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Prevention of hyperglycemia in Zucker diabetic fatty rats by exercise training: effects on gene expression in insulin-sensitive tissues determined by high-density oligonucleotide microarray analysis

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Abstract

Exercise training (ET) causes metabolic improvement in the prediabetic and diabetic states. However, only little information exists on the changes to ET at the transcriptional level in insulin-sensitive tissues. We have investigated the gene expression changes in skeletal muscle, liver, fat, and pancreatic islets after ET in male Zucker diabetic fatty (ZDF) rats. Eighteen ZDF rats (7 weeks old) were divided in a control and ET group. Exercise was performed using a motorized treadmill (20 m/min 1 hour daily for 6 days a week). Blood glucose, weight, and food intake were measured weekly. After 5 weeks, blood samples, soleus muscle, liver, visceral fat (epididymal fat pads), and islet tissue were collected. Gene expression was quantified with Affymetrix RG-U34A array (16 chips). Exercise training ameliorates the development of hyperglycemia and reduces plasma free fatty acid and the level of glucagon-insulin ratio ($P < .05$). In skeletal muscle, the expression of 302 genes increased, whereas that of 119 genes decreased. These changes involved genes related to skeletal muscle plasticity, Ca^{2+} signals, energy metabolism (eg, glucose transporter 1, phosphorylase kinase), and other signaling pathways as well as genes with unknown functions (expressed sequence tags). In the liver, expression of 148 genes increased, whereas that of 199 genes decreased. These were primarily genes involved in lipogenesis and detoxification. Genes coding for transcription factors were changed in parallel in skeletal muscle and liver tissue. Training did not markedly influence the gene expression in islets. In conclusion, ET changes the expression of multiple genes in the soleus muscle and liver tissue and counteracts the development of diabetes, indicating that ET-induced changes in gene transcription may play an important role en the prevention of diabetes.

1. Introduction

The development of type 2 diabetes is counteracted by lifestyle modifications such as weight loss, dietary changes, and exercise [1,2]. Exercise training (ET) is associated with an improvement of insulin sensitivity, amelioration of hyperlipidemia, and reduction in cardiovascular disease [3].

The mechanisms underlining the metabolic improvement during training have been studied previously. After ET, the glucose clearance and insulin action are increased in healthy [4] as well as in type 2 diabetic subjects [5]. Recent studies [6-8] have focused on the importance of gene expression modifications in the adaptation to ET, especially in skeletal muscle. However, the impact at the molecular level in the various insulin-sensitive tissues has only been scarcely described in diabetic and prediabetic states.

Prospective studies assessing the physiological and biochemical effects of physical training in humans can be difficult to carry out in a controlled setting. A suitable animal model may provide a unique research tool for such studies. The Zucker diabetic fatty (ZDF) rats have a mutation in the gene encoding the leptin receptor, which results in the expression of nonfunctional receptor, and

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develop obesity, insulin resistance, and type 2 diabetes. The progression into overt diabetes is quite similar to the development of human type 2 diabetes [9,10].

In the present study, we have investigated in an animal model of type 2 diabetes, using male ZDF rats, the effect of 5 weeks' training on the metabolism and gene expression in soleus muscle, liver, pancreatic islets, and adipose tissue using the oligonucleotide microarray gene chip technology.

Our hypothesis was that ET causes alterations at the gene expression level in insulin-sensitive tissues that lead to an improvement in the glucose metabolism in type 2 diabetes. The primary objective was to compare changes in the expression of genes involved in metabolic (glucose and lipid) and signaling pathways in skeletal muscles, liver, pancreatic islets, and visceral adipose. The second objective was to identify possible new genes involved in the prevention of type 2 diabetes after ET.

2. Research design and methods

2.1. Animals

Male ZDF (ZDF/Gmi-*fa/fa*) rats were purchased from Genetic Models (Indianapolis, IN). Animals were housed in individual cages in a vivarium, which maintains a constant temperature and an artificial 12-h light-dark cycle. Animals were fed with commercial chow (Purina Formulab 5008, Ralston Purina Corp, St Louis, MO).

2.2. Experimental protocol

After acclimatization, 18 animals at the age of 7 weeks were randomly divided into 2 groups: control (n = 9) and ET (n = 9). The animals were maintained on an ad libitum diet. The ET animals ran 1 hour daily at 20 m/min on a motorized treadmill (Columbus Instruments, Columbus, OH). The exercise was performed in the morning 6 days a week. In the first week, exercise intensity and duration were gradually increased to ensure compliance and to reduce stress and injury. The 2 groups were studied in parallel for a period of 5 weeks (from 7 to 12 weeks of age). All institutional guidelines for care and use of animals were followed. One ET animal was excluded and subsequently killed after injury in the second week. Animals were weighed in the morning once per week, and glucose was measured in blood from the tail vein after 4 hours without access to food. Food intake was measured. At the end of the fifth week, 24 hours after the last exercise bout and after 4 hours without access to food, the animals were anesthetized with pentobarbital (50 mg/kg IP), and capillary blood samples were obtained from the retro-ocular plexus using a 75-μL heparinized capillary tube. Successively, the pancreas, liver, skeletal muscle (soleus), and visceral fat tissues (epididymal fat pads) were isolated. Liver, muscles, and fat tissue were rapidly frozen and stored at -80°C. The remains of the animals were also stored at -800°C for further body composition measurements.

2.3. Biochemical measurements

The weekly control of blood glucose concentration was carried out using One Touch Instrument (Lifescan, Milpitas, CA). Other blood samples were placed on ice and centrifuged (10 minutes, 4°C, 4000 rpm). Total cholesterol was analyzed with the cholesterol CHOD-PAP method (Roche, Mannheim, Germany). Triglyceride was analyzed with the triglyceride GPO-PAP method (Roche). Free fatty acid was analyzed with the nonesterified fatty acid (NEFA) C kit, ACS ACOD method (Wako, Neuss, Germany). We analyzed plasma insulin and glucagon by radioimmunoassay kits (Linco Research, St. Charles, MO).

2.4. Isolation of pancreatic islets

Islets were isolated by the collagenase digestion technique [11] with minor modifications. In brief, when the animals had become anesthetized, a midline laparotomy was performed. The pancreas was retrogradely filled through the pancreatic duct with 9 mL ice-cold Hanks balanced salt solution (Sigma Chemicals, St. Louis, MO) supplemented with 0.9 mg/mL collagenase P (Boehringer Mannheim, Mannheim, Germany). The pancreas was subsequently removed and incubated for 17 minutes at 37°C in a shaking water bath. After rinsing in ice-cold Hanks balanced salt solution, the islets were handpicked under a stereomicroscope and stored in 1.5 mL Trizol (Gibco BRL, Paisley, UK).

2.5. Messenger RNA preparation and analysis

2.5.1. RNA extraction and samples pool

Total RNA was isolated from the different tissues using Trizol. For gene chip analysis, an equal amount (10 μg) of total RNA from a given tissue was pooled in 2 subgroups (RNA from 4 to 5 animals in each subgroup).

2.5.2. Gene expression chip analysis

RNA labeling, array hybridization, and scanning were preformed as previously described [12] according to the Affymetrix (Santa Clara, CA) technical manual. All arrays were visualized using Affymetrix Genechip 5.0 software. The Rat Genome U34 A monitors the expression of more than 8000 genes and expressed sequence tag (EST) clusters. The present microarray has been largely evaluated and used in similar experiment [12-18].

2.5.3. Data analysis

A total of 16 gene chips were used, 2 biological replicas for each tissue and condition. After global scaling, the signal, the detection (P, present; A, absent), the signal log ratio (SLR; ie, the logarithm in base 2 of the fold change), and the different call change (I, increase; D, decrease; M, moderate I or D) were calculated. For every tissue, we compared the gene expression of the intervention groups vs the control group in a double-cross analysis (concordance analysis), for a total of 4 comparisons. Moreover, for every gene or EST, we calculated the concordance on the different call change (I + MI, increase and moderate increase; D +