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Anti-Obesity Effects of Androgens, Dehydroepiandrosterone (DHEA) and Testosterone

Kazuo Kajita, Ichiro Mori, Masahiro, Takahide Ikeda,
Hiroyuki Morita and Tatsuo Ishizuka

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1. Introduction

Despite considerable research, the relationships between obesity and metabolic disorders have yet to be fully understood. Recent evidence has revealed that fat depots, rather than the volume of fat, are essential in determining systemic insulin sensitivity. Adipose tissue is classified into visceral adipose tissue, including epididymal, mesenteric and perirenal fat, and subcutaneous adipose tissue according to its anatomical location. Increases in visceral adipose tissue are considered to be linked to insulin resistance [1, 2]. Especially, mesenteric fat is postulated to relate more closely to metabolic disorders, as mesenteric fat secretes free fatty acids and other substances directly into the portal vein [3]. Although the mechanisms regulating fat distribution remain obscure, sex hormones are unquestionably one of the determinants.

Since men tend to accumulate much more visceral fat than women, androgens have been postulated to promote insulin resistance. In practice, low serum testosterone levels promote obesity. Numerous studies have demonstrated that androgen deprivation therapy (ADT) increases the risk of obesity, metabolic syndrome, type 2 diabetes and cardiovascular disease in patients with prostate cancer [4-8]. Basaria *et al*, pointed out that high fat mass, as well as low bone density and anemia, was observed in men with prostate cancer treated with ADT compared with ones treated without it. They concluded that patients receiving ADT are at enhanced risk for insulin resistance and cardiovascular disease. Katznelson *et al*, reported that percent body fat was greater in acquired hypogonadal men compared with eugonadal controls, which was improved by testosterone replacement therapy [9].

Recently, a high prevalence of hypogonadism in men with obesity, metabolic syndrome and type 2 diabetes has been recognized. Dhindsa *et al*, reported that total testosterone and free

testosterone inversely relate to BMI and fat mass [10] in type 2 diabetic men. Kapoor *et al*, in a cross-sectional study of 355 type 2 diabetic subjects, found overt and borderline hypogonadism in 42%, with 42 of these men having free testosterone levels <0.255 nM [11]. Although the Framingham Heart Study concluded that sex hormone-binding globulin (SHBG), but not testosterone, is significantly associated with metabolic syndrome [12], both low SHBG and low free testosterone may contribute to low serum total testosterone level in obese and diabetic men [13]. Another issue currently of interest is whether low testosterone is a cause or result [13, 14]. Weight loss induced by diet or surgery has been demonstrated to increase testosterone level and sexual function [15-17]. Probably, low testosterone and metabolic disorders worsen each other. The results of clinical studies of testosterone replacement therapy were reviewed by Grossmann [13]. RTC was performed in 10 trials in obese men with borderline low testosterone levels. Although reduced fat mass was commonly observed, improved insulin sensitivity was detected in only 2 trials. Six RCTs in diabetic patients similarly demonstrated beneficial changes in body composition. However, reduction of HOMA-R was detected in 3 trials, and decreased HbA1c in one. These data suggested the limited efficacy of testosterone replacement. Although only one meta-analysis noted that combined prostate events including prostate cancer, elevated PSA and prostate biopsies were more frequent in testosterone-treated men [18], there is no clear evidence that testosterone replacement increases the incidence of prostate cancer. However, the possibility remains that the study was too small to detect significant results. In contrast, a significantly increased risk of cardiovascular events has been associated with testosterone therapy [19, 20], emphasizing that its potential risks should not be ignored.

Dehydroepiandrosterone (DHEA) and its sulfate ester, dehydroepiandrosterone-sulphate (DHEA-S) are referred as a weak androgen produced in adrenal gland (90%) and testis (10%) in men [21]. DHEA is an intermediate product, which is synthesized from pregnenolone, and converted to testosterone and estrogen. DHEA is one of the most abundantly secreted steroids, although its precise physiological roles remain uncertain. DHEA exerts 0.1-2% of the activity of testosterone on the genital organs [22], and 42% on bone formation in mice [23]. Since no specific nuclear receptor for DHEA or DHEA-S has been identified, these hormones are regarded as precursors of more active androgens, such as testosterone and 5 α -dihydrotestosterone (DHT), or estrogens. In addition, DHEA and DHEA-S can be converted to more active forms subcellularly in target tissues, the underlying mechanism of which was referred to as "intracrine" by Labrie [24].

Both serum DHEA and testosterone levels decline during the aging process [25, 26]. Hence, low serum DHEA level has been assumed to be involved in the development of age-related diseases and shortening of the life span. Such studies suggest an association between high serum DHEA-S level and longevity. However, numerous studies have reported that serum DHEA(-S) exhibits positive, negative or no relation to adiposity, cardiovascular disease and mortality in men and women [27]. Recent longitudinal and cross sectional studies support the favorable effects of DHEA-S on cardiovascular disease and all-cause mortality in both sexes [28-30].

Like the case of testosterone, inconsistent results of DHEA replacement have been published. DHEA replacement decreased fat mass and elevated bone mineral density (BMD) [31], whereas, opposite results were obtained [32] in elderly men and women with DHEA deficiency. Recently, Corona *et al*, conducted a meta-analysis study of 25 RTC trials of DHEA supplementation in elderly men. They observed no significant effects on the levels of glucose, insulin, total cholesterol or BMD with DHEA, while a small but significant reduction of fat mass was detected in the supplemented group [33].

Production of testosterone in the testis is regulated by gonadotropin, while that of DHEA in the adrenal gland is by ACTH. Low free testosterone is correlated positively with LH in diabetic men, and therefore, hypogonadotropic hypogonadism is common in these patients [34]. However, the pathogenesis of low DHEA level has been unclear. Both serum testosterone and DHEA levels decline with aging. Although some studies have published data on testosterone and DHEA in elderly persons [35, 36], to our knowledge, no research has focused on individual relationships among testosterone, DHEA and metabolic disorders. Theoretically, low testosterone level might be compensated for by DHEA via an intracrine mechanism in men having low testosterone and normal DHEA level. The opposite can also be supposed. Therefore, we speculate that severe metabolic impairment might be observed in men with low testosterone and low DHEA levels. Further study is necessary to help clarify this issue.

In animal studies, extensive research has elucidated the physiological and pharmacological roles of androgens. However, few papers have compared testosterone and DHEA. The hormonal actions of testosterone and DHEA are mediated via the androgen receptor (AR), and so the difference in biological activity between these hormones may be caused by the efficacy of steroid converting enzymes mediated by an intracrine mechanism. In addition, numerous cell surface receptors for testosterone and DHEA have been identified [37, 38]. Differences in the biological responses to testosterone and DHEA may be derived from these membrane receptors. Anagnostopoulou *et al*, reported opposite effects of DHEA and testosterone on the apoptosis of prostate and colon cancer [39]. They concluded that the differential effects of these hormones on nerve growth factor receptors in cancer cells accounted for these results. Piñeiro *et al*, showed that DHT, DHEA-S, stanozolol (non-aromatizable androgen), and androstenedione, but not testosterone, suppressed leptin secretion in cultured adipocytes sampled from female omental adipose tissue [40]. The authors presumed that aromatization of testosterone might result in effects opposite to those of other androgens. Sato *et al*, considered that testosterone increased the expression level of Glut4 more potently than DHEA in cultured skeletal muscle, which was abrogated by a DHT inhibitor [41]. These results suggested that DHT, a metabolite of testosterone and DHEA, finally acts as an androgen in skeletal muscle. In this article, we outline our research investigating the impact of androgens, testosterone and DHEA, on adiposity and glucose metabolism, and the results of our recent study.

2. Materials and Methods

2.1. Animals

Male Wistar rats and C57/black mice at 8 wk of age were fed with or without (control) 0.4% testosterone or 0.4% DHEA containing food in CE2 powder (carbohydrate 51.4%, protein 24.9%, fat 4.6%, fiber 3.7%) for 4 wk. Individual food consumption was determined by subtracting the food remaining from that supplied every 2-3 days, with the averages of these values in one week expressed as the weekly food consumption. The animals were housed in a specific pathogen-free facility with a 12-h light/12-h dark cycle. After sacrifice white adipose tissue (epididymal fat), skeletal muscle (gastrocnemius muscle) brown adipose tissue (BAT) and liver were collected. All procedures for animal care were carried out in accordance with protocols approved by the University of Gifu's Institutional Animal Care Committee.

O₂ consumption (VO₂), CO₂ production (VCO₂) and locomotor activity in mice were measured individually by indirect calorimetry using an Oxymax apparatus (Columbus Instruments, Columbus, OH) as described previously [42]. Respiratory exchange rate (RER) was calculated as VCO₂/VO₂. Heat generation was calculated as caloric value $(3.815 + 1.232 \times \text{RER}) \times \text{VO}_2$.

2.2. Cell culture

3T3-L1 preadipocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Upon confluence, 3T3-L1 preadipocytes were differentiated with differentiation medium containing insulin, dexamethasone and IBMX for 3 days followed by incubation with DMEM again. At 5 days after the differentiation, cells were stimulated with 50 nM DHEA or 50 nM testosterone for 48 hr. The content of triglyceride was visualized with Oil Red O (Santa Cruz Biotechnology, Santa Cruz, CA) according to the manufacturer's instruction.

F442A preadipocytes were cultured in DMEA with supplement as described above. When confluence was reached (0d), 50 nM DHEA or testosterone was added to the medium to evaluate the effects of these hormones on spontaneous differentiation of F442A cells into mature adipocytes without differentiation medium.

C₂C₁₂ myoblasts were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. When cells reached 90% confluence, the medium was exchanged for DMEM containing 4% horse serum (differentiation medium). After incubation with the differentiation medium for 7 days, cells were morphologically determined to complete the differentiation into C₂C₁₂ myotubes, and then these cells were treated with various concentrations of testosterone for 48 hr

2.3. Real time PCR

Real time PCR was performed to measure mRNA expression levels of PPAR γ , fatty acid binding protein 4 (FABP4), lipoprotein lipase (LPL), adiponectin, SREBP-1, fatty acid synthase

(FAS) and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) in 3T3-L1 adipocytes, and PGC1 α , cytochrome C and G3PDH in C₂C₁₂ myotubes, as described previously [43-45]. All data were normalized to the expression level of G3PDH.

2.4. Triglyceride content in liver and skeletal muscle

Liver and gastrocnemius muscle were homogenized in KRP buffer, and the triglyceride in the homogenate was extracted with chloroform-methanol, and assayed using a LabAssay Triglyceride kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan) as described previously [43].

2.5. Western blot

The cell lysate was mixed with Laemmli sample buffer and boiled for 3 min. Equal amounts of cell lysate were subjected to SDS-PAGE, and transferred onto nitrocellulose paper. The paper was blocked with 1% BSA, and incubated with anti-PPAR γ antibody, anti-adiponectin antibody or anti-actin antibody (Santa Cruz). Protein bands were visualized with an ECL system.

2.6. Statistics

All experimental results were calculated as means $\pm$ SE. Statistical comparisons were performed by Student's t-test or ANOVA. Significance was defined as $P < 0.05$.

3. Results

3.1. Body weight and plasma glucose level

Treatment with testosterone or DHEA containing food reduced weight gain in both rats (Fig. 1A) and mice (Fig. 1B). Administration of testosterone and DHEA reduced body weight equivalently. The dose response study showed that food containing both testosterone and DHEA at 0.4% significantly suppressed body weight gain (Fig.1C). Our previous study [43] indicated that treatment with 0.4% testosterone for 4 wk resulted in an increase of serum testosterone and DHEA-S levels up to 674% and 1040%, respectively (note that serum DHEA-S level is very low in rodents due to the lack of 17 α hydroxylase in adrenal glands), whereas treatment with DHEA increased testosterone and DHEA-S levels up to 310% and 6420%, respectively. The fact that these androgens are convertible to each other, partially explains the similar results obtained with administration of these hormones. Administration of testosterone and DHEA did not influence fasting plasma glucose level in rats (Fig. 1D), while testosterone suppressed it a little but significantly in mice (Fig. 1E). Food consumption was not influenced by the administration of either hormone in rats (Fig. 1F).

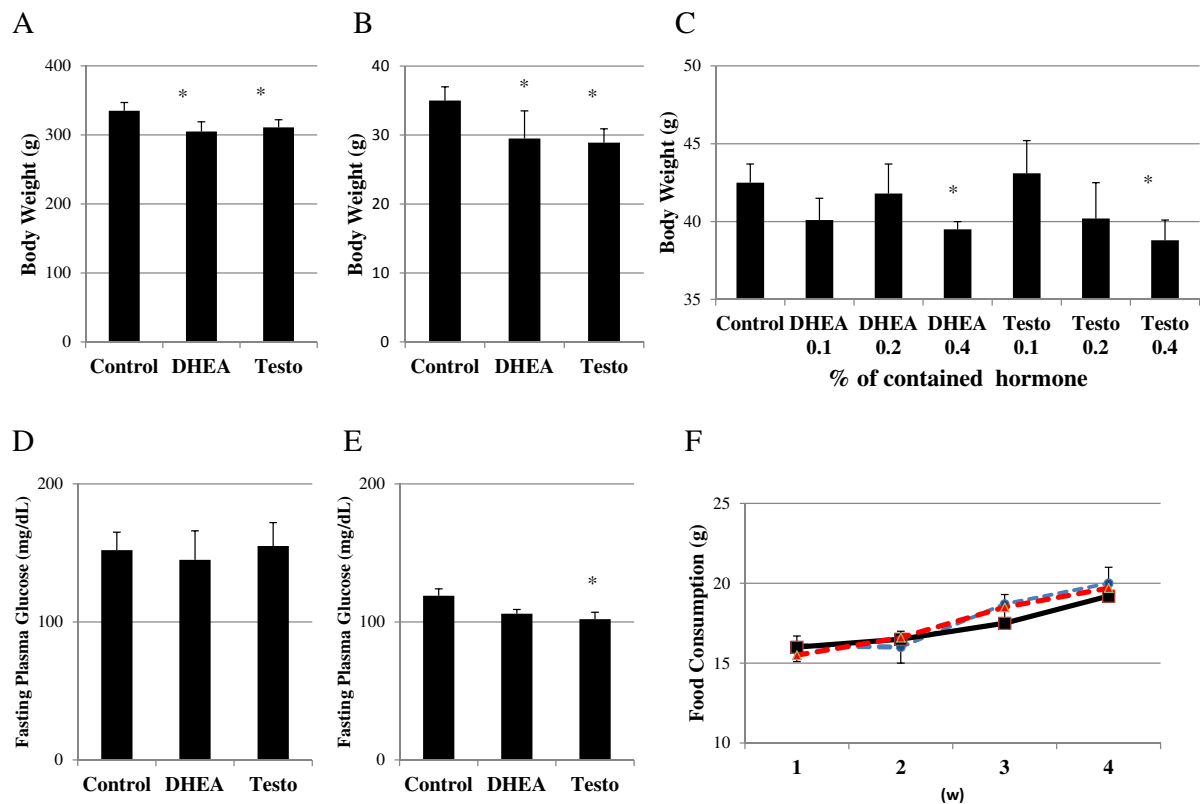


Figure 1. Effects of administration of DHEA and testosterone on body weight. Effects of treatment with 0.4% DHEA or testosterone containing food for 4wk on body weight in Wistar rats (n=6) (A) and C56/black mice (n=4) (B) at 8 wk of age are shown. *: $p < 0.05$ vs control. Effects of 0.1%-0.4% DHEA or testosterone containing food for 4wk on body weight in C57/black mice (n=4) (C) are shown. *: $p < 0.05$ vs control. Effects of treatment with 0.4% DHEA or testosterone containing food for 4wk on fasting plasma glucose level in Wistar rats (n=6) (D) and C56/black mice (n=4) (E) are shown. *: $p < 0.05$ vs control. Effects of DHEA or testosterone administration on food consumption in mice (n=4) are shown. Black solid line: Control, Red broken line: DHEA, Blue broken line: testosterone. *: $p < 0.05$ vs control.

3.2. Effect of DHEA and testosterone on adipocytes

Administration of DHEA or testosterone suppressed fat weight, including that of subcutaneous, epididymal and mesenteric fat (Fig. 2A). In addition, both DHEA and testosterone decreased adipocyte size equivalently (Fig. 2B). We found that treatment with DHEA reduced the expression of PPAR γ in adipocytes in both *in vivo* and *in vitro* [42]. Treatment with DHEA and testosterone similarly reduced the expression level of PPAR γ in adipose tissue isolated from Wistar rats and 3T3-L1 adipocytes (Fig. 2C, D). Genes regulated by PPAR γ , such as FABP 4, LPL and adiponectin were equally down-regulated by DHEA and testosterone in 3T3-L1 adipocytes. Neither hormone influenced the expression levels of genes, which are not directly regulated by PPAR γ , such as SREBP-1 and FAS (data not shown). Administration of DHEA or testosterone decreased triglyceride content in liver and skeletal muscle to the same degree in rats (Fig. 2 E, F).

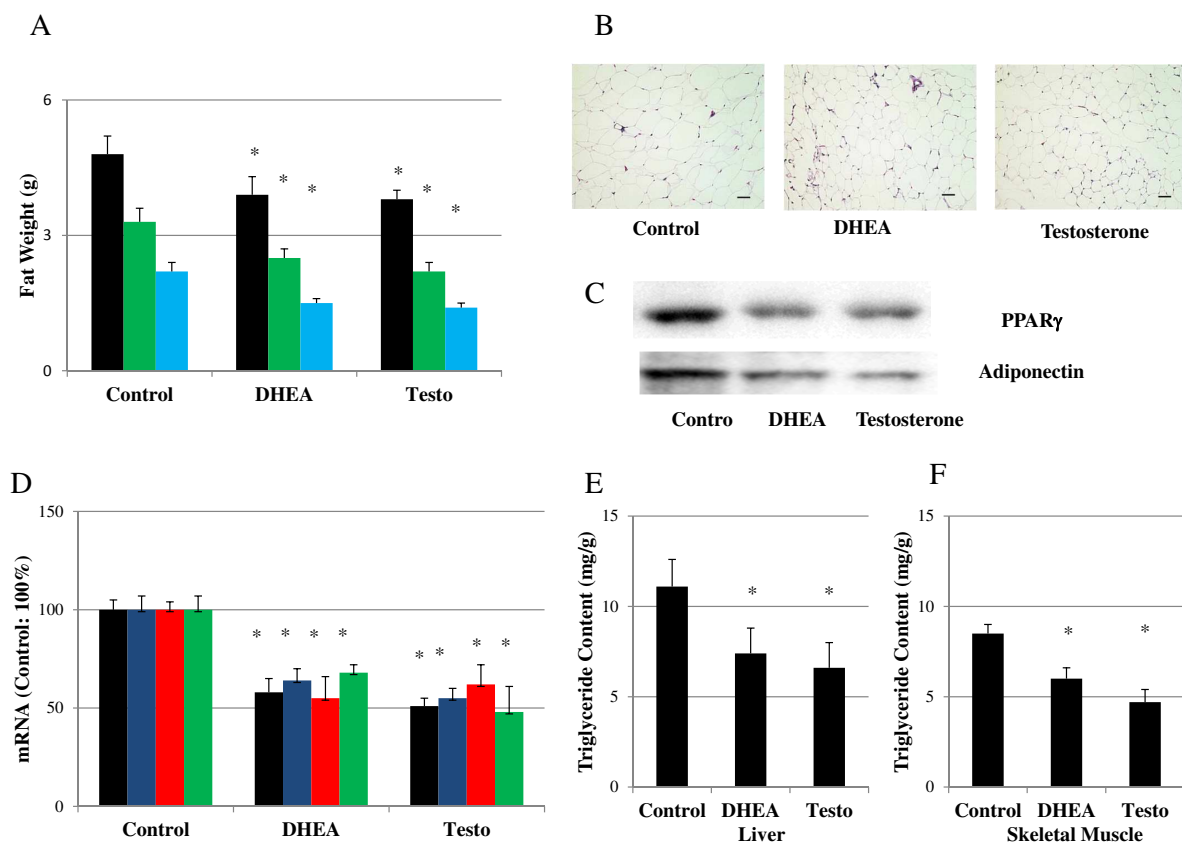


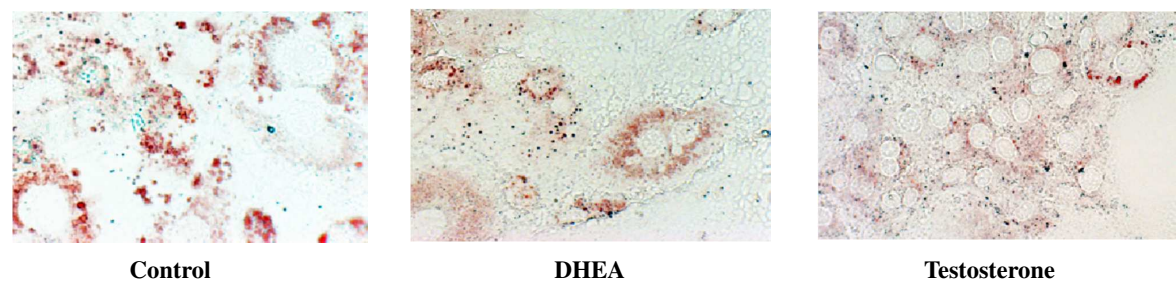
Figure 2. Effects of administration of DHEA and testosterone on adipocytes. Effects of treatment with DHEA or testosterone for 4wk on fat weight (black: subcutaneous, green: epididymal, blue: mesenteric fat, $n=6$) (A), and histological findings (B) are shown. *: $p<0.05$ vs each control. Effects of treatment with DHEA or testosterone on the protein level of PPAR γ and adiponectin in adipose tissue in Wistar rats were evaluated. A typical result was shown (C). Effects of treatment with DHEA or testosterone on the expression of adipocyte specific genes in 3T3-L1 adipocytes are shown (D). Fully differentiated 3T3-L1 adipocytes were incubated with 50 nM DHEA or testosterone for 48 hr. Expression levels of PPAR γ (black), FABP4 (blue), LPL (red), adiponectin (green) were shown ($n=6$). *: $p<0.05$ vs each control. Effects of treatment with DHEA or testosterone on triglyceride content in liver ($n=5$) (E) and skeletal muscle ($n=5$) (F) in Wistar rats are presented. *: $p<0.05$ vs control.

Next, we examined the effects of these hormones on adipocyte differentiation. We observed the differentiation of F442A cells, since they spontaneously differentiate into mature adipocytes when they reach confluence. DHEA and testosterone suppressed the accumulation of triglyceride (Fig. 3A) and the appearance of PPAR γ and FABP4 mRNA during the differentiation process. These data indicated that DHEA and testosterone similarly suppress adipocyte differentiation.

3.3. Effect of DHEA and testosterone on mitochondrial biogenesis

As noted above, since the administration of neither DHEA nor testosterone influenced food consumption, we speculated that these hormones elevate energy expenditure. Hence we examined the effects of testosterone administration on energy production. Mice were treated with or without testosterone for 4 wk, and then, oxygen consumption and locomotor activity were measured by indirect calorimetry. O_2 consumption and CO_2 production were increased

A



B

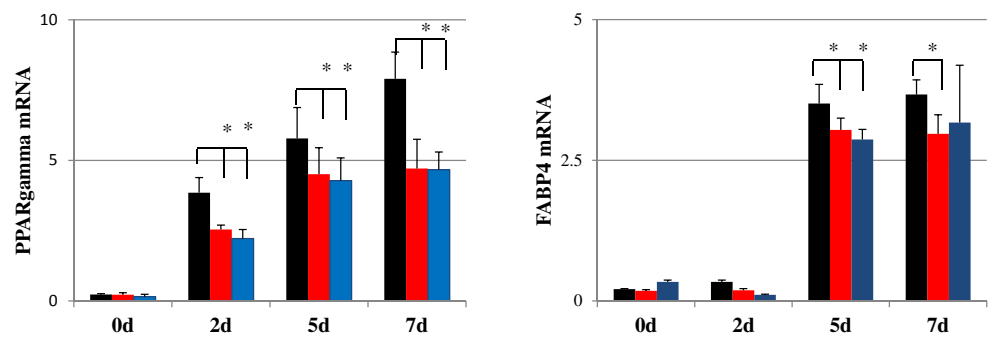


Figure 3. Effect of treatment with DHEA and testosterone on the differentiation of F442A adipocytes. F442A preadipocytes were cultured in DMED. When cells reached confluence as judged by the morphological findings (0d), 50nM DHEA or testosterone was added to the medium, followed by subsequent incubation for the indicated period. Triglyceride accumulation was assessed with oil-Red staining at 7d (A). Expression levels of PPAR γ and FABP4 were measured with real time PCR on the indicated day (n=4) (B). *:p<0.05 vs each control.

significantly in testosterone-treated mice, regardless of whether the values were normalized by body weight or not (Fig. 4B-E). In addition, heat production, the values of which were normalized by body weight, was elevated in testosterone-treated mice (Fig. 4G). No difference was detected in respiratory exchange rate between control and testosterone-treated mice (Fig. 4H). To our surprise, administration of testosterone suppressed locomotor activity (Fig. 4I).

These results indicate that administration of testosterone increases the basal metabolic rate. Therefore, we evaluated the effects of administration of these androgens on mitochondrial biogenesis and its upstream regulator, PGC1 α . Expression of mitochondrial protein, Cox4, and PGC1 α was elevated in skeletal muscle, but not brown BAT or liver, isolated from testosterone-treated rats (Fig. 5A). The increase of Cox4 in skeletal muscle induced by DHEA administration was less than that induced by testosterone (Fig. 5B). The testosterone-induced increases in mRNA levels of PGC1 α and cytochrome C were greater than the DHEA-induced ones in C₂C₁₂ myotubes (Fig. 5C). These results show that increased mitochondrial biogenesis by these hormones leads to up-regulation of energy expenditure, which may result in reduced adiposity.

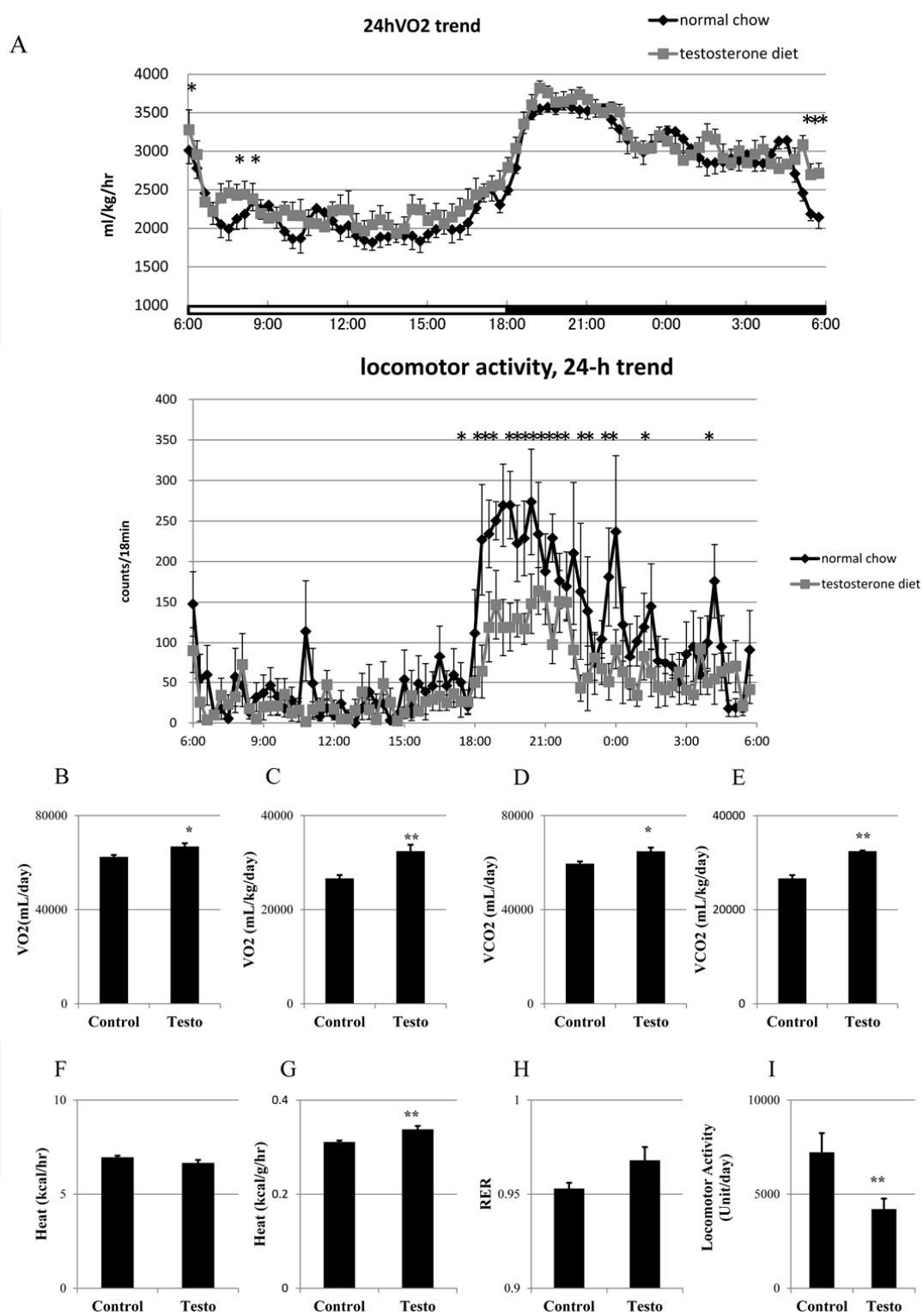


Figure 4. Effects of treatment with testosterone on oxygen consumption, heat production and locomotor activity. C56/ black mice at 8 wk of age were treated with testosterone for 4 wk, and individual oxygen consumption and locomotor activity were determined by indirect calorimetry (A). Cumulative O₂ consumption for 24 hr (B) and normalized values by body weight (C), CO₂ production (D) and normalized values by body weight (E), heat production for 24 hr (F) and normalized values by body weight (G) are shown. Values of RER (H) and locomotor activity (I) for 24 hr are also shown. *: $p < 0.05$ vs control, **: $p < 0.01$ vs control

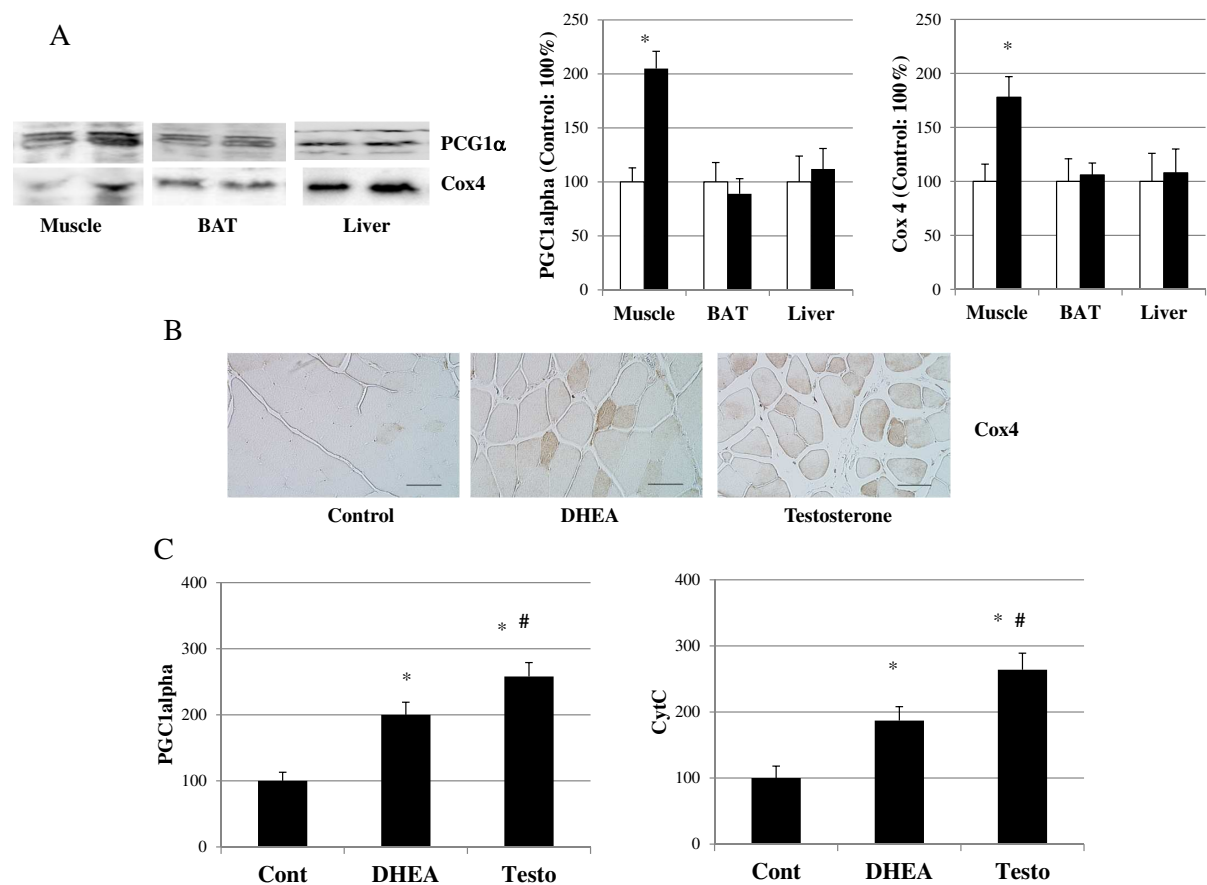


Figure 5. Effect of treatment with DHEA and testosterone on mitochondrial biogenesis. Wistar rats were treated with DHEA or testosterone for 4 wk. Effects of treatment with testosterone on the expression of PGC1α and Cox4 in skeletal muscle, BAT and liver are shown (A). Typical results of western blot are shown in the left panel, and quantified results are shown in the right (n=4). White: Control, Black: Testosterone-treated. *:p<0.05 vs control. Representative image of immunohistochemistry of skeletal muscle isolated from control, DHEA-treated and testosterone-treated rats are shown (B). Effects of incubation with 10 nM DHEA or testosterone for 48 hr on the expression of PGC1α and cytochrome C mRNA in C₂C₁₂ myotubes (n=4) are shown (C). *: p<0.05 vs control, #: p<0.05 vs DHEA.

4. Discussion

Coleman *et al.*, demonstrated that administration of DHEA reduces blood glucose level in db/db mice [46]. We found that administration of DHEA improved blood glucose in OLETF rats, a model of obese diabetes, but not GK rats, a model of lean diabetes [47]. Accordingly, we presumed that DHEA-induced weight reduction might contribute to improving blood glucose levels. Although administration of DHEA consistently suppresses body weight and fat weight, significant improvement of blood glucose is detected only in extremely obese animals [44]. We noted that DHEA and testosterone reduce the expression of PPARγ in adipocytes [43, 44]. Heterozygous PPARγ deficient mice are protected from insulin resistance under a high-fat diet [48], and reduced receptor activity of PPARγ by Pro12Ala substitution leads to lower body mass index in man [49], suggesting that modest suppression of PPARγ activity may help to

prevent obesity and resultant insulin resistance. However, the production and secretion of adiponectin are positively regulated by PPAR γ in adipocytes [50], and inhibition of PPAR γ may result in insulin resistance due to low plasma adiponectin level. In this study, we showed that DHEA and testosterone decrease PPAR γ , as well as adiponectin (Fig. 2C, D). This result is consistent with the fact that despite their obese phenotype, glucose homeostasis remained intact because of a high plasma adiponectin level in androgen receptor null mice (ARKO) [51]. These data explain the results of the numerous clinical studies described above in which administration of DHEA or testosterone consistently reduced adiposity, despite which numerous studies have failed to find proof of any beneficial effect on glucose metabolism.

This study confirmed that administration of DHEA and testosterone reduced body weight and fat weight equally, as described in our previous study [44]. If this conclusion is applied to men for weight reduction, supplementation of DHEA would be more desirable than that of testosterone given the smaller possibility of adverse effects. Our study also reveals that DHEA and testosterone attenuate proliferation of 3T3-L1 preadipocytes in a similar concentration dependent manner [44]. In addition, we showed that these hormones decrease the expression levels of PPAR γ , LPL and FABP4, but not SREBP-1, at common concentrations and in a time dependent manner [44]. The possibility that fat content increased in other organs in compensation for the decrease in fat mass, was ruled out by the fact that fat content in liver and skeletal muscle decreased similarly both in DHEA and testosterone-treated rats [44], which was confirmed in this experiment. The findings that neither DHEA nor testosterone increased glycerol release in 3T3-L1 adipocytes and administration of these hormones decreased serum free fatty acid concentration in rats, rule out the possibility that these hormones reduce adiposity by increased lipolysis [41]. In this study, we revealed that both DHEA and testosterone suppress differentiation of adipocytes using F442A. Both DHEA and testosterone equivalently inhibited spontaneous differentiation of cells. Recently, concurrent results have been published with regard to 3T3-L1 preadipocytes, C3H 10T1/2 pluripotent cells and human preadipocytes [52-55]. Singh *et al*, reported that formation of androgen receptor/ β -catenin and T-cell factor 4 complex and activation of Wnt signaling are involved in androgen-induced inhibition of adipogenesis [54].

To clarify the mechanisms underlying androgen-induced weight reduction, we analyzed the effect of testosterone administration on energy expenditure. Administration of both DHEA and testosterone increased the rectal temperature in rats [44]. Although an abnormally high body temperature was not detected, elevated O₂ consumption and CO₂ production was observed in testosterone-treated mice (Fig. 4A-D). Although heat production was increased in testosterone-treated mice, it was not significant when these values were not normalized by body weight (Fig. 4E). We have no data on lean body mass or water. If lean body mass is not influenced by testosterone, testosterone-induced reduction of adiposity could not result from an increase in energy expenditure. On the other hand, our results indicate that basal metabolic rate increases in testosterone-treated mice since heat production in these mice did not decrease despite suppressed locomotor activity. The result of suppressed locomotor activity in testosterone-treated mice was unexpected, since lower locomotor activity was also reported in

ARKO [51]. We are not yet able to explain this discrepancy, probably because change in locomotor activity may not occur in parallel with an androgen signal.

Next, we speculated that testosterone might increase mitochondrial activity to explain the increased basal metabolic rate. As shown in Fig. 5A, increased Cox4, a mitochondrial protein, as well as PGC1 α , an up-stream regulator of mitochondrial biogenesis, was recognized in skeletal muscle isolated from testosterone-treated rats. Similar results were noted in mice [45]. In addition, treatment with testosterone up-regulates the expression levels of genes contributing to mitochondrial biogenesis, such as nuclear respiratory factor-1 (NRF-1), NRF-2 and mitochondrial transcriptional factor A (Tfam), as well as mitochondrial DNA (mitDNA) in skeletal muscle [44]. Although DHEA and testosterone exhibit similar effects on adipocytes, administration of DHEA resulted in less increase in Cox4 than that of testosterone in skeletal muscle. This result was confirmed by the experiment showing that the testosterone-induced increase in mRNA of PGC1 α and cytochrome C was greater than the DHEA-induced ones (Fig. 5C) in C₂C₁₂ myotubes. These results are consistent with data published by Sato *et al.* [41]. These differences in the response to DHEA and testosterone between adipocytes and myocytes may be attributable to differences in the efficacy of subcellular steroid converting enzymes. Although we did not assess the effect of androgens on total skeletal muscle volume, androgens have been reported to enhance the differentiation into skeletal muscle [53]. Therefore, the conclusion derived from our experiment should be further explored by increasing the whole skeletal muscle mass. In addition, we found that expression of PGC1 α and mitochondrial genes was reduced in skeletal muscle isolated from ARKO [45].

The results of our studies were summarized in Fig. 6. DHEA and testosterone equally suppressed proliferation of preadipocytes, differentiation of adipocytes and expression of PPAR γ and its down-stream genes including adiponectin in adipocytes. Both DHEA and testosterone up-regulated PGC1 α and mitochondrial biogenesis, more actively in the latter than the former in skeletal muscle. Which organ plays the main role in the androgens-induced reduction of adiposity remains an interesting problem. Our results suggest that reduced adiposity in testosterone-treated animals may be derived from decreased expression of PPAR γ and suppressed differentiation into adipocytes. Moderate suppression of PPAR γ activity by its antagonist HX531 resulted in decreased fat mass and increased oxygen consumption [56], and therefore androgen-induced reduction of PPAR γ expression may be able to influence systemic energy metabolism.

Whole body silencing of AR results in late-onset obesity [51, 56]. Recent technology has facilitated the generation of organ specific deletion of a gene. Adipocyte specific AR deficient mice showed identical body weight and adiposity with wild type at 20 wk of age in one study, although the authors did not show the data of older mice [57]. Since late obesity after 20 wk of age is the distinguishing feature in ARKO, this point is important. Conversely, mice lacking AR in the central nervous system develop late onset obesity and insulin resistance [59]. Although several investigations have reported that myocyte specific AR knockdown did not influence body weight and adiposity [60, 61], myocyte specific AR overexpression resulted in an increased metabolic rate and fat body mass [62]. These results suggest that skeletal muscle and brain might be responsible organs for androgen-induced reduction of adiposity. However,

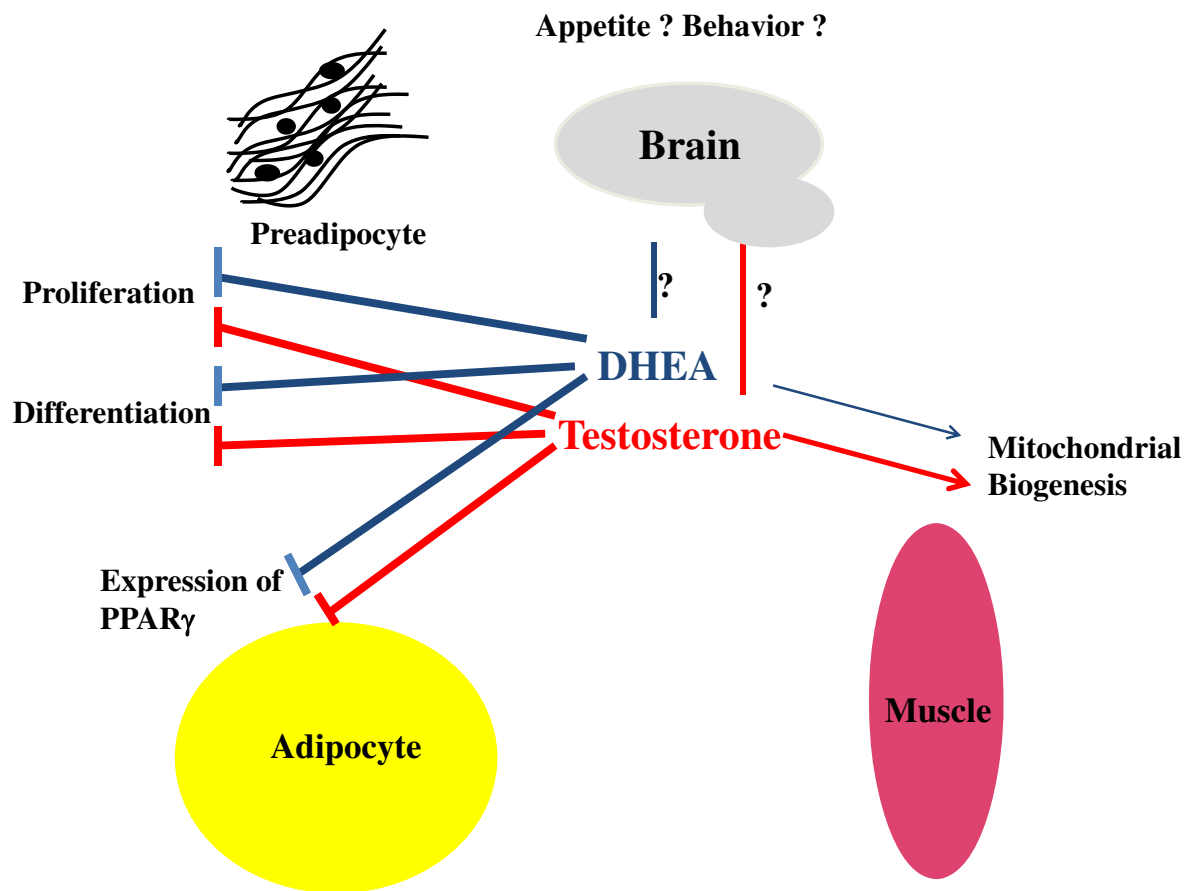


Figure 6. Effect of DHEA and testosterone on brain, adipocytes and skeletal muscle

the role of AR in adipocytes in systemic insulin sensitivity cannot be ruled out at present. Further experiments will be required to help clarify these issues.

Author details

Kazuo Kajita^{1*}, Ichiro Mori¹, Masahiro¹, Takahide Ikeda¹, Hiroyuki Morita¹ and Tatsuo Ishizuka²

*Address all correspondence to: kkajita@gifu-u.ac.jp

1 Department of General Internal Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu, Japan

2 Department of General Internal Medicine and Rheumatology, Gifu Municipal Hospital, 7-1 Kashima-cho, Gifu, Japan

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