 3; *P* < 0.05). The addition of cytochrome c did not result in significant increases in O₂ flux, ensuring the intactness of the outer mitochondrial membrane. Citrate synthase activity increased 61% after 4 months of training from 12.0 ± 4.3 to 19.3 ± 5.0 μmol/g wet weight/min (*P* < 0.05). When O₂ flux data was normalized by citrate synthase activity training effects disappeared suggesting that the training induced increase in oxygen flux was attributable to an increase muscle mitochondrial content (Fig. 2b).

**Relapse after one month of detraining**

After one month of detraining subjects maintained their average reduction in body weight (1.7 ± 0.4 kg), waist circumference (4.1 ± 0.4 cm) and trunk fat losses (0.8 ± 0.2 kg). One month of detraining returned blood pressure to the 3 month level (Table 2; Fig. 3a). However, HDL-c returned to the levels observed after 2–3 months of training. Resting insulin and HOMA returned towards pre-training values (Table 2 and Fig. 3b). The following exercise

<table>
<thead>
<tr>
<th>Table 1 Evolution of metabolic syndrome factors and compound Z score with training and detraining.</th>
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<tbody>
<tr>
<td><strong>Pre-training</strong></td>
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<tr>
<td>Waist circumference (cm)</td>
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<tr>
<td>HDL-c (mmol/l)</td>
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<tr>
<td>Glucose (mmol/l)</td>
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<td>Triglycerides (mmol/l)</td>
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<tr>
<td>MetS Z score</td>
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<td>Subjects with ≤3 MetS factors</td>
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</tbody>
</table>

Values are the mean ± s.e. for 48 MetS subjects.

* Significantly different from pre-training.

* Significantly different from the previous month.

* Significantly different than 2 months (all *P* < 0.05).

<table>
<thead>
<tr>
<th>Table 2 Change in anthropometric, blood and exercise variables after 4 months of training and 1 month of detraining.</th>
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<tbody>
<tr>
<td><strong>Pre-training</strong></td>
</tr>
<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Body fat (%)</td>
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<tr>
<td>Trunk body fat (kg)</td>
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<tr>
<td>Leg fat-free mass (kg)</td>
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<tr>
<td>Blood variables</td>
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<tr>
<td>Insulin (pmol/l)</td>
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<tr>
<td>HbA1c (%)</td>
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<td>HOMA</td>
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<td>Apo B (g/l)</td>
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<td>hs CRP (mmol/l)</td>
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<tr>
<td>Exercise parameters</td>
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<tr>
<td>FCMAX (g/min)</td>
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<tr>
<td>VO2peak (ml kg⁻¹ min⁻¹)</td>
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<tr>
<td>WMAX (watts)</td>
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<td>HRMAX (bpm)</td>
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</table>

Values are mean ± s.e. for 48 Mets subjects.

* Significantly different from pre-training.

* Significantly different from the previous month.

* Significantly different than 2 months (all *P* < 0.05).
parameters were also sensitive to training discontinuation, \( W_{\text{MAX}} \) returning to the 3 months value, \( VO_2\text{peak} \) to the 2 month value and \( FOM_{\text{MAX}} \) to pre-training values (Table 2 and Fig. 3b).

**Discussion**

We monthly followed the evolution of the risk factors that compose the metabolic syndrome (MetS) with aerobic interval training (AIT) and its relapse with subsequent detraining. We observed a dose-response relationship between exercise training duration and improvements in waist circumference (surrogate of abdominal obesity), blood pressure (systolic and diastolic) and HDL-c. As a consequence of these improvements 29% of our subjects reduced the number of MetS factors below three (Table 1). Thus, our data suggest that in previously sedentary MetS patients 4 months of an AIT program could reverse MetS in roughly one third of this population. Of note, this outcome is obtained when patients do not alter their regular medication or their self-reported caloric intake, although body weight was reduced by 2.1 ± 0.4%. Of note, after 4 months of the AIT program, we did not observe a plateau response in the factors that training improved (Fig. 3). This suggests that an AIT program prolonged beyond 4 months could reverse to normal clinical values three important MetS components in most patients.

The most salient and consistent benefit of AIT was the reduction in blood pressure that reached 12% (Table 1, Fig. 3a) which to our knowledge, is unmatched in the training literature in MetS patients [29]. A recent review analyzing the effects of high-intensity interval training suggests that 3 months of AIT are required for lowering blood pressure with effects only visible in people not taking antihypertensive medication [15]. In contrast, 67% of our sample were under antihypertensive medication and a large portion of the blood pressure reduction took place only in the high-intensity interval training group, although this latter finding is not statistically significant (Fig. 3a).

![Figure 2](image1.png)

**Figure 2** Effects of 4 months of training on (a), mitochondrial respiration in vastus lateralis (O2 flux per mg wet weight tissue) when adding different substrates and (b) mitochondrial respiration when normalized for mitochondrial content (CS activity). Data are mean ± s.e. for 7 volunteers. * Significantly higher than Pre-training (\( P < 0.05 \)).

![Figure 3](image2.png)

**Figure 3** Percent change from pre-training on (a) variables persistent after 1 month of detraining and (b) variables that relapse with detraining. Data are mean ± s.e. for 48 MetS subjects. * Significantly different from Pre-training (\( P < 0.05 \)).
place after only 1 month of AIT (Fig. 3a; \( P < 0.05 \)). Thus, our data suggest that the hypertension associated with the MetS can be rapidly and substantially improved with AIT. This improvement in conjunction with the increased HDL-c lowers the cardiovascular disease mortality risk in MetS patients that follow this AIT program.

Another of the variables most markedly improved with AIT was cardio respiratory fitness with a 21 \( \pm 2 \)% increase in VO\(_{2}\)peak. It has been proposed that the type of training that result in larger increase in VO\(_{2}\)max is the one associated with the removal of more MetS factors [9]. Furthermore, in a prospective study in 1226 men and women it was found that one standard deviation increase in VO\(_{2}\)max raised the likelihood to resolve MetS 1.8 times [13]. We observed in our subjects that a 1.3 SD improvement in their MetS Z score was associated with a 1.4 SD raise in VO\(_{2}\)peak. Moreover, the improvements in cardio respiratory fitness were accompanied by a 31 \( \pm 8 \)% increased capacity to oxidize fat during exercise (FO\(_{\text{max}}\); Fig. 3b). Our data support that with AIT MetS patients experience the habitual cardiovascular adaptations that improve VO\(_{2}\)peak and the metabolic adaptations that increase the reliance on fat as energy substrate during exercise.

In a subset of our subjects (only seven) we studied mitochondrial function \textit{in vivo} within hours of vastus lateralis muscle collection. The reason to perform this measurement is that it has been suggested that mitochondrial dysfunction may be a central cause of insulin resistance in MetS patients as it is in T2DM patients [25]. However, our MetS subjects greatly increased vastus lateralis oxygen flux after 4 months of training (Fig. 2a). Those increases were mostly due to increased mitochondrial density since when normalize by CS activity (surrogate of mitochondrial proliferation) the differences disappeared (Fig. 2b). Obese and type 2 diabetic subjects [18] have reduced mitochondrial density in comparison to lean controls but not \( O_2 \) flux when normalized per mitochondria unit [17]. Rather than mitochondrial dysfunction, these sedentary populations (e.g., our metabolic syndrome patients) seem to have underdeveloped muscle mitochondrial mass that however proliferates normally upon stimulation with AIT.

Our AIT program did not lower blood glucose below 100 mg \textpergdash{} dL\(^{-1}\) (Table 1). However, HbA1c, an index of the average plasma glucose concentration over prolonged periods of time significantly improved after AIT (Table 2). Some studies propose the inclusion of HbA1c as an additional factor in the definition of MetS [24]. HbA1c reduction with the progression of training suggests that training decreased the amount and/or magnitude of daily blood hyperglycemic peaks. We could not detect a reduction in carbohydrate ingestion in subject’s dietary records that could account for the lower presence of glucose in blood. Thus, the reduced glucose presence in hemoglobin suggests improved glucose clearance from blood. In addition, the improvement in HOMA (Fig. 3b) suggests that peripheral tissue insulin sensitivity was improved by our AIT program. We observed a significant reduction in hsPCR with exercise training along with the early improvement in HOMA (Table 2). Our data may support the theory of an inflammatory origin to the carbohydrate metabolism disarrangements in MetS [30].

Our 4 months of AIT did not lower subject’s blood TG, Apo B, HDL-c or total cholesterol. However, AIT increased the capacity to oxidize fat during exercise and lowered body fat by 0.9 kg in the trunk (Table 2). In addition, HDL-c increased with exercise training in a progressive fashion (Table 1) which has been shown to reduce coronary heart diseases risk [6]. Some investigators have found triglycerides resiliency to be reduced after 12–16 weeks of AIT [15,29], while others find a lowering effect when a Mediterranean hypocaloric diet is combined with exercise training [8]. We have recently reported in overweight subjects that exercise does not lower plasma triglycerides when the diet is high in saturated fat [22]. Our training program was neither accompanied by a reduction in dietary energy intake nor by restrictions in saturated fat intake. It is possible that the combination of an exercise training program with dieting be required to significantly reduce blood TG and LDLC in MetS patients.

While the improvements in body composition and blood pressure resisted the effects of 1 month of detraining, HOMA, HDL-c, VO\(_{2}\)peak and FO\(_{\text{max}}\) returned to the 1–2 month training values (Fig. 3b). After 6 months of a demanding training program in overweight individuals, 15 days of detraining does not completely revert the improvements in insulin sensitivity and HDL-c [2,27]. Either due to our longer detraining period (1 month) or to our shorter training program (4 months) we could not observe remaining effects of training on insulin sensitivity. However, our data coincides on the persistent elevation of 2–3 mg\textpergdash{}dL\(^{-1}\) in HDL-c despite detraining. In the cited studies as well as in the present study, subjects did not regain body mass or body fat during detraining. It seems that if body fat is not restored after short-term detraining (15–30 days) some of the keys training adaptations are preserved (i.e., blood pressure).

In conclusion, four months of aerobic interval training progressively reduces body weight, trunk fat, blood pressure and increases HDL-c which in combination greatly lowers cardiovascular disease risk. MetS does not seem to impede the exercise related muscle and systemic adaptations to improve oxygen and fat consumption. However, blood glucose and triglycerides did not improve and combinations of exercise training with diet and medication should be sought. Lastly, the detraining data suggest the importance of not regaining abdominal fat to preserve the reductions in blood pressure.

**Acknowledgments**

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