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| 著者  | Author(s) | Sato, Koji / Fujita, Satoshi / Yamauchi, Hiideki / Shiroya, Yoko / Kitamura, Hiromi / Minato, Kumiko / Iemitsu, Motoyuki |
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The Exercise-Induced Improvement in Hyperglycemia is Mediated by DHT Produced in the Skeletal Muscle of Zucker Diabetic Fatty Rats

Koji Sato1, Satoshi Fujita1, Hideki Yamauchi2, Yoko Shiroya2, Hiromi Kitamura2, Kumiko Minato3 and Motoyuki Iemitsu1*

1Ritsumeikan University, Kusatsu, Shiga, Japan
2The Jikei University School of Medicine, Tokyo, Japan
3Department of Life Science, Wayo Women’s University, Japan

Abstract

The ability of exercise to improve hyperglycemia by enhancing glucose metabolism in the skeletal muscle of type 2 diabetic patients is well established. We reported sex steroid hormones can be locally synthesized in skeletal muscle and decrease fasting blood glucose levels in obese rats. Here, we determined whether exercise-induced production of sex steroid hormones in skeletal muscle could directly reverse hyperglycemia in the Zucker diabetic fatty rat model using osmotic mini pump.

Thirty Zucker diabetic fatty rats were randomly assigned to the following groups: control, exercise, or exercise with continuous infusion of 5α-reductase inhibitor.

The results indicated 6 weeks of exercise significantly reduced serum insulin and fasting glucose levels compared to control group. Dehydroepiandrosterone, 5α-dehydrotestosterone, and 5α-reductase levels were all significantly higher in skeletal muscle of the exercise group. Moreover, exercise increased glucose transporter-4 translocation with a concomitant upregulation of phosphorylated phosphoinositide 3-kinase, protein kinase B and C-ζ/λ. Furthermore, significant correlations were observed between fasting glucose and muscular DHT levels.

These results indicated the exercise-induced improvements in glucose metabolism signaling and glucose levels may be directly attributed to the increased levels of sex steroid hormones within skeletal muscles.

Keywords: Exercise training; Sex steroid hormones; Muscle glucose metabolism

Abbreviations: GLUT-4: Glucose Transporter 4; PI3-Kinase: Phosphatidylinositol 3-Kinase; Akt: Protein Kinases B; PKCζ/λ: Protein Kinases C ζ/λ; DHEA: Dehydroepiandrosterone; DHT: 5α-Dehydrotestosterone; HSD: 3β-Hydroxysteroid Dehydrogenase

Introduction

The beneficial effects of exercise on type 2 diabetes have been well-documented, including restoration of glycemic control [1-3]. At the molecular level, an increase in the translocation of glucose transporter 4 (GLUT-4) following the activation of phosphatidylinositol 3-kinase (PI3-kinase), and protein kinases B (Akt) and C ζ/λ (PKCζ/λ) improves glycemic control [4,5]. Thus, chronic exercise prevents or improves type 2 diabetes.

Dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEA-S) are precursors of sex steroid hormones. DHEA is converted to testosterone by 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD, and is converted to 5α-dehydrotestosterone (DHT) by 5α-reductase in peripheral tissues such as the ovary, testis, brain and bone [6]. Recently, we demonstrated that DHEA is metabolized to testosterone and DHT in cultured skeletal muscle, suggesting that skeletal muscle is capable of locally synthesizing sex steroid hormones [7,8]. Blood DHEA and DHEA-S levels in patients with type 2 diabetes are reduced [9]. Moreover, DHEA and DHEA-S levels are lower in both serum and skeletal muscle in obese and type 2 diabetic model rats as compared to controls [10,11]. DHEA and DHT levels in skeletal muscle are significantly correlated with blood glucose levels [11], and in type 1 diabetic model rats, DHEA administration reduced blood glucose levels. Interestingly, this effect was prevented by pre-administration of a DHT inhibitor [12]. Accordingly, these steroid hormones may be part of the mechanism through which exercise leads to improve hyperglycemia.

Exercise elevates blood steroid hormone levels [11]. We demonstrated that acute and chronic exercise significantly increases muscular DHEA and DHT levels, and upregulates enzymes involved in steroidogenesis in normal and obese rats [10,11,13,14]. Furthermore, DHEA and DHT activate Akt and PKCζ/λ/GLUT-4 pathways in skeletal muscle [8]. Exercise-induced alleviation of insulin resistance was correlated with increased DHEA and DHT levels and activated Akt and PKCζ/λ/GLUT-4 pathways in the skeletal muscle of obese rats [11,12]. However, in spite of these correlations, there has been no direct evidence addressing whether sex steroid hormones can directly modulate hyperglycemia in type 2 diabetics.

To address this, we elected to use an animal model of type 2 diabetes, the Zucker diabetic fatty rats. These animals lack functional leptin receptors, and develop obesity, and type 2 diabetes. Their...
progression into overt diabetes is similar to the development of type 2 diabetes in humans [15,16]. Therefore, to test our hypothesis, we used Zucker diabetic fatty rats allowed to exercise and infusion of a 5α-reductase inhibitor to evaluate the effects of exercise-induced increases in sex steroid hormones on glucose metabolism and signaling through the Akt and PKCζ/GLUT-4 pathways.

**Methods**

Approval for the study was obtained from the Committee on Animal Care at the Ritsumeikan University. Male Zucker diabetic fatty (Crlj:ZUC-Lepr+/−) and Zucker lean (Crlj:ZUC-Lepr+/+) rats (110–140 g, 5 weeks old) were obtained from Charles River Japan (Kanagawa, Japan) and cared for according to *The Guiding Principles for the Care and Use of Animals* based on the Declaration of Helsinki. Rats were housed individually under controlled conditions (12:12-h light–dark cycle), and were maintained on an *ad libitum* diet. After 1 week, Zucker rats were randomly assigned to one of four groups: lean *(n=10)*, control *(n=10)*, exercise *(n=10)*, or exercise with 5α-reductase inhibitor *(n=10).* The animals exercised by voluntary running on a wheel. Animals were housed in cages with free access to a running wheel for 6 weeks. Total wheel revolutions were recorded daily by a magnetic switch. The total exercise performed each day was determined by multiplying the number of wheel rotations by the circumference of the wheel [17]. The 5α-reductase inhibitor (dutasteride; Avodart GG745, GalaxoSmithKline, NJ, USA) was continuously delivered at 0.15 µl per hour for 42 days via an implanted osmotic mini pump [Model 2006; Alzet, Cupertino, CA].

The inhibitor (2 mg/kg) was dissolved in sesame oil and 200 µl was loaded into the pump. Then, the pump was inserted subcutaneously, and the skin closed with wound clips [18]. To avoid the effect of acute exercise, forty eight hours after the last exercise session [15], blood was taken from the tail vein to assess fasting glucose levels, and the soleus and gastrocnemius muscles were quickly removed, weighed, rinsed in ice-cold saline, and frozen in liquid nitrogen. These muscle samples were stored at -80°C until use.

**Immunoblot analysis**

Muscle specimens were homogenized with 20 mM Tris-HCl pH 7.8, 300 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM dithiothreitol (DTT), 2% nonidet P-40 (NP-40), 0.2% sodium lauryl sulphate (SDS), 0.2% sodium deoxycholate, 0.5 M phenylmethylsulphonyl fluoride (PMSF), 60 µg/ml aprotinin, and 1 µg/ml leupeptin. The homogenate was gently mixed for 30 min at 4°C, and then centrifuged at 12,000 × g for 15 min. The supernatant was centrifuged at 50,000 rpm for 1 h. The resulting supernatant was spun for 1 h at 55,000 rpm. The resulting supernatant was then used as the cytosolic fraction. GLUT-4 protein levels were measured in both the membrane and cytosolic fractions. Translocation was determined as the difference in protein levels between the cytosol and membrane fractions [8].

**Measurement of citrate synthase activity**

Soleus muscle (50 mg) was homogenized on ice in 10 volumes of 250 mM sucrose, 1 mM Tris-HCl pH 7.4, and 130 mM NaCl with a Teflon homogenizer. The homogenate was centrifuged at 9,000×g for 20 min at 0°C, and the pellet was resuspended in homogenate buffer and centrifuged at 600×g for 10 min at 0°C. The supernatant was centrifuged at 8,000×g for 15 min at 0°C, and the pellet was resuspended in 250 mM sucrose. To assess citrate synthase activity, a rate-limiting enzyme in the tricarboxylic acid cycle, 50 µl aliquots were incubated for 2 min at 30°C in 900 µl of incubation mixture [100 mM Tris-HCl pH 8.0, 1 mM MgCl₂, 2 mM magnesium acetate, and 2 mM acetyl-CoA]. The reaction was initiated by adding 50 µl of 10 mM oxaaloacetate and activity was measured by absorbance at 412 nm for 3 min [19].

**Sandwich-Enzyme Immunoassay (EIA)**

P3-kinase activity assay was a competitive EIA in which the signal was inversely proportional amount of PI(3,4,5)P₃ produced. After the P3-kinase reactions were complete, reaction products were added to the PI(3,4,5)P₃-coated microplate, a PI(3,4,5)P₃ detector protein then added to competitive binding. A peroxidase-linked secondary detector and colorimetric detection was used to detect PI(3,4,5)P₃, detector binding to the plate. The colorimetric signal was inversely proportional to the amount of PI(3,4,5)P₃ produced by P3-kinase (Echelon, Biosciences Inc, Salt Lake City, UT). DHEA and DHT levels in skeletal muscle extracts were determined using a sandwich-EIA kit according to the manufacturer’s instructions (Assay Designs Inc., Ann Arbor, MI). Polyclonal anti-DHEA and -DHT antibodies were used for...
immobilization, while the secondary horseradish peroxidase–coupled antibody was monoclonal. Optical density at 450 nm was determined using a microplate reader (Biolumin 960; Molecular Dynamics, Tokyo, Japan). All samples were assayed in duplicate.

**Statistical analysis**

All values represent the mean ± SE. Statistical evaluation of the data was performed using a one-way ANOVA. A post-hoc comparison test was used to correct for multiple comparisons (Bonferroni test) when analyses revealed significant differences. For the ANOVA, p < 0.05 was considered to be significant; p < 0.01 was considered to be significant for the post-hoc test. Relationships between sex steroid hormone concentrations and blood glucose levels, and level of GLUT-4 translocation were determined using Pearson correlation coefficients.

**Results**

As compared to the control rats, body mass was significantly decreased, and muscle mass significantly increased after 6 weeks of exercise (Table 1). The rats that exercised and received the 5α-reductase inhibitor also decreased their body mass, but without a corresponding increase in muscle mass. Fasting glucose levels were significantly lower in the rats that exercised alone; however, rats that exercised with administration of the 5α-reductase inhibitor showed no improvement in fasting blood glucose levels. Citrate synthase activity in the soleus muscle was significantly higher (p < 0.01) in both exercise groups as compared to controls (Table 1). The average of wheel running distance was 2872 ± 721 m per day in exercise group and 2765 ± 563 m per day in exercise plus inhibitor group (data not shown) and no significant difference between groups.

**Expression of sex steroid hormones and steroidogenic enzymes**

DHEA and DHT levels in skeletal muscle were significantly lower in the Zucker control group. However, these levels significantly increased in the Zucker rats after exercise. Administration of the 5α-reductase inhibitor suppressed the exercise-induced increase in DHT levels (Figure 1). Moreover, exercise induced an increase in the expression of steroidogenic enzymes such as 5α-reductase type 1, 3β-HSD, and 17β-HSD (Figure 2). Interestingly, 5α-reductase expression was suppressed after administration of the 5α-reductase inhibitor, whereas expression of 3β-HSD and 17β-HSD was not affected.

**PI3-kinase activity, Akt and PKC-ζ/λ phosphorylation**

PI3-kinase activity was significantly lower in Zucker control group (Figure 3A, p < 0.01). On the other hand, exercise induced significant increase of PI3-kinase activity, but 5α-reductase inhibitor suppressed exercise-induced increase of PI3-kinase activity. As similarly, Akt phosphorylation was significantly lower (p < 0.01) in Zucker control animals. Conversely, Akt phosphorylation levels in the exercise group were similar to those in the lean rats. This exercise-induced enhancement of Akt phosphorylation was suppressed by the 5α-reductase inhibitor (Figure 3B). Additionally, PKC-ζ/λ phosphorylation was significantly increased (Figure 3C, p < 0.01) in the exercise group, but decreased in the exercise plus inhibitor group, as compared to the Zucker control rats.

**GLUT-4 expression and translocation**

GLUT-4 expression was significantly lower (p < 0.01) in the Zucker control group (Figure 4). GLUT-4 translocation, which was evaluated by assessing the difference between the cytosolic and membrane GLUT-4 protein levels, was significantly enhanced (p < 0.01) with exercise but suppressed with administration of the 5α-reductase inhibitor.

**Relationship between sex steroid hormone and glucose metabolism indices**

Significant correlations were detected between fasting glucose and skeletal muscle DHT levels (r = −0.78, p < 0.001; Figure 5). Moreover, GLUT-4 translocation was significantly correlated with skeletal muscle DHT levels (r = 0.73, p < 0.001). The correlation analysis was conducted with Zucker rats, which excluded healthy lean rats.

**Discussion**

This study demonstrated that 6 weeks of exercise significantly increased DHEA, DHT, and 5α-reductase type 1 protein levels, as well as GLUT-4-regulated signaling, in skeletal muscle of Zucker diabetic fatty rats. Significant lower in fasting insulin and blood glucose levels with exercise were also observed. Furthermore, fasting blood glucose levels and GLUT-4 translocation were significantly correlated with the concentrations of DHT in skeletal muscle. Interestingly, we demonstrated for the first time that exercise-induced improvements in GLUT-4-regulated signaling and hyperglycemia were suppressed by chronic inhibition of 5α-reductase. Thus, exercise-induced increases in the intramuscular synthesis of DHT were necessary to achieve glycemic control through the upregulation of GLUT-4-regulated signaling in fatty diabetic hyperglycemic rats.

Patients with type 2 diabetes and metabolic syndrome generally present with lower than average serum DHEA and DHEA-S levels [20,21]. The decreased DHEA levels may result from insulin-induced

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**Table 1: Animal characteristics.**

|                          | Lean (n=10) | Control (n=10) | EX (n=10) | EX+in (n=10) |
|--------------------------|------------|----------------|-----------|--------------|
| Body mass (g)            | 308.6±80†  | 491.6±23.3     | 352.6±10.6▼ | 436.7±7.2    |
| Fasting glucose (mmol/l) | 8.8±1.1†   | 17.3±4.1       | 13.4±2.3*  | 14.8±5.1     |
| Fasting insulin (pmol/l) | 5.42±1.0*  | 12.2±2.2       | 8.41±1.8*  | 13.5±1.5     |
| CS activity (umol/l/min) | 13.7±1.2†  | 9.09±1.3       | 21.8±2.0*  | 19.2±2.2     |
| Tissue Weight (g/body weight) |           |                |           |              |
| Gastrocnemius muscle     | 3.24±0.63  | 2.65±0.81      | 4.12±0.52* | 3.46±0.66†   |
| Soleus muscle            | 0.46±0.03† | 0.26±0.02      | 0.36±0.05† | 0.23±0.04    |
| Abdominal fat            | 4.49±1.13† | 15.28±1.54     | 14.0±2.21† | 14.6±1.43    |

CS: Citrate Synthase
EX: Exercise Training
EX+in: Exercise training + 5α-reductase inhibitor administration
* p<0.01, vs. Zucker-control
† p<0.01, vs. Zucker-EX
‡ p<0.01, vs. Zucker-EX + in
inhibition of adrenal 17,20-lyase activity, a key enzymatic step in adrenal androgen synthesis [7]. In our previous studies, muscular DHEA and DHT concentrations declined in obese rats and type 1 diabetic rats [11,12]. In accord with those data, in the present study we observed reduced muscular DHEA and DHT concentrations in Zucker diabetic fatty rats. Steroidogenic enzymes that can synthesize testosterone and DHT from DHEA, i.e., 3β-HSD, 17β-HSD, and 5α-reductase, have been detected in skeletal muscle [7,8]. We observed decreased expression of these steroidogenic enzymes in skeletal muscle from type 2 diabetic rats. Therefore, the reduction in skeletal muscle DHT levels may be due to decreased DHEA secretion from the adrenal glands and/or local downregulation of steroidogenic enzymes. Furthermore, in Zucker diabetic fatty rats, the correlation coefficient between muscular DHT and fasting glucose levels was 0.61 (data not shown). These observations suggested that steroid hormones contributed to changes in hyperglycemia.

Six weeks of exercise led to elevated skeletal muscle DHT and steroidogenic enzyme levels in a rat model of type 2 diabetes. We previously reported that the effect of exercise on skeletal muscle DHT secretion was equal to the effect induced by administration of 50 mg/kg/day of DHEA, and that these outcomes were correlated with fasting glucose levels in obese rats [11]. In the current study, continuous inhibition of DHT synthesis abrogated the exercise-induced upregulation of DHT synthesis in skeletal muscle, as indicated by lowered 5α-reductase protein and DHT levels. Consequently, exercise-induced improvement in glucose metabolism was also attenuated in animals receiving the 5α-reductase inhibitor. Thus, an exercise-induced normalization of DHT synthesis in skeletal muscle partly contributes to the amelioration of hyperglycemia observed in this model of type 2 diabetes.

The mechanisms underlying the relationship between skeletal muscle levels of DHT and glucose remain to be elucidated. The known signaling mechanisms impacting glycemic control include GLUT-4–related pathways, e.g., PI3-kinase–associated Akt and PKC activation via insulin and IRS-1 [3]. GLUT-4 signaling is impaired in type 2 diabetes patient and type 2 model rats [16,22], but can be at least partially restored through exercise [1,3]. In the present study, 6 weeks of exercise improved the activation of PI3-kinase, Akt and PKCζ/λ-GLUT-4 pathways in the skeletal muscle of type 2 diabetic rats. Interestingly, a significant relationship was observed between both GLUT-4 translocation and fasting glucose levels and skeletal muscle DHT levels. Moreover, the exercise-induced PI3-kinase, Akt and PKCζ/λ signaling and reduced blood glucose levels were abrogated by chronic administration of a 5α-reductase inhibitor. Previously, we demonstrated that DHT activated glucose metabolism signaling...
pathways in skeletal muscle cell cultures and skeletal muscle from rats [8,11]. In a study using a rat model of type 1 diabetes, a single dose of DHEA resulted in increased DHT levels and greater GLUT4 translocation in skeletal muscle, which led to reduction of blood glucose levels. Interestingly, this effect was inhibited by pre-administration of a DHT inhibitor [12]. Together, the data demonstrate that exercise-induced increases in locally synthesized DHT improved hyperglycemia by activating the PI3-kinase, Akt and PKCζ/λ-GLUT-4 pathways in the skeletal muscle of fatty diabetic rats.

Exercise has been shown to improve glycemic control through activation of insulin-mediated GLUT-4-related signaling in skeletal muscle [2,5]. In the present study, significant decreases in fasting blood glucose levels observed with exercise were partially suppressed by chronic inhibition of 5α-reductase. Therefore, the effect of exercise on hyperglycemia may be achieved through a combination of insulin and DHT signaling pathways. In addition, the exercise-related decrease in fasting insulin levels was completely reversed to control levels by the DHT inhibitor. It could be that the attenuated effect of exercise on hyperglycemia observed with the DHT inhibitor led to a compensatory adaptation to elevate insulin secretion. It has been well documented that exercise training induced the improvement of insulin sensitivity, which is measured by the hyperinsulinemic-euglycemic clamp technique [23,24]. In the present study, exercise training improved fasting blood glucose and insulin levels through the activation of Akt and PKCζ/λ signaling pathway in our Zucker fatty obese rats. However, it should focus on whether exercise training-induced increase of sex steroid hormone contributes improvement of insulin sensitivity with using hyperinsulinemic-euglycemic clamp technique for future study.

Here, we observed that exercise increased skeletal muscle levels of sex steroid hormones and accelerated steroid metabolism. Rats allowed
individuals might not influence diabetic risk factors. However, 5α-reductase inhibitor blocked systemically, so it should focus on the effect of 5α-reductase inhibition for these tissues.

In conclusion, exercise increased sex steroid hormone levels in skeletal muscle and improved hyperglycemia through the activation of GLUT-4-regulated signaling. A DHT inhibitor blocked these effects, suggesting that the exercise-related improvements in hyperglycemia resulted from increased skeletal muscle and improved insulin resistance. These results might suggest new therapeutic targets for the restoration of impaired insulin signaling in skeletal muscle for patients with insulin resistance and hyperglycemia.

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Figure 5: Correlations exist between skeletal muscle DHT levels and fasting glucose/GLUT-4 translocation in Zucker obese fatty rats. (A) Correlations were observed between skeletal muscle DHT and fasting glucose levels, (B) and between skeletal muscle DHT and GLUT-4 translocation in Zucker obese fatty rats.
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