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Effects of an Anabolic Steroid, Nandrolone Phenylpropionate, on Reductions in Body and Muscle Proteins Under the Dietary Regimens of Feeding a Low-Protein Diet and of 50% Food Restriction in Rats

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ABSTRACT

The aim of the present investigation was to see whether an anabolic steroid, nandrolone phenylpropionate (NPP), exerts protien-anabolic effects under such adverse nutritional conditions as protein deficiency and protein-energy malnutrition in male rats. Feeding on a low-protein (8% casein) diet resulted in a marked reduction in body weight gain that was associated with reductions in body protein and protein content of gastrocnemius muscle. Administration of NPP (4 mg/kg body weight) did not alter muscle and body protein depletion induced by a low-protein diet. 50% food restriction caused reductions in body protein and in protein content of gastrocnemius muscle. These reductions were partially prevented by NPP (4 mg/kg body weight). Food restriction did not affect plasma concentration of corticosterone, insulin, or tetosterone plus dihydrotestosterone. On the other hand, neither plasma concentration of corticosterone nor insulin were affected by NPP. The present results show that anabolic steroids do not express anabolic effects under conditions of protein deficiency, but in protein-energy malnutrition, anabolic steroids exert their anabolic effects even in male rats.

KEY WORDS : anabolic steroid · nandrolone phenylpropionate · low-protein · food restriction.

INTRODUCTION

Analogues and synthetic derivatives of testosterone, commonly called anabolic steroids, are known to possess anabolic effects on muscle protein. They have been shown to improve nitrogen balance and body protein in muscle-wasting conditions such as surgical trauma,[1-3] accidental injury,[4] and myotonic dystrophy.[5] However, normal men and intact male animals do not respond to anabolic steroids.[6] It might be possible that in normal males the anabolic effect is already fully expressed by the endogenous androgens.

In man and animals, inadequate intake of energy or protein results in a reduction in body weight that is generally associated with a reduction in body protein and with unpredictable changes in body fat.[7-9] These nutritional insults cause a shift in hormonal profile and metabolism. Glucocorticoids, catabolic hormones on protein,[10] may be elevated[11] and in males, androgens may be suppressed.[12] These two hormones are thought to be at least partly responsible for the reduction in protein deposition. Therefore, anabolic steroids might exert their action by restoring the loss of androgens and/or suppressing the effects of glucocorticoids under conditions of inadequate energy or protein intake.

In the present study, the effects of the anabolic steroid nandrolone phenylpropionate (NPP, Durabolin, Δ^4-estr-en-17β-ol-3-one phenylpropionate) on growth, body composition, and muscle protein in male rats were investigated under two adverse nutritional conditions : a low protein diet and restricted amounts of food.

METERIALS AND METHODS

1. Animals

Male Sprague-Dawley rats (45d old) were housed singly at 24℃ with a 12-hour light/12-hour dark cycle, and fed a semi-synthetic diet for three days before the commencement of the experiments. At the begining of each experiment, animals were divided into three groups of six animals according to body weight gain during the period of adaptation.

In the low-protein study, one group was maintained on a normal diet and the other two groups were fed a low-protein diet (Table 1). One of the low-protein groups also received daily subcutaneous injections of 4 mg/kg body weight nandrolone phenylpropionate (NPP, Organon Ltd) suspended in carboxymethylcellulose (CMC) vehicle between 10.00 and 11.00 hours. The other two groups received CMC vehicle. The period of treatment was 10 days.

Table 1. Composition (g/kg) of normal and low-protein diets

Constituent	Normal	Low-protein
Casein	250	80
DL-methionine	2	1
Sucrose	280	365
Corn starch	280	365
Corn oil	100	100
α-cellulose	30	30
Vitamin mix[1)]	20	20
Mineral mix[2)]	40	40

1) The vitamin mix provides (per kg of diet) 10 mg retinol acetate ; 1 mg cholecalciferol ; 75 mg tocopherol acetate ; 1 mg menadione ; 10 mg thiamin HCl ; 10 mg pyridoxine HCl ; 10 mg riboflavin ; 60 mg nicotinic acid ; 40 mg calcium pantothenate ; 5 mg folic acid ; 1 mg biotin ; 0.05 mg cyanocobalamine ; 75 mg ascorbic acid and 1.8 g choline bitartrate.
2) The mineral mix provides (per kg of diet) 13g $CaHPO_4$; 8 g $CaCO_3$; 8 g KCl ; 7.5 g Na_2HPO_4 ; 180 mg $MgSO_4 \cdot H_2O$; 174 mg $C_8H_5O_7Fe \cdot 3H_2O$; 15 mg $CuSO_4$; 30 mg $ZnCO_3$ and 1 mg KIO_3.

In the food-restriction study, one group received a normal diet ad libitum and served as control. Rats of the remaining two groups were fed at 50% of ad libitum intake of their paired-controls. Food was provided by meal feeding between 17.00 and 17.30 hours. One of the food-restricted groups also received daily subcutaneous injection of 4 mg/kg body weight nandrolone phenylpropionate (NPP, Organon Ltd) suspended in carboxymethylcellulose (CMC) vehicle[13] between 10.00 and 11.00 hours. The other two groups received CMC vehicle. The period of treatment was 7 days.

At the end of the treatments, rats were killed between 10.00 and 11.00 hours by decapitation. Each rat's gastrocnemius muscle, heart, liver and seminal vesicles were removed. Blood was collected into EDTA tubes and the resulting plasma was stored at −20℃ until required for analysis.

2. Measurement of body composition

The carcasses were dried at 105℃ to constant weight. Body protein and fat contents were determined on dried homogenized carcasses by the Kjeldahl method (N×6.25) and petroleum ether extraction, respectively.

3. Measurement of muscle protein

Gastrocnemius muscle, which is a representative skeletal muscle, was homogenized in 0.3M-NaOH. Protein content was then measured by the method of Lowry *et al.*[14] using bovine serum albumin as a standard.

4. Measurement of plasma corticosterone, insulin and testosterone+dihydrotestosterone

Plasma corticosterone was measured by the methods of Lambert *et al.*[15] and Scott *et al.*[16] using high performance liquid chromatography (HPLC). Cortisol was used as an internal standard and isocratic elution was performed on a C_{18} reversed-phase column (Nova Pak C_{18}, Waters) at room temperature using a mixture of methanol and water (60 : 40, v/v). The flow rate was 0.7 ml/min. The absorbance of the column effluent was monitored at 254 nm.

Plasma insulin and testosterone+dihydrotestosterone were measured by radioimmunoassay (RIA) technique using the Amersham (England) kits. The antibody supplied for the determination of testosterone was not specific for testosterone and does cross-react with dihydrotestosterone significantly. The dihydrotestosterone level is, however, relatively low compared with the testosterone level. For example, the dihydrotestosterone level in adult male rats is about 7% that of testosterone.[17] Furthermore, the cross-reactivity of dihydrotestosterone for the antibody supplied is about 45−50% (Amersham information). Considering the cost of the RIA kit along with the facts, in the present study, total testosterone+dihydrotestosterone was measured and considered as the representative testosterone level.

5. Statistical analysis

The data was expressed as mean±SEM. Statistical significance was analyzed by one-way and two-way ANOVA for the low-protein and food restriction studies, respectively. Significance of the differences between two groups was determined by least significant difference (LSD) using a threshold probability of 5%.

RESULTS

Dietary protein deficiency did not affect energy intake but resulted in a marked reduction in weight gain associated with reductions in body protein and protein content of gastrocnemius muscle (Table 2). However, body fat content was not changed. Administration of NPP had no effects on body protein and protein content of gastrocnemius muscle but food intake, weight gain, and body fat were significantly reduced by NPP (Table 2).

50% food restriction alone caused a reduction in weight gain associated with reductions in body protein and body fat (Table 3). Protein content of gastrocnemius muscle was also reduced by food restriction (Table 4). Weight of heart and liver were observed to be reduced. However, magnitude of reductions in weight of these organs exceeded that of gastrocnemius muscle, the severity observed in liver being especially noticable (Table 4). NPP had no

Table 2. Effects of nandrolone phenylpropionate (NPP, 4 mg/kg) on food intake, body composition, and protein content of gastrocnemius muscle in male rats fed a low-protein diet

	Normal	Low-protein	Low-protein+NPP
Initial body weight (g)	196 ± 2^{a}	196 ± 1^{a}	197 ± 2^{a}
Final body weight (g)	287 ± 4^{a}	246 ± 3^{b}	232 ± 4^{c}
Weight gain (g)	91 ± 3^{a}	50 ± 3^{b}	35 ± 4^{c}
Food intake (g)	$249\pm8^{a,b}$	$253\pm4^{a,c}$	$234\pm3^{b,d}$
Body protein (g)	49.7 ± 0.7^{a}	38.6 ± 0.6^{b}	37.9 ± 0.4^{b}
Body fat (g)	$46.9\pm2.5^{a,b}$	$51.9\pm3.4^{a,c}$	$41.4\pm1.6^{b,d}$
Gastrocnemius muscle			
Weight (g)	1.32 ± 0.03^{a}	1.07 ± 0.02^{b}	1.01 ± 0.03^{b}
Protein content (mg)	221 ± 4^{a}	180 ± 6^{b}	169 ± 5^{b}

Values are means for six rats, with their standard errors. Values within a column with different superscript letters were significantly different($p<0.05$).

Table 3. Effects of nandrolone phenylpropionate (NPP, 4 mg/kg) on food intake and body composition in male rats on 50% food restriction

	Normal	Food restriction	Food restriction+NPP
Initial body weight (g)	214 ± 1^{a}	215 ± 2^{a}	216 ± 1^{a}
Final body weight (g)	293 ± 1^{a}	228 ± 3^{a}	235 ± 2^{a}
Weight gain (g)	79 ± 2^{a}	13 ± 5^{b}	19 ± 3^{b}
Food intake (g)	189 ± 7	94.5	94.5
Body protein (g)	49.0 ± 0.4^{a}	41.6 ± 1.0^{b}	42.7 ± 1.0^{b}
Body fat (g)	43.5 ± 3.2^{a}	20.1 ± 1.4^{b}	19.1 ± 1.6^{b}

Values are means for six rats, with their standard errors. Values within a column with different superscript letters were significantly different ($p<0.05$).

Table 4. Effects of nandrolone phenylpropionate (NPP, 4 mg/kg) on weight and protein content of gastrocnemius muscle, and on weight of heart, liver, and seminal vesicles in male rats on 50% food restriction

	Normal	Food restriction	Food restriction+NPP
Gastrocnemius muscle			
Weight (g)	1.25 ± 0.05^{a}	1.09 ± 0.02^{b}	1.14 ± 0.01^{c}
Protein content (mg)	203 ± 5^{a}	171 ± 4^{b}	186 ± 3^{c}
Heart (g)	1.01 ± 0.02^{a}	0.87 ± 0.02^{b}	0.92 ± 0.04^{b}
Liver (g)	16.8 ± 0.3^{a}	11.1 ± 0.5^{b}	11.1 ± 0.5^{b}
Seminal vesicles (g)	0.44 ± 0.03^{a}	0.39 ± 0.03^{b}	0.82 ± 0.04^{c}

Values are means for six rats, with their standard errors. Values within a column with different superscript letters were significantly different($p<0.05$).

effect on body protein but the weight and protein content of gastrocnemius muscle were significantly increased (Tables 3 and 4). On the other hand, body fat was not affected by NPP. NPP did not change the weights of heart and liver. Weight of seminal vesicles was significantly reduced by food restriction whereas NPP caused more than a two-fold increase (Table 4). Food restriction did not affect plasma concentration of corticosterone, insulin, or testosterone+dihydrotestosterone (Table 5). Neither plasma concentration of corticosterone nor insulin were affected by NPP.

Table 5. Effects of nandrolone phenylpropionate (NPP, 4 mg/kg) on the plasma concentration of corticosterone, insulin, and androgens in male rats on 50% food restriction

	Normal	Food restriction	Food restriction+NPP
Corticosterone (ng/ml)	108 ± 30^{a}	174 ± 46^{a}	91 ± 17^{a}
Insulin (μU/ml)	99 ± 16^{a}	92 ± 17^{a}	76 ± 28^{a}
Testosterone+ Dihydrotestosterone (ng/ml)	5.07 ± 1.27^{a}	4.56 ± 0.90^{a}	ND

ND, not determined

Values are means for six rats, with their standard errors. Values within a column with different superscript letters were significantly different ($p<0.05$).

DISCUSSION

In the low-protein study, interpretation of whether NPP had any anabolic effect on protein deposition is complicated by a decrease in food intake. This problem might be overcome by calculating protein conversion efficiency (g protein gain/g protein intake $\times 10^2$). The finding that NPP reduced food intake without affecting body protein might imply an improvement in protein conversion efficiency. However, no difference in this parameter was observed (27.6 ± 4.2 and 26.4 ± 5.0 for low-protein group and low-protein plus NPP group, respectively). Thus, it can be concluded that NPP had no effect on protein deposition under conditions of a low-protein dietary regimen.

Although there is much controversy on the benefits of anabolic steroids to nitrogen balance, one consistent finding is that when intakes of both protein and energy are lower than required, anabolic steroids act to promote nitogen balance. The present animal model of 50% food restriction might not be representative of malnutrition in clinical patients since these animals were still depositing protein, whereas net breakdown of body protein is a common feature in those patients. Despite this fact, NPP exerted significant anabolic effects in rats on 50% food restriction in the present study.

Early demonstrations of the anabolic effects of testosterone were derived from the classical nitrogen balance study in castrated male,[18][19] normal male,[20] and normal female[21] rats. In these nitrogen balance studies, rats were given a period of restricted amount of food and thereafter food intake was further reduced gradually until body weight stabilized and nitrogen balance was established. Obviously, those animals were suffering from protein and energy malnutrition. The present study together with those mentioned above, is strongly indicative of the expression of anabolic effects of anabolic steroids under a

restrictive dietary protein and energy regimen.

The administration of NPP decreased food intake and fat deposition in low-protein-fed rats. This might be due to aromatization of NPP to estrogens[22] which have been shown to reduce fat deposition[23] and inhibit adipose tissue lipoprotein lipase activity.[24] However, Choo[25] observed a tendency for body fat to be reduced in low-protein fed male rats by the administration of 1 mg/kg body weight of a nonaromatizable anabolic steroid, stanozolol, with food intake being unaffected. This suggests that apart from the indirect effect on fat deposition by aromatization to estrogens of aromatizable anabolic steroids, there might also exist a common direct effect on fat metabolism that all anabolic steroids share.

It is now widely accepted that the clinical picture associated with protein-energy malnutrition is mediated by changes in hormonal pattern, with the role of glucocorticoids and insulin being especially emphasized. Malnourished patients commonly suffer from muscle wasting,[26] and the relative importance of insulin[27] and glucocorticoids[28] on muscle protein has been well documented. Therefore, low insulin and elevated glucocorticoid levels might be expected in malnourished patients with muscle wasting. However, the work on experimental animal models of protein-energy malnutrition has yield conflicting data. In their study, Coward *et al.*[10] and Lunn *et al.*[29] observed that low-protein diets (3.2% and 0.5%) and 50% food restriction of normal diet increased corticosterone and decreased insulin levels in rats. On the other hand, Anthony and Edozien[30] reported decreases in both insulin and corticosterone levels after feeding rats a low-protein (0.5% lactalbumin) diet. However, Edozien *et al.*[31] observed that 25 and 50% restriction of food increased serum insulin whereas serum corticosterone was reduced. These workers also found that corticosterone values varied directly with dietary fat and showed a biphasic response to changes in dietary protein levels between 2 to 50%. Thus, some of the controversy concerning effects of low-protein and food restriction on circulating levels of glucocorticoids and insulin migh have been avoided by specifying level of dietary fat and/or protein used.

In the present study, 50% food restriction and NPP administration did not result in any significant changes in plasma concentrations of insulin. This observation implies that the anabolic effects of anabolic steroids do not involve insulin as a secondary hormone. This suggestion is further supported by the study of Wright and Kochakian,[32] who observed that administration of testosterone propionate to alloxan-induced diabetic castrated rats still improved nitrogen balance. This finding might also suggest that insulin does not act as a permissive hormone on the action of anabolic steroids.

Neither 50% food restriction nor NPP altered plasma concentrations of corticosterone. However, values varied within each group. This variation might have been due to such stressess as daily injections, handling, and restricted-feeding schedules. It should also be mentioned that in the study of Sharpe *et al.*,[33] restriction of food to an amount that stopped growth did not change total cortisol levels in sheep but free levels were increased due to a decrease in binding protein. Therefore, it would be unwise to arrive at a conclusion from the present finding that a dietary regimen of 50% food restriction and NPP had no effect on the functional potency of glucocorticoids.

Testicular atrophy and reductions in plasma testosterone levels have been reported in men subjected to protein and energy insufficiency[34] and in protein-deficienct monkeys.[35] On the other hand, Nduka *et al.*[36] reported no change in plasma testosterone levels in rats fed a low-protein (3%) diet in spite of a significant reduction in testes weight. As they suggested, this might be due to reduced plasma clearance rates or increased secretion. The present study also observed no effect of 50% food restriction on plasma concentrations of testosterone+dihydrotestosterone. These disparate findings imply that the rat might be more resistant than man to the effects of nutritional insults on testosterone levels. NPP increased the weight of an androgen target tissue, seminal vesicle, more than 2 times, implying the high sensitivity of this sexual tissue to anabolic steroids. This observation suggests the overriding importance of hormonal levels rather than nutritional factors, in regulating these tissues.

In conclusion, the results of the present study show that NPP does not improve protein deposition in rats fed a low-protein diet but exerts its anabolic effects under conditions of restrictive dietary protein along with energy restriction.

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