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Effects of an Anabolic Steroid, Nandrolone Phenylpropionate, on Insultin-Mediated Increase in Fat Deposition and Fasting-Induced Lipolysis in Female Rats

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Introduction

It has been known for over 40 years that a group of synthetic analogues and derivatives of testosterone, the so-called anabolic steroids, increase muscle protein content under certain circumstances[1)2)]. Besides their muscle protein-anabolic effects, anabolic steroids have often been shown to reduce fat deposition, though this effect is dependent on dose and the nature of the steroid concerned. Orchidectomy causes a significant increase in body fat[3)], and high doses of testosterone[4)] or the anabolic steroid, nandrolone phenylpropionate[5)], result in substantial loss of body fat in female rats whereas another anabolic steroid, trenbolone acetate, exhibits this body fat-suppressive effect at a dose of only 800μg/kg[6)].

Androgens have been suggested to cause insulin resistance[7)8)9)] and there is an association between diabetes and hyperandrogenism in women[10)]. From these observations it can be speculated that the body fat-suppressive effect of anabolic steroids is brought about by inhibiting the action of insulin on fat deposition. On the other hand, body fat suppression by anabolic steroids might be a result of stimulation of lipolysis. Therefore, the present study aimed to investigate the effect of an anabolic steroid, nandrolone phenylpropionate, on fat deposition stimulated by exogenous insulin, and on fasting levels of free fatty acids in female rats.

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Materials and Methods

Female Sprague-Dawley rats were housed singly at 24℃ with a 12-hour light-dark cycle and fed a semi-synthetic diet(see table 1 for composition) for 3 days before the commencement of the experiments. At the begining of each experiment, rats weighing 150-160g were divided into three groups of eight rats matched for weight.

In experiment 1, two groups received daily subcutaneous injections of isophane insulin, the initial dose was 20U/kg body weight and this was increased by 10U/kg every three-day interval to 50U/kg over a 12-day period. One of these groups also received daily subcutaneous injections of an anabolic steroid nandrolone phenylpropionate (NPP) suspended in CM-cellulose(CMC) vehicle(11) at a dose of 2mg/kg body weight. The remaining group served as a control and received daily injections of 0.9% saline and CMC vehicle. All injections were carried out separately at 16 : 00~17 : 00 for 12 days. Daily food intake was

recorded, taking into account spillage. At the end of the experiment animals were killed between 10 : 00 and 10 : 30 by decapitation and gastrocnemius muscle was removed.

In experiment 2, animals were allocated into three groups and fed a semi-synthetic diet ad libitum for three days. During this period, one of these groups received daily subcutaneous injections of 4mg/kg body weight of NPP while the other two groups received CMC vehicle. On the fourth day, the food pot was removed from the NPP-treated group and one of the CMC vehicle-treated group at 09 : 00 whereas food was still accessible to the remaining group. During this time, the dose of NPP was increased to 10mg/kg to augment any existing effect. On the same day, between 21 : 00 and 22 : 00, rats were killed by decapitation.

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	17.5KJ/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 10 mg ; 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 7.5 g ; $MgSO_4 \cdot H_2O$ 180mg ; $C_8H_5O_7Fe \cdot 3H_2O$ 174mg ; $CuSO_4$ 15mg ; $ZnCO_3$ 30mg ; KIO_3 1 mg.

In both experiments, blood was collected into chilled EDTA tubes and centrifuged immediately at 4℃. Plasma was removed and stored at −20℃ until analysis. Plasma glucose concentration was measured by a Glucose oxidase method, using commercial kit supplied by Boehringer(Germany). The level of free fatty acids was determined by summing up the individual concentration of each fatty accid measured by Gas Liquid Chromatography after conversion to methyl esters with diazomethane. Fatty acids are extracted with the Dole mixture(Isopropanol : heptane : 0.5M-H_2SO_4=40 : 10 : 1) and are then isolated by converting them to sodium salts which are water soluble. Tissue protein content was measured by the method of Lowry et al.[12], using bovine serum albumin as a standard. Body protein and fat contents were determined by the Kjeldahl method and Soxhlet extraction(petroleum ether), respectively, after drying at 105℃ to constant weight.

Results are expressed as mean± SEM. Statistical significance was analyzed by using the one-way analysis of variance(ANOVA) followed by least significant differences(LSD) comparison at a threshold probability of 5% when significant differences were observed by the one-way ANOVA[13].

Results

The administration of insulin caused marked increases in food intake and body weight gain (Table 2). The increase in body weight gain was entirely due to an increase in body fat. Insulin had no effect on either body protein or gastrocnemius muscle protein content(Table 2). Although an insufficiency of insulin has been well documented to be associated with a reduction in muscle mass, for example in diabetic rate[14)15)], from the present observations it appeared that the admini-

Table 2. Effects of nandrolone phenylpropionate(NPP) on body weight gain and composition, and muscle protein content. in insulin-treated female rats(Experiment 1).

	Control	Insulin	Insulin+NPP
Body weight gain(g)	58.3± 4.4[a]	88.0± 3.9[b]	106.9± 4.3[c]
Food intake(g)	206± 8[a]	282± 13[b]	271± 10[b]
Body fat(g)	34.0± 1.9[a]	57.2± 3.3[b]	47.0± 2.2[c]
Body protein(g)	34.0± 0.8[a]	34.5± 0.5[a]	40.9± 0.8[b]
Gastrocnemius muscle protein content(mg)	156± 3[a]	160± 4[a]	180± 4[b]

Mean values± SEM(n=8)

Values in rows which share the same superscript are not significantly different at the 5% level.

stration of relatively large doses of exogenous insulin to normal rats did not have any additive effect on either body protein or muscle protein content. However, insulin still exerted its potent effect on fat deposition.

The simultaneous administration of an anabolic steroid nandrolone phenylpropionate(NPP) with insulin caused a further increase in body weight gain with no alteration in food intake(Table 2). The increase in body weight gain was associated with an increase in body protein and a reduction in body fat. Consistent with an increase in body protein, NPP caused a significant increase in gastrocnemius muscle protein content (Table 2).

The administration of insulin resulted in a dra-

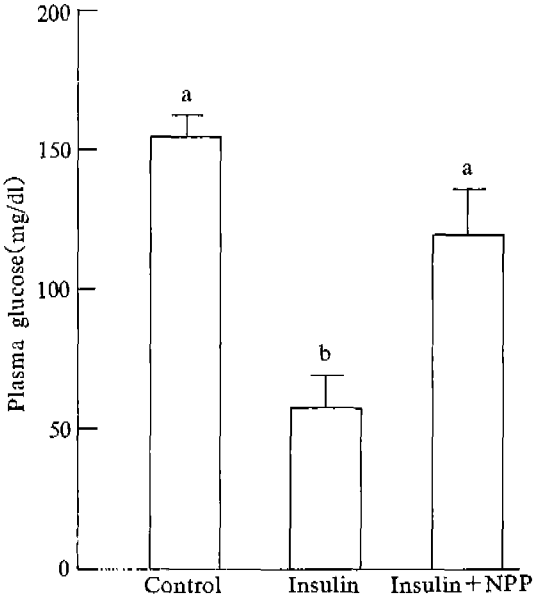

Fig. 1. Effects of nandrolone phenylpropionate(NPP) on plasma glucose levels in insulin-treated female rats(Experiment1). Results are means ± SEM(n=8). Groups sharing the same letter, indicated on the top of error bars, are not significantly different at the 5% level.

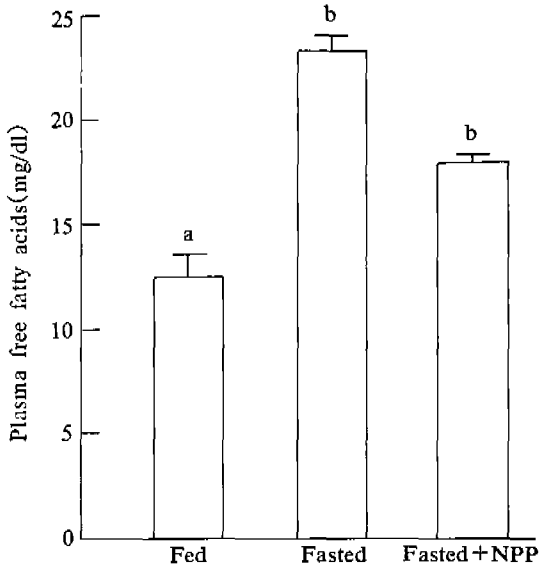

Fig. 2. Effects of nandrolone phenylpropionate(NPP) on the plasma level of free fatty acids in 12-hour fasted female rats(Experiment 2). Results are means± SEM(n=8). Groups sharing the same letter, indicated on the top of error bars, are not significantly different at the 5% level.

matic reduction in plasma glucose levels(Fig. 1). On the other hand, the simultaneous administration of NPP with insulin prevented much of the reduction in plasma glucose levels.

A 12-hour fast caused a significant increase in plasma levels of free fatty acids(Fig. 2). The administration of NPP had no effect on this parameter.

Discussion

There are many regulatory sites in fat metabolism alterations of which can result in changes in fat deposition. These can be broadly divided into four processes : the extraction of fatty acids from plasma lipoprotein triacylglycerol, fatty acid synthesis, esterification and lipolysis. There is evidence for the involvement of insulin in all four processes, always acting to cause a net increase in fat deposition[16-19]. Suprisingly, Torbay et al.[20] observed no increase in the activities of lipogenic enzymes, citrate cleavage enzyme and fatty acyl synthetase, in epididymal fat pad of male rats treated with protamine-zinc insulin. They ascribed the increase in body fat to the possible enhancement of lipoprotein lipase activity, thus shunting more dietary fat into the fat depots. However, in the present study increases in body fat far exceeded the increase in fat intake, with a 23.2g increase in body fat associated with only 7.6g increase in fat intake. Therefore, along with the remarkable reduction in plasma glucose levels, these observations suggest that there was an increase in de novo synthesis of fatty acids.

An anabolic steroid nandrolone phenylpropionate(NPP) prevented much of the increase in fat deposition associated with insulin treatment. This phenomenon was accompained by partial restoration of the reduced plasma glucose levels. It is very likely that NPP inhibited the action of insulin on glucose disposal, thus reducing the utilization of glucose for fat deposition. Insulin resistance is commonly found in women with elevated circulating testosterone levels[21][22]. A very recent study of Buffington et al.[23] showed that hypertestosteronemic women patients had 7- to 10-fold greater than normal basal and glucose-challenged insulin values, blunted sensitivity to insulin, and in vitro defects in insulin binding. In animal studies, testosterone administration has been shown to inhibit glycogen synthesis and 2-deoxy-D-glucose transport in muscles of female rats[9].

Xu et al.[24] have reported that testosterone stimulates catecholamine-induced lipolysis in vivo by increasing the number of β-adrenoceptors as well as the activity of adenylate cyclase in rat adipocytes. In contrast, in the present study, despite the use of a high dose(10mg/kg), NPP had no effect on the fasting level of free fatty acids in 12-hour fasted rats. The observation can be interpreted as NPP does not have any effect on lipolysis. However, it might be possible that the rate of lipolysis was already operating at the maximum level in fasted rats which negated any existing lipolytic effect of NPP.

It has often been suggested that the muscle protein-anabolic effect of anabolic steroids might be through increasing levels of anabolic hormones such as insulin and growth hormone[25]. NPP improved body protein and gastrocnemius muscle protein content whereas exogenous insulin itself had no effect on any of these parameters. This obervation clearly rules out the possibility of insulin as a secondary hormone to anabolic steroids in promoting protein deposition.

In summary, the anabolic steroid nandrolone phenylpropionate decreases fat deposition possibly through inhibiting the action of insulin on glucose disposal, but its effects on protein meta-

bolism are not mediated by altering the activity of insulin.

Literature cited

1) Kochakian CD. Definition of androgens and protein anabolic steroids. *Pharmac Therap Bull 1(2) : 149-177, 1975*
2) Galbraith H, Topps JH. Effect of hormones on the growth and body composition of animals. *Nutr Abstr Rev 51(8) : 521-540, 1981*
3) Fletcher JM, Lobley GE, Connell A. Effects of growth and body composition of androgen deprivation by castration or autoimmunization to LH-releasing hormone in the male rat under conditions of controlled food intake. *J Endocrinol 110 : 97-102, 1986*
4) Hervey GR, Hutchinson I. The effect of testosterone on body weight and composition in the rat. *J Endocrinol 57 : xxiv, 1973*
5) Choo JJ, Emery PW, Rothwell NJ. Dose-dependent effects of an anabolic steroid, nandrolone phenylpropionate(Durabolin), on body composition and muscle protein metabolism in female rats. *Ann Nutr Metab 35 : 141-147, 1991*
6) Vernon BG, Buttery PJ. The effect of trenbolone acetate with time on the various responses of protein synthesis of the rat. *Br J Nutr 40 : 563-572, 1978*
7) Woodard RL, Burghen GA, Kitabchi AE. Glucose intolerance and insulin resistance in aplastic anemia treated with oxymetholone. *J Clin Endocrinol Metab 53 : 905-908, 1981*
8) Cohen JC, Hickman R. Insulin resistance and diminished glucose tolerance in powerlifters ingesting anabolic steroids. *J Clin Endocriol Metab 64 : 960-963, 1987*
9) Holmang A, Svedberg J, Jennische E, Bjorntorp P. Effects of testosterone on muscle insulin sensitivity and morphology in female rats. *Am J Physiol 259 : E555-560, 1990*
10) Stein IF, Lebenthal ML. Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol 20 : 181-187, 1935*
11) Tomas FM, Munro H, Young VR. Effect of glucocorticoid administration on the rate of muscle protein breakdown in vivo in rats, measured by urinary excretion of 3-methylhistidine. *Biochem J 178 : 139-146, 1979*
12) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem 193 : 265-275, 1951*
13) Snedecor GW, Cochran WG. Statistical methods, pp258-298, The Iowa State University Press, Ames, 1981
14) Odedra BR, Millward DJ. Effect of corticosterone treatment on muscle protein turnover in adrenalectomised rats and diabetic rats maintained on insulin. *Biochem J 204 : 663-672, 1982*
15) Tomas FM, Murray AJ, Jones LM. Interactive effects of insulin and corticosterone on myofibrillar protein turnover in rats as determined by 3-methylhistidine excretion. *Biochem J 220 : 469-479, 1984*
16) Cryer A, McDonald A, Williams ER, Robinson DS. Colchicine inhibition of the heparin-stimulated release clearing-factor lipase. *Biochem J 152 : 717-720, 1975*
17) Saggerson ED, Greenbaum AL. The regulation of triglyceride synthesis and fatty acid synthesis in rat epididymal adipose tissue. Effects on insulin, adrenaline and some metabolites in vitro. *Biochem J 119 : 193-219, 1970*
18) Saggerson ED. Hormonal regulation of biosynthetic activities in white adipose tissue. In : Cryer A, Van RLR, ed. New perspectives in adipose tissue, pp87-120, Butterworths, London, 1985
19) Belfrage P. Hormonal control of lipid degradation. In : Cryer A, Van RLR, ed. New perspectives in adipose tissue, pp121-144, Butterworths, London, 1985
20) Torbay N, Bracco EF, Geliebter A, Stewart IM, Hashim SA. Insulin increases body fat despite control of food intake and physical activity. *Am J Physiol 248 : R120-124, 1985*
21) Smith S, Ravnikar VA, Barbieri RL. Androgen and insulin response to an oral glucose challenge in hyperandrogenic women. *Fertil Steril 48 : 72-77,*

1987

22) Stuart DA, Peters EJ, Prince MJ, Richards G, Cavallo A, Meyer WJ. Insulin resistance and acanthosis nigricans : the roles of obesity and androgen excess. *Metabolism 35 : 197-205, 1986*

23) Buffington CA, Givens JR, Kitabchi AE. Opposing actions of dehydroepiandrosterone and testosterone on insulin sensitivity. *Diabetes 40 : 693-700, 1991*

24) Xu X, De Pergola G, Bjorntorp P, Testosterone increases lipolysis and the number of β-adrenoceptors in male rat adipocyte. *Endocrinology 128(1) : 379-382, 1991*

25) Buttery PJ. Hormonal control of protein deposition in animals. *Proc Nutr Soc 42 : 137-148, 1983*

아나보릭 스테로이드인 Nandrolone phenylpropionate가 암컷 쥐에서 인슐린에 의한 지방축적 증가 및 절식에 의한 지방분해에 미치는 영향

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= 국문초록 =

아나보릭 스테로이드인 nandrolone phenylpropionate(NPP)가 인슐린에 의한 지방축적의 증가 및 절식시 혈중 유리지방산 농도에 미치는 영향을 암컷쥐에서 조사하였다. 인슐린 투여는 식이 섭취량을 유의적으로 증가시켰는데 이는 인슐린에 의한 혈당 저하에 의한 것으로 보인다. 지방축적은 인슐린 투여에 의해 유의적으로 증가하였으나 체단백질이나 근육단백질 함량은 변화하지 않았다. 인슐린과 NPP를 동시에 투여하였을 때 NPP는 인슐린에 의한 저혈당증과 지방축적의 증가를 부분적으로 완화시켰으며 체단백질과 근육단백질 함량을 유의적으로 증가시켰다. 반면 NPP는 절식시킨 쥐의 혈중 유리 지방산 농도에는 영향을 미치지 않았다. 이러한 결과를 통해 아나보릭 스테로이드가 단백질 대사에 미치는 영향은 인슐린과 무관하며, 아나보릭 스테로이드의 지방축적 억제효과는 아나보릭 스테로이드가 지방분해에는 영향을 미치지 않고 인슐린에 의한 지방합성으로의 당이용도를 저해함으로서 발휘된다는 것을 알 수 있다.