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Elevated mitochondrial biogenesis in skeletal muscle is associated with testosterone-induced body weight loss in male mice

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ABSTRACT

Androgen reduces fat mass, although the underlying mechanisms are unknown. Here, we examined the effect of testosterone on heat production and mitochondrial biogenesis. Testosterone-treated mice exhibited elevated heat production. Treatment with testosterone increased the expression level of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α), ATP5B and Cox4 in skeletal muscle, but not that in brown adipose tissue and liver. mRNA levels of genes involved in mitochondrial biogenesis were elevated in skeletal muscle isolated from testosterone-treated male mice, but were down-regulated in androgen receptor deficient mice. These results demonstrated that the testosterone-induced increase in energy expenditure is derived from elevated mitochondrial biogenesis in skeletal muscle.

1. Introduction

Testosterone deficiency leads to increased fat mass and insulin resistance [1]. Epidemiological studies demonstrate that low testosterone level is associated with type 2 diabetes and metabolic syndrome [2,3]. A double-blind placebo-controlled study reveals that testosterone replacement therapy reduces HOMA index, fasting glucose, HbA1c and waist circumference in type 2 diabetic patients with hypogonadism [4]. Our previous study demonstrated that treatment with dehydroepiandrosterone (DHEA) and testosterone equally reduces adiposity in rats [5]. Both DHEA and testosterone equally suppress 3T3-L1 preadipocyte proliferation and expression of PPARγ in 3T3-L1 adipocytes. In this study, we found that these effects of DHEA are mediated via the androgen receptor (AR). However, the mechanism of weight reduction is unclear. Since neither DHEA nor testosterone suppresses food consumption, we hypothesized that they may increase energy expenditure. This prompted us to examine the effect of treatment with testosterone on oxygen consumption. As we found that an increase in serum testosterone level enhances heat production without increasing locomotor activity, we investigated the effect of testosterone on mitochondrial biogenesis and the expression of its key regulator, peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α).

2. Materials and methods

2.1. Animals

C57/Black male mice at 8 weeks of age were fed CE2 powder with (testosterone group) or without (control group) 0.4% testosterone ad libitum for 4 weeks. They were housed in a specific pathogen-free facility with a 12-h light/12-h dark cycle. Androgen receptor deficient mice (ARKO) were established with Cre-lox system as described previously [6,7]. They were fed CE2 ad libitum. After the treatment with testosterone, the animals were decapitated to collect epididymal fat (WAT), brown adipose tissue (BAT), liver and gastrocnemius muscle. In addition, these organs were harvested at 8 and 25 weeks of age in ARKO.

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All procedures for animal care were carried out in accordance with protocols approved by the University of Gifu's Institutional Animal Care Committee.

2.2. Measurement of oxygen consumption and locomotor activity

O_2 consumption, CO_2 production and locomotor activity were measured by indirect calorimetry as described previously [8].

2.3. Cell culture

C_2C_{12} myoblasts were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Cells were incubated at 37 °C in a humid atmosphere of 5% CO_2 in air. When cells reached 90% confluence, the medium was exchanged for DMEM containing 4% horse serum (differentiation medium). After the incubation with differentiation medium for 7 days, cells were morphologically determined to differentiate C_2C_{12} myotubes. C_2C_{12} myotubes were treated with various concentrations (0, 1, 10 and 100 nM) of testosterone for 48 h.

2.4. Assay for serum testosterone concentration

Serum testosterone concentration was measured with DPC Total Testosterone Kit (Diagnostic Products Corporation).

2.5. Real time PCR

Total RNA from gastrocnemius muscle isolated from mice treated with testosterone and ARKO, and C_2C_{12} myotubes were extracted using TRIzol, and purified as described previously [9]. Reverse transcription was performed using a PrimeScript Reverse Transcriptase (TAKARA) genes were established using SYBR Premix Ex Taq Kit (TAKARA) according to the manufacturer's instructions. 20 μl of the reaction solution consisted of 2 μl of the template, 10 μl of SYBR Premix Ex Taq, 0.4 μl of 10 μM of each primer and 0.4 μl of ROX Reference Dye. PCR amplification was performed as follows: predenaturation for 1 cycle at 95 °C for 30 s, and 40 cycles at 95 °C for 5 s, 60–62 °C for 30 s using a Thermal Cycler Dice (TAKARA, Ohtsu, Japan). Expression levels, calculated as copy numbers in each sample, were normalized to the expression level of GAPDH. Oligonucleotide primers were designed, based on sequences from the GeneBank database (Supplemental Table).

2.6. Quantification of mitochondrial DNA (mitDNA)

Total DNA containing nuclear DNA and mitDNA was extracted using ZymoBead™ Genomic DNA kit (Zymo Research Corp, Irvine, CA). Real time PCR was performed as described above to quantify mitDNA (16S rRNA: CCGCAAGGGAAAGATGAAAGAC (f) and TCGTT

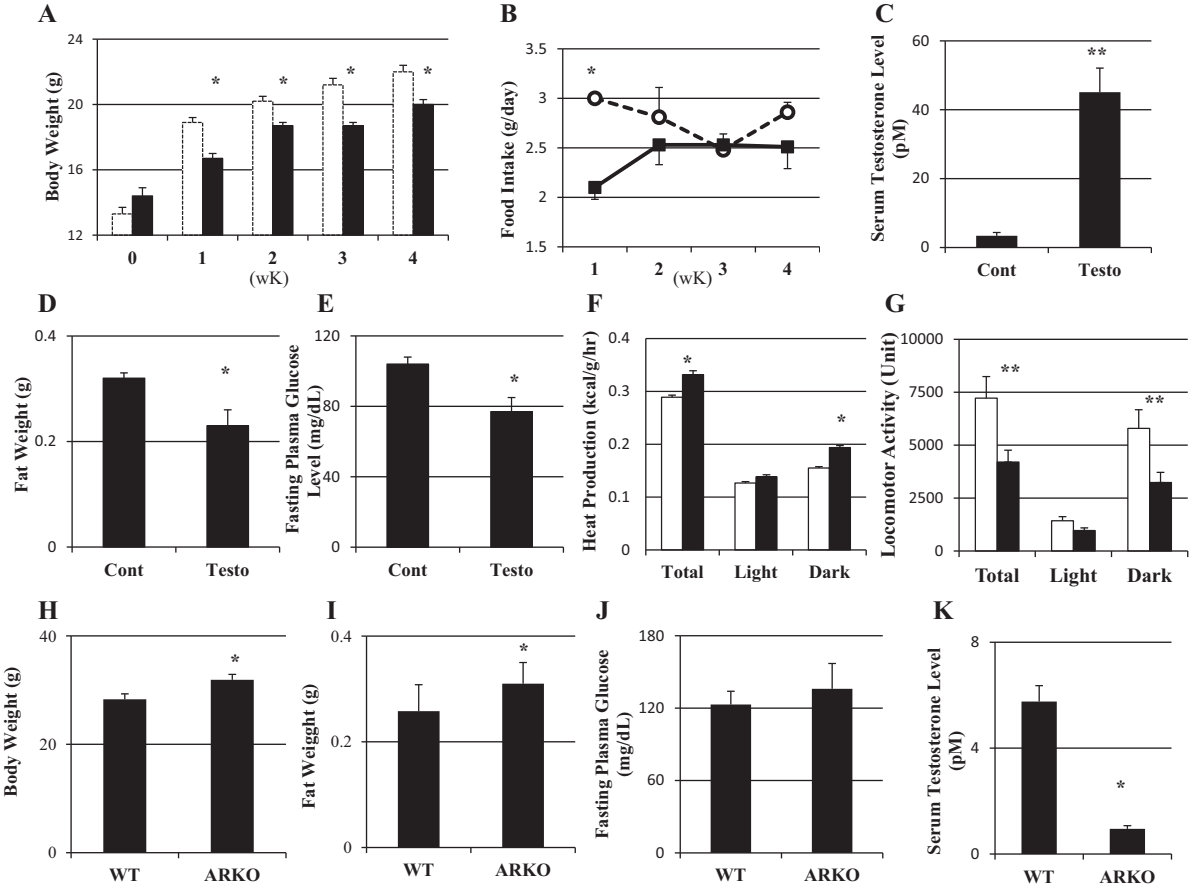

Fig. 1. Body weight, fat weight, plasma glucose level in testosterone-treated mice and ARKO. (A) C57/black mice were treated with food containing 0.4% testosterone for 4 weeks. Body weight in control (white) and treated (black) mice ($n = 16$, *: $P < 0.05$ control vs treated). (B) Food consumption in control (open circle-dotted line) and treated (closed square-solid line) mice ($n = 16$, *: $P < 0.05$ control vs treated). (C) Serum testosterone level in control and treated mice ($n = 8$, **: $P < 0.01$ control vs treated). (D) Epididymal fat weight in control and treated mice ($n = 16$, *: $P < 0.05$ control vs treated). (E) Fasting plasma level in control and treated mice ($n = 8$, *: $P < 0.05$ control vs treated). (F) Calculated heat production in control (white) and treated (black) mice during light phase (12 h), dark phase (12 h) and total (24 h) ($n = 7$, *: $P < 0.05$ control vs treated). (G) Locomotor activity in control (white) and treated (black) mice during light phase (12 h), dark phase (12 h) and total (24 h) ($n = 7$, *: $P < 0.05$ control vs treated). (H) Body weight in wild type and ARKO mice at 24 weeks of age ($n = 3$, *: $P < 0.05$ wild type vs ARKO). (I) Epididymal fat weight in wild type and ARKO mice at 24 weeks of age ($n = 3$, *: $P < 0.05$ wild type vs ARKO). (J) Plasma glucose levels in wild type and ARKO ($n = 3$, *: $P < 0.05$ wild type vs ARKO). (K) Serum testosterone levels in wild type and ARKO ($n = 3$, *: $P < 0.05$ wild type vs ARKO).