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Effects of Testosterone on Body Composition and Muscle Protein Synthesis in Female Rats

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ABSTRACT

The effects of varying doses(1, 4 and 10mg/kg body weight/day) of testosterone propionate (TP) on body weight gain and composition, and energy and muscle protein metabolism were investigated in female rats. TP had no effect on food intake at any dose, but injection of 1mg/kg resulted in an increase in body weight gain which was associated with increases in body protein and fat. At higher doses(4 and 10mg/kg), body protein content was still increased but body fat was not affected. Increases in energy gain and gross energetic efficiency were observed at a dose of 1mg/kg but neither parameter was affected at other doses. The mass, protein and RNA content of gastrocnemius muscle were increased by TP but the ratio of RNA to protein and the rate of muscle protein synthesis measured *in vivo* were not affected at any dose of TP. The results indicate that the effects of testosterne on body composition are highly dose-dependent and the anabolic action of testosterone is not through stimulation of protein synthesis.

KEY WORDS : testosterone · androgens · body composition · muscle protein synthesis.

Introduction

In most species, males have larger body size and greater musculature than females. These sex differences thought since ancient times to be due to the presence of testes are now known to result from the presence of androgens such as testosterone in males and much attention has been focused on the possible use of androgens as muscle protein-anabolic agents[1]. Indeed, until the mid 1980s androgen analogues and derivatives, collectively anabolic steroids, were widely used to promote growth and carcass protein in animal production[2)3)4)], and have been shown to improve nitrogen balance and body protein in muscle wasting conditions such as surgical trauma[5)6)7)], accidental injury[8)] and myotonic dystrophy[9)]. However, the mechanisms by which these agents increase muscle protein content are unclear and their effects on muscle protein turnover are variable. For example, muscle protein synthesis has been shown to be increased and decreased by an anabolic steroid stanozolol[10)] and trenbolone acetate[11)], respectively, in female rats.

In addition, testosterone and its derivatives have been reported to exert dose-dependent effects on body weight[12)], and reduce body fat at high doses[13)]. However, the changes in body fat and protein in

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relation to changes in body weight at various doses have not been systematically documented. Therefore, in the present study effects of varying doses of testosterone on body composition, and energy and muscle protein metabolism have been investigated in female rats.

Materials and Methods

Female Sprague-Dawley rats were divided into five groups of six and fed a semi-synthetic diet(see table 1 for composition) ad libitum. Three groups of animals received daily subcutaneous injections of 1, 4 or 10mg/kg body weight of testosterone propionate suspended in a CM-cellulose vehicle[14] for 10 days. Equivalent volume of only vehicle was given to another group which acted as a control. The remaining group was killed on day 0 and their body composition was determined and used to calculate initial body energy content of the other groups using

Table 1. Composition of diet

Component	g/kg
Casein	250
DL-methionine	2
Sucrose	280
Corn starch	280
Corn oil	100
Solka floc	30
Vitamin mix[1)]	20
Mineral mix[2)]	40
Protein content	195
Gross energy	17.5 kJ/g

1) The vitamin mix provides(per kg of diet) retinol acetate 10mg ; cholecalciferol 1mg ; tocopherol acetate 75mg ; menadione 1mg ; thiamin HCl 10mg ; pyridoxine HCl 10mg ; riboflavin 10mg ; nicotinic acid 60mg ; calcium pantothenate 40mg ; folic acid 5mg ; biotin 1mg ; cyanocobalamine 0.05mg ; ascorbic acid 75mg ; choline bitartrate 1.8g.

2) The mineral mix provides(per kg of diet) $CaHPO_4$ 13g ; $CaCO_3$ 8g ; KCl 8g ; Na_2HPO_4 7.5g ; $MgSO_4 \cdot H_2O$ 180mg ; $C_8H_5O_7Fe \cdot 3H_2O$ 174mg ; $CuSO_4$ 15mg ; $ZnCO_3$ 30mg ; KIO_3 1mg

23.5 and 39.0kJ/g for gross energy density of protein and fat, respectively[15].

Body protein and fat were determined by Kjeldahl method(N×6.25) and petroleum ether extraction, respectively, after drying at 105℃ until constant weight. Body energy gain was determined as the difference between final and initial body energy contents. Total energy expenditure was calculated from the difference between metabolizable energy intake [gross energy intake (measured by bomb calorimetry)×0.95] and body energy gain. Gross energetic efficiency was calculated as body energy gain per metabolizable energy intake.

At the termination of the experiment animals were injected at 10 : 00~11 : 00 hours with L-[2, 3-^{3}H]phenylalanine (50μCi/100g body weight) via the lateral tail vein. After 10 min animals were killed by decapitation and gastrocnemius muscles were rapidly dissected, frozen in liquid nitrogen. The fractional rate of protein synthesis (Ks) in muscle was determined as described by Garlick et al.[16], involving the measurement of specific radioactivities of free (Sa) and protein-bound (Sb) [^{3}H]phenylalanine. Protein synthetic rates were expressed as % per day which was calculated as follows :

$$Ks = \frac{Sb}{Sa \times t} \times 100$$

where t is the incorporation time in days.

Muscle protein content was measured by the method of Lowry et al.[17], using bovine serum albumin as a standard and RNA content by the UV method as described by Munro and Fleck[18].

Results are expressed as mean values with their standard errors. The significance of differences between control and treated groups was determined by Student's unpaired t-test using two-tailed probability levels. The significance of the relationship between dose and certain variables was tested by linear regression analysis.

Results

Daily injections of testosterone propionate (TP) to female rats for 10 days did not affect food intake or body weight gain at the higher doses(4 and 10 mg/kg), but caused a significant increase in body weight gain at a dose of 1mg/kg(Table 2). This latter effect was associated with increases in body fat and protein whereas only body protein content was increased at doses of 4 and 10mg/kg compared to controls. Energy expenditure was reduced by a dose of 4mg/kg, and gross energetic efficiency was increased by the 1mg/kg dose(Table 2).

The masses of gastrocnemius muscle, heart and kidney was generally increased by testosterone propionate treatment compared to controls but that of liver was not affected at any dose(Table 3). The increase in the weight of kidney was dose-related ($r=0.63$, $p<0.01$, $y=0.93+0.02x$). In accordance with the increase in weight, both protein and RNA content of gastrocnemius muscle were also increased by TP. The magnitude of the increases in these two parameters was similar so that the ratio of RNA

Table 2. Body weight gain and composition, and gross energetic efficiency of female rats treated with varying doses of testosterone propionate (TP) for 10 days

	Control	TP (1m/kg)	TP (4mg/kg)	TP (10mg/kg)
Initial body weight (g)	157 ± 1	157 ± 1	157 ± 1	158 ± 1
Final body weight (g)	207 ± 5	223 ± 5*	218 ± 5	211 ± 5
Weight gain (g)	50 ± 4	66 ± 5*	61 ± 5	53 ± 4
ME intake (kJ/day)	286 ±10	305 ±11	280 ±12	268 ± 5
Body fat (g)	27.8± 2.4	35.6± 1.2*	30.7± 2.2	25.2± 1.8
Body protein (g)	33.5± 0.4	35.4± 0.7*	36.0± 0.7*	35.3± 0.6*
Energy gain (kJ/day)	59 ±10	93 ± 5*	76 ± 9	53 ± 7
Energy expenditure (kJ/day)	228 ± 6	212 ± 9	205 ± 7*	217 ± 7
Gross energetic efficiency (%)	20.2± 2.7	30.5± 1.4**	26.6± 2.4	19.2± 2.6

Mean values± SEM(n=6), *$p<0.05$, **$p<0.01$ vs control

Table 3. Weight of tissues of female rats treated with varying doses of testosterone propionate (TP) for 10 days

	Control	TP (1m/kg)	TP (4mg/kg)	TP (10mg/kg)
Gastrocnemius muscle (g)	0.94± 0.01	1.03± 0.03*	1.04± 0.02**	1.03± 0.03*
Heart (g)	0.62± 0.01	0.68± 0.02*	0.67± 0.02	0.68± 0.02*
Kidney (g)	0.88± 0.03	0.98± 0.02*	1.03± 0.02**	1.07± 0.05**
Liver (g)	10.4 ±0.5	11.3 ±0.4	11.1 ±0.4	10.5 ±0.4

Mean± SEM(n=6), *$p<0.05$, **$p<0.01$ vs control

Table 4. Protein and RNA content, and fractional rate of protein synthesis of gastrocnemius muscle of female rats treated with varying doses of testosterone propionate (TP) for 10 days

	Control	TP (1m/kg)	TP (4mg/kg)	TP (10mg/kg)
Protein content (mg)	155± 2	169± 6*	176± 5**	175± 5*
RNA content (mg)	1.61± 0.03	1.81± 0.06*	1.84± 0.06**	1.88± 0.09
RNA/Protein (mg/mg×10^3)	10.3 ±0.1	10.8 ±0.3	10.5 ±0.2	10.7 ±0.2
Protein synthetic rate (%/day)	10.3 ±0.6	10.5 ±0.8	10.7 ±0.4	10.1 ±0.6

Mean± SEM, *$p<0.05$, **$p<0.01$ vs control

to protein was not affected by any dose of TP(Table 4). The measurement of the fractional rate of protein synthesis *in vivo* revealed that TP did not affect muscle protein synthesis at any dose(Table 4).

Discussion

A number of studies have investigated the effect of androgens on body weight and it has generally been concluded that testosterone or its derivatives exert dose-dependent effects on body weight[12)19)]. However in most studies, changes in body composition have not been monitored simultaneously with those in body weight. In the present study treatment with 1mg/kg testosterone propionate (TP) caused an increase in body weight gain. This was associated with significant increases in body protein and also body fat. At higher doses, TP did not affect body weight gain, because although body protein was increased, body fat was reduced with increasing doses. Thus changes in body weight were not indicative of the relative changes in mass of fat and lean tissue.

Treatment of TP at a dose of 1mg/kg increased body fat. However, when dose was increased, body fat gradually returned to control values. There was a significant inverse relationship between body fat and dose within TP treatments($r=-0.73$, $p<0.001$, $y=36.1-1.13x$). It has been suggested that reductions in body weight and fat at high dose of testosterone or its derivatives are due to aromatization of testosterone to oestrogens[3)12)13)] which have been shown to reduce fat deposition[20)]and inhibit adipose tissue lipoprotein lipase activity[21)]. The supportive evidence was provided by Gentry and Wede[20)] who observed no modification by even very high dose(20mg/d) of 5α-dihydrotestosterone, a non-aromatizable androgen, of body weight. Therefore the depletion of body fat at higher doses of TP relative to the stimulated body fat at a dose of 1mg/kg could be the result of such aromatization. If this was the case, the increase in body fat caused by 1mg/kg may have resulted from a direct action of testosterone itself which might have been counteracted by the oestrogen-mediated effect at higher doses.

Energy expenditure can be reliably assessed by the comparative carcass technique which involves determination of initial and final body energy content, and this is particularly useful in chronic studies in laboratory animals. The method is, however, critically dependent on the accuracy of assessment of initial body energy, and this is usually obtained by analysis of a representative group of animals at the begining of experiment. In the present study the coefficient of variation of initial body weight was less than 2%, and that of body energy in the six animals killed on day 0 was less than 5%. Treatment of TP at a dose of 4mg/kg reduced energy expenditure, but other doses of TP had no effect. However, when data were expressed on the basis of metabolic body size (kJ/body weight$^{0.75}$/d) energy expenditure was reduced by TP in animals treated with 1mg/kg as well as 4mg/kg, but was the same as control animals in the group given the dose of 10mg/kg(control, 821± 19 ; 1mg/kg, 736± 26 ; 4mg/kg, 719± 22 ; 10mg/kg, 770± 24).

An increase in body protein by TP at all doses used was associated with increases in the weights of gastrocnemius muscle, heart and kidney, implying a broad range of responsive tissues. The effect on kidney was particularly remarkable, and the increase was significantly correlated with dose. Castration causes a reduction in the weight of kidney as well as secondary sex organs such as the seminal vesicles and the prostate gland in male rats and these returns to normal following the administration of testosterone[1)22)]. In intact male rats the weight of these tissues is increased by TP in a dose-related manner whereas muscle and many other tissues are not affected(Choo, unpublished data). Thus it

appears that the kidney is more sensitive than other non-sex-related tissues to the action of androgens.

Muscle protein synthesis was unaffected by any dose of TP indicating that stimulation of protein content was achieved by reduction in protein degradation. This finding is consistent with the data of Lobley et al.[23], who observed unaltered muscle protein synthesis by testosterone in wether lambs. In contrast, Martinez et al.[19] reported that TP increased both protein synthesis and to a lesser extent, protein degradation in female rats at a dose of 1 mg/kg. However there are a number of differenes between the experimental protocols, such as age and strain of animals, which might have been responsible for these discrepancies. The study of Martinez et al.[19] performed on female Wistar rats weighing 90g whereas female Sprague-Dawley rats weighing 150g, and thus sexually mature, were used in the present study. Younger animals exhibit higher rates of muscle protein turnover[24], and there also exist strain-differences in muscle protein turnover in the rat[24]. Amongst Synthetic anabolic steroids, stanozolol has been shown to increase muscle protein synthesis without affecting protein degradation[10], and nandrolone phenylpropionate also increases protein synthesis in female rats but in a dose-dependent manner[25] whereas trenblone acetate decreases both protein synthesis and, to a greater extend, protein degradation in female rats[11] and lambs[26].

An increase in total RNA content of muscle was in proportion to an increase in protein content in TP treatments, thus leaving the ratio of RNA to protein(an index of translation capcity) being unchanged. No changes in muscle protein synthesis also imply that TP had no effects on RNA acivity (translation efficiency). This is again in contrast to the effect of either trenbolone acetate, or nandrolone phenylpropionnate. Trenbolone acetate has been shown to decrease[11] and nandrolone phenylpropionnate to increase[25] RNA activity while stanozolol has no effect on this parameter[10]. It seems therefore that androgens and anabolic steroids may act on muscle by more than one mechanism, with different effects predominating under different circumstance.

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=국문초록=

Testosterone이 암컷 쥐의 체구성성분 및 근육단백질 합성율에 미치는 영향

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남성호르몬인 testosterone 투여가 정상 암컷쥐의 체중, 체구성성분(체단백질, 체지방), 에너지 대사 및 근육단백질 대사에 미치는 영향을 조사하였다. Testosterone propionate를 체중 1kg당 1mg(1mg/kg)으로 10일간 투여했을 때 식이섭취량은 변화하지 않았음에도 체중 및 체단백질, 체지방은 유의적으로 증가하였다. 반면 testosterone propionate를 4 또는 10mg/kg으로 투여시에는 체단백질만 유의적으로 증가하였고 체지방은 영향을 받지 않았다. Testosterone propionate에 의한 체내 에너지축적(energy gain) 및 에너지 이용율(gross energetic efficiency) 증가는 1mg/kg의 투여량에서만 관찰되었다. 근육조직(gastrocnemius muscle)의 무게, 단백질 및 RNA 함량은 모든 투여량에서 유의적으로 증가하였으나 단백질 합성율은 어느 투여량에서도 영향을 받지 않았다. 이러한 결과를 통해 testosterone이 체중 및 체단백질, 체지방에 미치는 영향은 투여량에 크게 의존하며 testosterone의 근육단백질 증진 효과는 단백질 합성율에는 영향을 미치지 않고 단백질 분해율을 저하시킴으로써 발휘된다는 것을 알 수 있다.