Effects of Long-Term Injection of Steroid Hormone on Soluble Protein in Rat Muscles and Organellae

Yoshibumi NAKAHARA, Chikako YABUKI, Toshitada YOSHIOKA and Shoichi NAKANO

Department of Physiology III, School of Medicine, Tokai University

(Received June 4, 1982)

Steroid hormone (dexamethasone, 0.1 mg/kg/day) was injected into Wistar strain rats, and its effect on the weight of organellae and skeletal muscles was studied. Steroid was injected for 20, 30 and 40 weeks, and a de-steroid group was obtained by interrupting the steroid injections for three weeks.

The body weight of the injected rats decreased by 40 to 60% from that of the control, even when food intake per body weight was increased by more than 20%. The weight of organellae (heart, liver and adrenal glands) apparently decreased. Nevertheless, the weight per body weight tended to recover to the control level in the de-steroid group. Muscle atrophy of the proximal muscles, the rectus femoris and the semimembranosus muscles was observed in the steroid group. Remarkable atrophy appeared in the semimembranous muscle, named type II fiber, with 40% to 60% of control at the 30th and 40th weeks and the recovery rate of this weight was determined in only 80% in the de-steroid group. A decrease of the 40,000 to 60,000 MW polypeptides of serum and muscle soluble protein fraction was shown in the 40-week group.

These results suggested that acceleration of glycolysis, glyconeogenesis and protein catabolism were induced after long-term steroid injection.

(Key Words: Steroid Myopathy, Proximal Muscle, Distal Muscle, Soluble Protein Fractions)

INTRODUCTION

There have been many reports (1, 5, 7, 18) on steroid myopathies. Since Cushing (3) reported that Cushing's syndrome was manifested by long-term oversecretion of adrenal glands cortex hormone, the induction of muscle weakness and atrophy in animals as a result of glucocorticoid treatment has been noted in the pathological state of steroid myopathy (36) and experimentally, using many species, including mice (6), rats (20, 21, 30, 32, 38) and rabbits (4, 23, 29, 33, 34).

In these numerous reports, the selective effects of glucocorticoid on muscle have been noted along with its differing action according to the fiber-type composition of the muscle (29, 34, 35), especially on type II muscle fiber (18).

Vignos et al. (35) noted that, after administration of dexamethasone, muscular atrophy leads primarily to changes in the composition of fast myofibrillar proteins.

However, the details of the mechanism of muscle waste occurring during glucocorticoid administration have not yet been clarified (31).
According to Koski et al. (14) and Heiner et al. (13), dexamethasone causes loss of the oxidative capacity of muscles, but mitochondrial defects do not represent the primary lesion in steroid myopathy.

To date no study has been made on the long-term administration of low concentrations of steroid hormone.

In this study, we attempted to clarify the effects of long-term steroid injection on organellae and polypeptide contents of soluble protein. Furthermore, the effects of three weeks interruption were also investigated.

Part of this report was presented at the 35th and 36th annual meetings of Physical Fitness and Sports Medicine in Japan.

MATERIALS AND METHODS

Twelve Wistar strain rats (two kinds both mother, three weeks old) were treated with dexamethasone (0.1mg/ml, Takeda Chemical Industries, Ltd., C_{22}H_{28}FNa_{2}O_{8}P., mol. wt.: 516.42) as a glucocorticoid at a dose of 0.1mg/kg of body weight/day in the buttocks and back alternately for 40 weeks after they were fed for a week under specified conditions. At the same time, food intake was measured.

After 20, 30 and 40 weeks, the rats were anesthetized, and the muscular tissue was completely and carefully dissected.

After removing the fasciae and tendons, the wet weight was determined. The lower limb muscles, the semimembranosus and rectus femoris muscles as proximal muscles and the tibialis anterior and gastrocnemius muscles as distal muscles were used. The weights of the heart, liver and adrenal glands were also ascertained.

The amount of soluble protein and its polypeptide contents in the muscles, liver and serum were measured by sodium dodecyl sulphate gel electrophoresis (Table 1).

The amounts and contents of polypeptide chains were analyzed by the dual-wavelength thin layer chromatography scanner (CS-900, Shimadzu Co.). These measurements and tests were also performed on the de-steroid group obtained by interrupting the steroid injections for three weeks after 20, 30 and 40 weeks.

RESULTS

In our earlier studies (20, 37, 38, 39), the decrease of body weight was significant in the steroid hormone treated group in comparison with the control group after five weeks of treatment. The changes of body weight and food intake of each group are shown in Fig. 1.

After 10 weeks of injection, the body weight in the steroid group showed a plateau, and its increase was slight (230-250g). On the other hand, the body weight of the control group kept increasing to about 580-600g at 40 weeks. Thus, that of the steroid group was only 40% to 60% that of the control group.

In the de-steroid group after 20, 30 and 40 weeks of treatment, the body weight tended to recover by 10 to 60g.

The daily food intake volume per body weight reversed at the fifth week, and, after this point, that of the steroid group remained at about
Table 1  The materials and methods used in this study.

<Materials>
- **Animals**: Wistar strain Rats (12 male Rats from same mother; 3 weeks old)
- **Muscles**: M. tibialis anterior, M. gastrocnemius, M. rectus femoris, M. semimembranosus
- **Organs**: Heart, Liver, Adrenal gland
- **Serum**: Human, Rats

<Methods>
1. **Injectioned**: The glucocorticoid dexamethasone (0.1 mg/ml) daily injection intramuscularly (0.1 mg/kg/day) for 20, 30 and 40 weeks (steroid G.) de-steroid G. (5w after interruption of steroid injections)
2. **Food intake**: daily food intake volume
3. **Fraction and determination of soluble protein**

\[
\begin{align*}
&\text{Muscles} \quad \text{Liver} \quad \text{\{wet weight\} } \rightarrow \text{homogenize} \rightarrow \text{centrifugation} \rightarrow \text{measured} \rightarrow \text{Electrophoresis} \\
&\quad \text{2000} \sim \text{3000} \quad \text{10000 rpm} \quad \text{rpm} \quad \times 30' \\
&\quad \times 5 \sim 10 \text{min} \quad (0 \sim 5\degree C) \\
&\quad (0 \sim 5\degree C) \quad +0.85\% \\
&\quad \text{physiol. saline} \\
&\text{Serum} \quad \text{Human; V. mediana cubiti} \quad \text{Rats; left ventricle} \quad \rightarrow \text{centrifugation} \\
&\quad \text{2500 rpm} \quad \times 25' \\
\end{align*}
\]

*Gel: gradient gel (Pharmacia PAA 4/30, 4.9 × 82 × 82mm)
*Electrophoresis Buffer: 0.04M tris, 0.02M sodium acetate, pH 7.4 with 2mM EDTA and 0.2% SDS
*Sample Buffer: 10mM tris-HCl pH 8.0, 1mM EDTA with SDS and β-Mercaptoethanol as indicated below
*Sample Treatment: HMW Calibration Kit Protein Mixture-1.0% SDS, 1.0% β-Mercaptoethanol, 60°C 15’
*Electrophoresis Conditions: Pre-electrophoresis (without samples); 70 volts for 1 hr

Apply Samples
Electrophoresis; 300 volts for 10 min.
Complete Electrophoresis; 150 volts for 5 or 16 hrs

*Fix Proteins and Remove SDS: Electrophoretically at 24 volts for 30 min in 50% isopropanol, 10% acetic acid

*Staining: Diffusion, overnight or 6 hrs in 0.02% Coomassie Blue R-250 (C_{48}H_{44}N_{3}NaO_{6}S_{2}), in 7% acetic acid or 30 min in 60 ~ 70°C

*Destaining: Electrophoretically at 24 volts for 2 hrs in 7% acetic acid

*Scanning: Dual-wavelength TLC Scanner, CS-900, Shimadzu Co.

*HMW Kit (Pharmacia Fine Chemical Co.)
Thyroglobulin mw 330,000; Ferritin mw 220,000; Albumin mw 67,000; Catalase mw 60,000; Lactate Dehydrogenase mw 36,000; Ferritin mw 18,500
20% more than that of the control.

Despite the fact that a greater volume of food intake per body weight was observed in the steroid group, the body weight was significantly decreased compared with the control group. This might be the effect of the steroid injection over such a long-term. Fig. 2 shows the changes in weight of organs and ratios to the control. The wet weight of the heart, liver and adrenal glands decreased by 40% to 50% as compared with the control group during each treatment period.

Fig. 1 Changes in body weight (top) and daily food intake (bottom). The open circles indicate the control group, the closed ones the steroid group and x the de-steroid group.

On the other hand, the weight per body weight was increased as compared with that of the control at each injection period. Clark et al. (2) have also observed an increase in the weight of the liver with a certain dosage of bethamethasone (0.3 mg/kg/day) administered for two weeks.

Although the body weight decreased significantly in the steroid group, the weight per body weight of the heart and liver increased. These results suggested that the organic damage was slight.

Fig. 3 shows the wet weight of the distal (upper part) and proximal (lower part) muscles. The reduction of the wet weight of each muscle was markedly in proportion to the length of the injection period. The ratio of decrease in the proximal muscles was higher than that in the distal muscles.
by approximately 10% to 15%.

However, the weight per body weight in the distal muscles tended to recover to the control level in the de-steroid group. On the contrary, this was not the case with the proximal muscles, especially the fast-twitch glycolytic semimembranosus muscle (22), which were decreased by 20% from the control muscles at each period.

In this study, muscle atrophy was obviously recognized in so-called type II muscle fibers and this finding is similar to those reported previously (18, 25, 29, 32, 34, 35).

Fig. 4 shows the soluble protein fractions of the serum as ascertained by SDS gradient gel electrophoresis at the 30th and 40th weeks of injection.

The fraction of the 40,000 to 60,000 molecular weight level tended to
decrease in the 40-week steroid injection group, but this was not the case in the de-steroid group. These results were related to the fact that the amount of total soluble protein of the steroid injection group decreased by 35% as compared to the control, but the ratio of decrease was slight in the de-steroid group.

Fig. 5 shows the soluble protein fraction of the semimembranosus muscle, which was the most significantly affected among the four muscles. The amount of total soluble protein of the serum was 5.6 g/dl at the 40th injection week, 60% of the control. In the de-steroid group, it was 9.2 g/dl, just the same as the control.

These soluble protein patterns were analyzed by the dual-wavelength TLC Scanner (CS-900, Shimadzu Co.) at least two times for each sample and the molecular weights of these fractions were calculated by an High Molecular Weight kit. Changes of polypeptide contents in the serum and the semimembranosus muscle on each period are shown in Fig. 6 and 7.

Fig. 3 The changes of the distal (top) and proximal (bottom) muscles as percent of control. Open bars represent the steroid group and shaded bars the de-steroid group.
Soluble Protein Fraction of Serum by SDS Gradient Gel Electrophoresis

Fig. 4 Soluble protein fraction in the serum as ascertained by SDS gradient gel electrophoresis at the 30th and 40th treatment week.

Soluble Protein Fraction of M. semimembranosus by SDS Gradient Gel Electrophoresis

Fig. 5 Soluble protein fraction in the semimembranosus muscle as ascertained by SDS gradient gel electrophoresis at 30th and 40th treatment week.
Fig. 6  The changes of soluble protein fraction in the serum were analyzed by the dual-wavelength analyzer at the 20th, 30th and 40th treatment week. The body weight and volume of total soluble protein (g/dl) are shown in the graph at the top for each treatment period. Solid lines show controls, broken lines the steroid group and dotted lines the de-steroid group.
Fig. 7  The changes of soluble protein fraction in the semimembranosus muscle. Control, steroid group and de-steroid group are shown in the same manner as in Fig. 6. Body weight (bar), muscle weight per body weight (circle) and total volume of soluble protein for each period are shown in the graph at the top.
The amount of total soluble protein had a slight tendency to decrease in the steroid injected group during each treatment period (Fig. 6).

There were no significant changes among the control, steroid and de-steroid groups at 20th and 30th treatment week. However, at the 40th treatment week, a slight decrease in the level of 40,000 to 60,000 molecular weight polypeptide chains was recognized in the steroid group, while in the de-steroid group, the polypeptide pattern was similar to the control (Fig. 6).

In the semimembranosus muscle (Fig. 7), a remarkable difference was found only at the 40th treatment week. With the exception of the 40th treatment week, there were no differences among the control, steroid and de-steroid groups in the pattern of the soluble protein fractions. In the steroid group at the 40th treatment week, the decrease in the level of the low-molecular-weight fraction as mentioned above was observed and an increase in the level of the high-molecular-weight fraction was noted as compared with the control. Although muscle wasting was observed in the de-steroid group as mentioned above and as show in Fig. 3, the pattern of the soluble protein fraction was no different from the control.

DISCUSSION

Many workers studying not only experimental animals such as rabbits, rats and mice, but also human beings, have reported that atrophy in the skeletal muscles induced directly or as a side effect of steroid treatment.

In fact, these effects vary according to the species of experimental animals and the type of steroid hormone.

Biochemical studies have shown increased protein breakdown and decreased protein synthesis (12, 15, 19, 27, 28, 31), a decrease of myofibrillar protein in type II muscle fiber (2), depressed actomyosin ATPase activity (25) and the inhibition of phosphorylase activity (29). Many of these results were obtained by the administration of large doses of steroid hormone during a 10-week treatment period.

In this study, small doses of steroid hormone (dexamethasone, 0.1 mg/kg/day) were administered for long-term periods of up to 40 weeks.

It is well known that the action of glucocorticoid hormone in vivo causes changes of the carbohydrate metabolism (16), and this result may be related to the increase in glycogenesis and glyconeogenesis (16, 24). Because of this, selective atrophy in type II muscle fiber is induced, but the details of the mechanism of this action have not yet been clarified.

Despite the fact that food intake/body weight/day in the steroid group was about 20% higher than that in the control group after the fifth treatment week, body weight was decreased by about 50% in the former as compared with the latter (Fig. 1). It is obvious that this characteristic change of body weight was due to systematic metabolism acceleration stemming from the physiological action of glucocorticoid (26), and, as a result, the content of adipose tissue in each organ declined in the steroid group.

Wet weight of the heart and liver in the steroid group was 20% to 40% less than in the control group at each period. However, these weights per body weight were more than the control, as mentioned above. These fin-
The findings suggest that steroid injection induced hypertrophy of the heart and liver, especially in these physiological functions. From the results, it was assumed that cardiac output and glycogenolytic action in the liver might be increased. The adrenal glands wet weight were decreased by more than 60% as compared with the control at 20th treatment week. The increase after the 30th treatment week is regarded as a compensatory adaptation to such long-term glucocorticoid administration. It is not clear why the weight increases or what the function of this increase is.

Previous studies have shown skeletal muscle wasting (9). In our study, muscle atrophy was recognized in the proximal and distal muscle after the 20th treatment week. However, this atrophy, except for that of the semi-membranosus muscle, was not significantly different from that seen in rabbit steroid myopathy (5, 10, 11, 29, 33, 35) up to the 30th treatment week.

The decrease of muscle weight per body weight is believed to be caused by a decrease in the amount of the connective or adipose tissue (38).

From the viewpoint of the changes in soluble protein fractions (Fig. 5, 7), especially the notable decrease of the mol. wt. 40,000 to 60,000 fraction, two points were assumed: that the damage to the muscle tissue was slight, and that the protein catabolism, glycogenesis and glycogenolysis were accelerated as a result of long-term steroid injection.

In fact, Grossie (10), Gruener (11) and Vignos et al. (35) have shown altered contractile proteins of the skeletal muscle in rabbits given glucocorticoid. Takács et al. (31) and Mayer et al. (17) reported that the reduction of protein synthesis and increase of protein breakdown caused the wasting of muscles.

The investigation of Simon et al. (28) has shown that muscle wasting in myopathies is associated with increased protein breakdown rather than a decrease in the rate of protein synthesis (13). The amount of extractable myofibrillar protein from type II muscle was decreased (2), and actomyosin ATPase activity was depressed (25).

On the functional changes, Vignos et al. (35) demonstrated that glucocorticoid treatment in rabbits caused a slower relaxation time in the fast-twitch glycolytic M. extensor digitorum longus while slow-twitch oxidative soleus (21) was unaffected. Subsequently, Gardiner et al. (8) reported similar findings.

From morphological studies, the accumulation of glycogen particles and an increase in lipid droplets were recognized in the glucocorticoid hormone injected group as opposed to the control group (24, 38). In addition to these results, rupture of mitochondrial cristae, swelling and enlarging tended to be more often observed as compared with the control (37, 38, 39). On the other hand, these specific results tended to recover to the control level within three weeks (de-steroid group) except in the 40-week treated group (40).

Therefore, based on our results and previous studies, it is believe that atrophy occurred as a result of long-term steroid injection at some site in the muscle fiber judging from the decrease of the muscle weight, although to prove this point further investigation is needed.
REFERENCES


