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Rapid down-regulation of mitochondrial fat metabolism in human muscle after training cessation is dissociated from changes in insulin sensitivity ☆

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ABSTRACT

The association between impairment in mitochondrial muscle fat oxidative capacity (OX_{FA}) and occurrence of insulin resistance was examined in 14 healthy trained men (age, 24 ± 4 yr) submitted to 4 weeks of training cessation. Training stop induced a significant decrease in mRNA levels of proteins involved in muscle fat metabolism, particularly PPARα (−58%, $P < 0.01$) and PGC-1α (−30%, $P < 0.05$), a 21% reduction in OX_{FA} ($P < 0.01$), and reduced fat oxidation during moderately intense exercise ($P < 0.05$). In contrast, there was no significant alteration in insulin sensitivity. In conclusion, decline in OX_{FA} is a rapid metabolic event following training cessation. It is involved in the regulation of whole body fat balance but not in the deterioration of insulin sensitivity.

1. Introduction

Insulin resistance in skeletal muscle precedes and predicts the development of type 2 diabetes [1,2]. Decreased muscle mitochondrial oxidative function [3] and fat oxidative capacity [4,5] has been considered as a risk factor for insulin resistance. Indeed, decreased fat oxidation and excess fat storage can promote the intramyocellular accumulation of fatty acid metabolites (e.g. diacylglycerols and ceramides) known to impede the insulin signal transduction pathway [6,7]. This assumption has been supported by several cross-sectional studies [3,4,8]. Furthermore, we previously reported that 8 weeks of progressive endurance training improved insulin sensitivity in parallel with muscle fat oxidative capacity (OX_{FA}) in healthy elderly people [8]. In contrast, recent studies have demonstrated that high-fat diet induced-insulin resistance can be dissociated from muscle mitochondrial function [9]. The present study aimed to tackle these controversies by examining whether any intervention known to induce a decline in OX_{FA} is or is not associated with impaired insulin sensitivity. For this purpose, we considered short-term training cessation as a pertinent model for investigating the early mechanisms responsible for decreased OX_{FA} [10,11] and its association with changes in insulin sensitivity. We studied healthy adults to determine the consequences of 4 weeks of training cessation on: (1) muscle fat oxidative capacity (both at skeletal muscle mitochondrial level (OX_{FA}) and whole body level), (2) mRNA expression of genes encoding major enzymes and proteins controlling the muscle fat oxidative pathway, and (3) the association between alterations in muscle fat metabolism and changes in insulin sensitivity.

Abbreviations: BMI, body mass index; VO_{2max}, maximal oxygen uptake; EE, energy expenditure; RER, respiratory exchange ratio; NEFA, non-esterified fatty acids; OX_{AG}, muscle fat oxidative capacity; HAD, beta-hydroxyacyl CoA dehydrogenase; CS, citrate synthase; COX, cytochrome *c* oxidase

☆ This work is dedicated to Prof. Mario Bedu, University Clermont 2, Laboratory of Exercise Biology.

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2. Materials and methods

2.1. Subjects

The intervention study involved 14 healthy men (23.7 ± 3.9 yr) who regularly practiced exercise >4 h/week (i.e. cycling and/or running). A control group was formed with six sedentary healthy

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men (25.5 ± 2.8 yr). All subjects were non-obese, weight-stable, non-smokers, not suffering from any diagnosed disease, and under no medication known to influence energy and lipid metabolism. Subjects were included in the study following a maximal aerobic power test performed to confirm the declared volume of sport exercise. Maximal oxygen consumption (VO_{2max}) averaged 53.0 ± 3.5 and 38.5 ± 3.2 ml/min/kg/body weight for the trained subjects and the sedentary individuals, respectively ($P < 0.0001$). The nature and potential risks of the study were fully explained, and written informed consent was obtained from each participant. The experimental protocol was approved by the Clermont–Ferrand ethical committee (No. AU533) and was performed according to the principles expressed in the Declaration of Helsinki, as revised in 2000.

2.2. General study design

2.2.1. Intervention study

The inclusion trial consisted of an activity questionnaire, a medical examination and a maximal aerobic power test. The measurement period started the first week with a moderate aerobic power test and heart rate recordings during 7 days in free-living conditions. The training cessation protocol lasted for 4 weeks (from week 2 to week 6). The subjects stopped sport exercise 2 days before initial metabolic measurements that included basal indirect calorimetric measurement, muscle biopsy, 3-h euglycemic–hyperinsulinemic clamp, and body composition determination. From two days before and throughout the metabolic measurements, the volunteers were placed on a controlled diet (35%, 50% and 15% of energy in the form of lipids, carbohydrates and proteins, respectively) [12]. Heart rate was re-recorded over 7 days in free-living conditions at the end of the training cessation period. Thereafter, the final metabolic measurements repeating the initial measurements were performed while diet was similarly controlled. Finally, 4 days after the final muscle biopsy, the volunteers underwent moderate and maximal aerobic power tests.

2.2.2. Control group

After the inclusion trial, the subjects were placed on a controlled diet and the metabolic measurements included a muscle biopsy, a 3-h euglycemic–hyperinsulinemic clamp, and body composition measurement.

2.3. Physical activity characterization

2.3.1. Maximal aerobic power test

The tests were all performed on the same cycloergometer (Ergomeca, Monark, Sweden) under cardiovascular supervision by a cardiologist, as described in [12].

2.3.2. Heart rate (HR) recording

HR was recorded minute-by-minute using telemetry (Polar protrainer™, Polar Electro Oy, Finland) during seven consecutive days. The time spent per day at specific activity intensities (<40%, 40–60% and >60% of VO_{2max}) was calculated using the individual relationship between HR and $\%VO_{2max}$ established during the maximal aerobic power test [12].

2.4. Body composition assessment

A transverse total body scan was performed using DEXA (Hologic QDR 4500 X-ray bone densimeter, Hologic, Waltham, MA) for determination of total and regional (arms, legs and trunk) body composition [13]. Appendicular muscle mass was calculated as the sum of the fat-free mass of both arms and legs.

2.5. Whole body substrate oxidation

2.5.1. Basal indirect calorimetric measurements

After an overnight fast, basal non-protein respiratory exchange ratio (RER) and energy expenditure (resting EE) were measured using open-circuit indirect calorimetry (Deltatrac, Datex, Geneva).

2.5.2. Moderate aerobic power test

The test was performed at least 3 h after the last meal and consisted of five sessions (at 25, 30, 40, 50 and 60%VO_{2max}) of 6 min per session on a cycloergometer [14]. RER was determined at each session taking into account the last 2-min values using indirect calorimetry (VIASYS, Oxycon Pro, JAEGER, Germany).

2.6. Daily energy expenditure (daily EE)

Daily EE was calculated from the 7 day-HR recordings in free-living conditions. The individual relationship between HR and EE from basal indirect calorimetry and maximal aerobic power test measurements was used [12].

2.7. Blood sampling

After an overnight fast, blood samples were collected and plasma was kept at −80 °C until further analysis. Plasma concentrations of glucose, insulin, triglycerides (TG) and non-esterified fatty acids (NEFA) were determined using commercial kits, as described in [8,13].

2.8. Insulin sensitivity

Insulin sensitivity was assessed after an overnight fast using a 3-h euglycemic–hyperinsulinemic clamp [15]. The steady-state period was reached during the last 60 min. The index of insulin sensitivity, i.e. $M/(G \times \Delta I)$ ratio, was calculated according to Katz et al. [15].

2.9. Muscle biopsy and assays

2.9.1. Muscle biopsy

After an overnight fast and before the insulin clamp was performed, percutaneous needle biopsies (180–250 mg) were obtained from the vastus lateralis muscle under local anesthetic (5 ml lidocaine 2%). Muscle biopsies were dissected free of blood, connective tissue and fat.

2.9.2. Muscle fat oxidative capacity

[1-^{14}C]palmitate oxidation by fresh muscle homogenate was performed using the method described in [13].

2.9.3. Muscle enzyme activities

Citrate synthase (CS), complex I, complex II, complex III, cytochrome *c* oxidase (COX) and beta-hydroxyacyl CoA dehydrogenase (HAD) activities were assayed spectrophotometrically on the above muscle homogenates (after storage at −80 °C) as described by Rustin et al. [16] and Morio et al. [13].

2.9.4. Preparation of total RNA

Total RNA was prepared from frozen muscle samples according to a procedure based on the method of Chomczynski and Sacchi [17]. Average yields of total RNA were 26 ± 2 μg/100 mg of muscle (wet weight) and were not significantly different before and after training cessation. Total RNA solutions were stored at −80 °C.