Myopathy resulted from the excessive long administration of Traimecinolone

H. K. Ulaiwi
College of Veterinary Medicine\ University of Al- Qadisyiah

Abstract

The study includes twenty four mice divided into two equal groups each group contain (12) animals, the first group represent the treated group which daily intraperitoealy injected with Traimecinolone at dose rate (0.5% mg/kg B.w) for 45 day, this group was subdivided according to days of killing of animals for histopathological study into three sub group (25 day group), (35 day group) and (45 day group) each group includes four animals. The second group represent control group which also subdivided to subgroups as in first group and daily injected with 0.5% normal saline. The blood was collected from the two groups for estimation of {Serum Creatinine (SC), Serum Aspartate transaminase (SAST) and Serum Alkaline Phosphatase (SALP)} after (25, 35 and 45 day) from the beginning of the study, then immediately after blood collecting four animals were killed at each periods for histopathological examination of muscle specimens. The conclusion were presence of significant differences between the concentration of SC of Traimecinolone group and control group after 35 and 45 day from the beginning of the study at ($P<0.05$), while the significant differences between the concentrations of SAST and SALP of Traimecinolone group and control group occur after 45 day from beginning of the study at ($P<0.05$), also the study revealed that the fibers of the muscle were swollen and have lost their striations and their nuclei and the muscle fibers are rounded and vary considerably in size, most relatively large(hypertrophic) and several much smaller (atrophic), and PAS(Periodic Acid Schiff) showed deposition of glycogen and glycogen storage myopathy with multiple subsarcolemmal vacuoles.

الاعتلال العضلي الناتج من الحقن طويل الأمد والمفرط لعلاج Traimecinolone

حسن خلف عليوي
كلية الطب البيطري/ جامعة القادسية

الخلاصة

شملت الدراسة 24 فأرة مختبرية وقسمت حيوانات التجربة إلى مجموعتين شملت المجموعة الأولى المجموعة المعاملة وعددها (12) فأراً والتي حقنت يومياً بعلاج Traimecinolone بجرعة o.5 mg/kg B.W ولمدة خمسة وأربعين يوماً والتي قسمت بدورها إلى ثلاثة مجاميع فرعية تضم كل مجموعة أربعة حيوانات بالاعتماد على الأيام التي تقتل فيه الحيوانات وهي (مجموعة 25يوماً) و(مجموعة خمسة وثلاثون يوماً) و(مجموعة 45يوماً) والمجموعة الثانية هي مجموعة السيطرة وعددها (12)فأراً ، والتي حقنت ولنفس الفترة وبنفس الجرعة بالمحلول الفسلجي الطبيعي

Normal saline والتي بدورها قسمت بنفس تقسيمات المجموعة الأولى، سحب الدم من حيوانات التجربة لتقييم مستويات (SC, SAST and ALP) في الفترا ت (25 و35 و45) تقتل المجموعة التي يتم سحب الدم منها في نفس اليوم لغرض الفحص النسيجي وتم وقد أخذت عينات من العضلات الهيكلية بعد التضحية بالحيوانات مباشرة قد وضعت في 10% فورمالين وبعد إجراء الطريقة الروتينية في تجفيف وترويق وطمر العينات صبغت بالهيماتوكسلين والايوسين وصبغة الـ PAS لتشخيص الكلايكوجين وفحصت باستخدام المجهر الضوئي.وقد خرجت الدراسة بالاستنتاجات التالية: تجمع الألياف الكبيرة في منطقة واحدة، وكذلك لوحظ وجود اختلاف في أحجام الألياف العضلية بين فرط التنسج وقلة التنسج وكما بينت الدراسة وجود اعتلال في العضلة و حالات شدة تقلص وكذلك تبين وجود فقدان في خطوط العضلات وقد أوضحت صبغة PAS وجود زيادة بنسبة ترسب الكلايكوجين في العضلات الهيكلية.

Introduction

Glucocorticoids are the most potent immunosuppressive and anti-inflammatory drugs and have been widely used in the treatment of autoimmune diseases. Glucocorticoid-associated adverse effects commonly develop and include hypertension (88%), Cushingoid features (66%), adrenal suppression (56%), myopathy (50%), osteopenia (46%), growth retardation (39%), obesity and hypercholesterolemia (30%), and cataracts (14%) (1). Several clinical studies have reported the occurrence of steroid-induced myopathy in intensive care unit patients after administration of high doses of glucocorticoids with or without neuromuscular junction blocking agents (2,3). Iatrogenic steroids, especially the 9-α-fluorinated ones like triamcinolone, betamethasone, or dexamethasone, can cause dose-dependent muscle wasting and weakness within weeks (4). This can be ameliorated by limitation of the steroid dose, alternate-day use, attention to exercise, and a high-protein diet. Electromyography shows myopathic features, whereas muscle biopsy reveals myopathy with selective loss of thick myosin filaments (rhabdomyolysis), necrosis, and atrophy of type II fibers (5,6).

Myopathies are diseases of skeletal muscle which are not caused by nerve disorders. These diseases cause the skeletal or voluntary muscles to become weak or wasted. There are many different types of myopathies, some of which are inherited, some inflammatory, and some caused by endocrine problems. Myopathies are rare and not usually fatal. Typically, effects are mild, largely causing muscle weakness and movement problems, and many are transitory. However, muscular dystrophy (which is technically a form of myopathy) is far more severe. Large groups of atrophic, denervated muscle fibers. Many hypertrophic fibers are also present. Reinnervation changes with fiber type grouping. Atrophic fibers are strongly over reactive for nonspecific esterase (7). The alteration in the fiber size, between atrophy and hypertrophy, in case of muscular dystrophic and myositis is due to block of the myostatin pathway which is a negative regulator of muscle growth; knockout of the myostatin gene or inactivation of its receptors result in increase muscle mass as well as muscular dystrophic mechanism may involve a delay in myofibers maturation and lack of hypertrophic response (8).Other myopathic findings include presence of different inclusions, vacuoles, glycogen or lipid storage or accumulations of abnormal material. Different patterns of inflammatory cell collections are associated with inflammatory myopathies (9).

Materials and Methods

This study included twenty four mice divided into two group, the first represent the treated group included twelve mice which were treated with Traimecinolone at dose rate (0.5 mg/Kg B.W) by daily intraperitoneal injection (I.P) for 45 days this group is subdivided according to day of killing into three groups, first subgroup represent the mice that killed after 25day from beginning of the study, second subgroup represent mice that killed after 35 day from the beginning of the study and the third subgroup represent mice that killed after 45 day from beginning of the study , each subgroup included four animals. The second group represented the control group which injected with normal saline at the same dose and subdivided as the first group above. The blood was collected from the animals for the estimation of serum Creatinine (SC), serum aspartate Transaminase (SAST) and Serum alkaline Phosphatase (SALP) immediately after blood collection the animals were killed for histopathological studies of (2 cm) specimens of skeletal muscles.

- **Estimation of Serum Creatinine (SC), SAST and SALP:-**

The SC was estimated by using Jeffers methods. (10). While SAST was estimated by using special kit prepared by (SPINREACT, S.A.Ctra.Santa Coloma, 7E-17176SANT ESTEVE DE BAS (GI) SPIN), and the SALP was estimated by using special kit prepared by.

- **Histopathological Procedures:-**

Muscle samples are taken at periods of (25, 35 and 45 day) and studied by using routine histological technique and staining of 5 μm with H&E, also PAS(Periodic Acid Schiff) Stain for carbohydrate staining was used and then examined by light microscope. (11, 12).

- **Statistical Analysis:-**

The data were expressed as mean ±standard deviation (SD) and analyzed using analysis of variance (ANOVA). Least significant difference (LSD) was used to test for differences among means for ANOVA indicated a significant ($P<0.05$), using computerized SPSS.

Results

The results showed that after 25 day from the beginning of the study there were no differences between the serum Creatinine concentrations in the treated and control groups, had the total average value of 1.05±0.30 mg/100 ml. the concentrations slightly increased in the treated group (3.37±1.77) but still at the normal range in the control group (1.05±0.19) after 35 day from the beginning of the study, while there are increased in the concentration of the treated group (9.15±4.03) after 45 day from beginning of the study , the concentration of the control group remain at the normal range (1.00±0.24). The result revealed that there are significant differences between the treated group and control group at ($P<0.05$) at period of 45 day (Fig.1 and Table 1).

The result revealed that after 25 day from the beginning of the study there are no differences between the serum AST concentrations in treated (10.95±2.59) and control groups, had the total average value of (5.58±1.59). After 35 day there is an elevation in the concentrations of the SAST in the treated group (21.9±7.62) while the concentrations in the control group were (5.33±1.50). After 45 day the concentrations in the treated group still elevated (32.57±15.27) as compared with control group (6.50±2.12). the result revealed that

there are a significant differences between the concentrations of SAST after 45 days and other periods of study in the treated group at (P<0.05) (Fig.2 and Table 1).

The result showed that the there are a significant differences between the concentrations of SALP of treated group () and control group () and in the treated group the concentrations in this period significantly differs from other periods at (P<0.05) (Fig.3 and Table 1).

- **Results of histopathological study:-**

It was clearly demonstrated that *triamcinolone* treatment resulted in loss of body and muscle mass. At 25 day, the changes were clustering of the largest fibers (Fig.4), variability of fiber size from smaller (atrophic) to most relatively large (hypertrophy), degenerating., hypercontraction of the myofibers and loss of muscle striations, on hematoxylin-eosin stain. At 35 day, there were two necrotic myofibers, characterized by densely eosinophilic staining, were shown. There were several muscle fibers. One of the fibers had lost its eosinophilic myofibrils, however, and is now a slender basophilic fiber which was attempting to regenerate. Other fibers are swollen and have lost their striations and their nuclei (Fig.5). At 45 day, the result revealed that the muscle fibers are rounded and vary considerably in size, most relatively large and several other are much smaller (Fig. 6). Multiple subsarcolemmal vacuoles containing PAS-positive material.

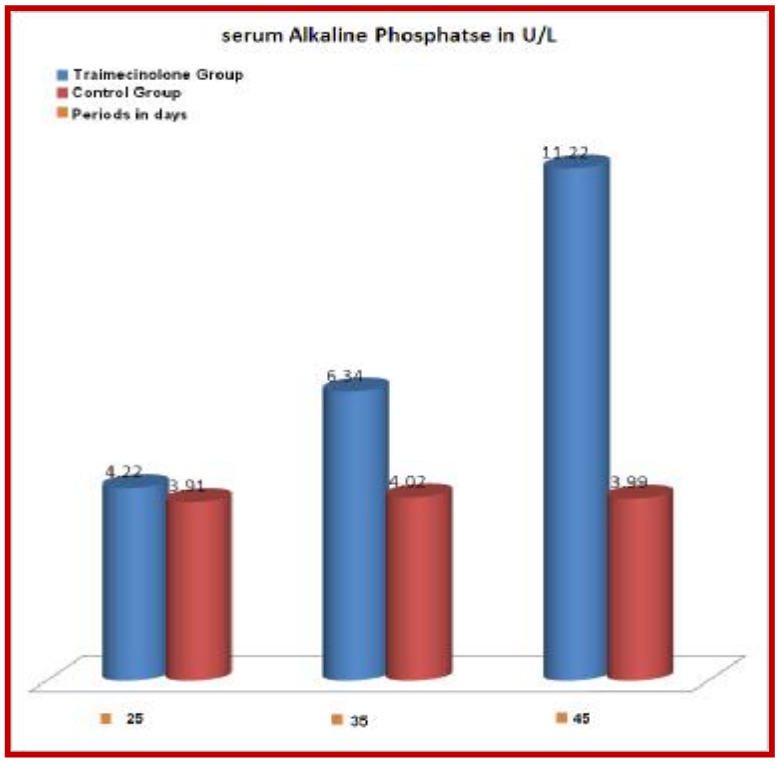

Fig. 1

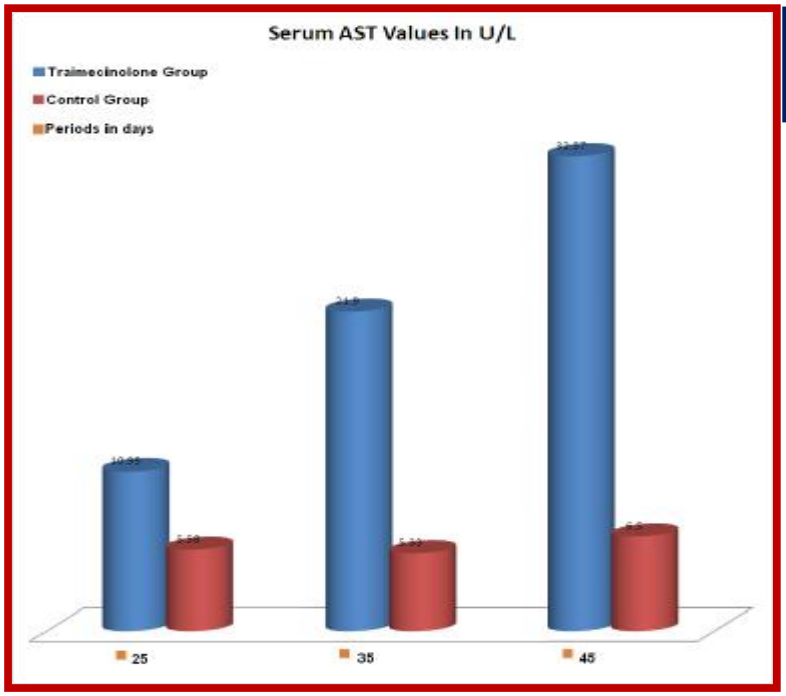
Serum AST Values In U/L
Trainecinolone Group
Control Group
Periods in days
25
35
45

Fig. 2

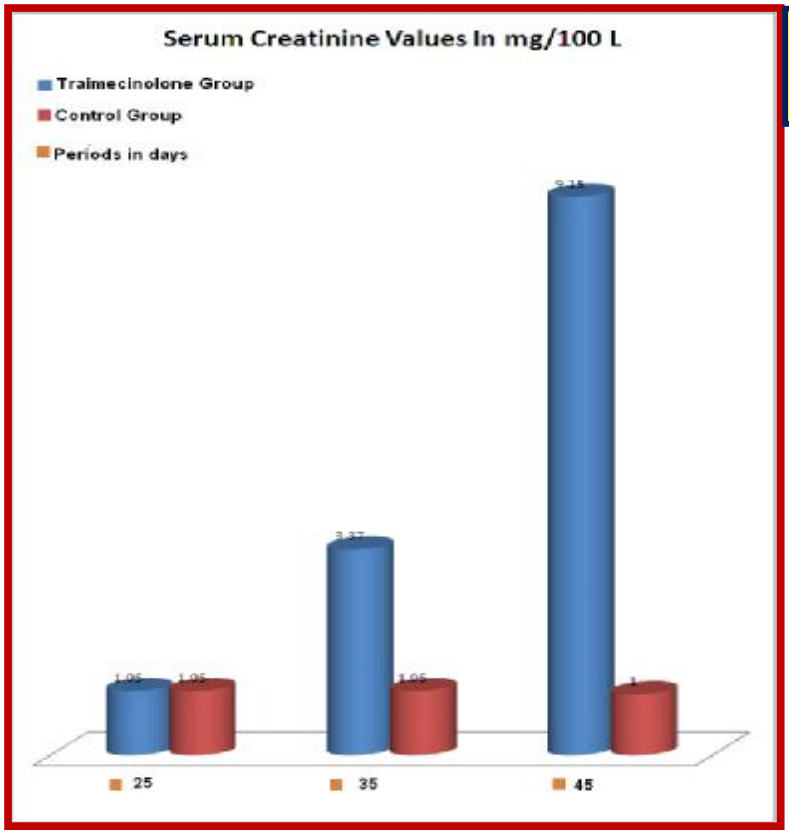
Serum Creatinine Values In mg/100 L
Trainecinolone Group
Control Group
Periods in days
25
35
45

Fig. 3

Table (1) The difference between the values of SC, SAST and SALP of Traimecinolone group and Control group during the different periods of study

D / G	25			35			45		
	SC	SAST	SALP	SC	SAST	SALP	SC	SAST	SALP
Traimecinolone G.	1.05± 0.10 Aa	10.95± 2.59 Aa	4.22± 0.3 Aa	3.37± 1.77 Bb	21.9± 7.62 Aa	6.34± 0.38 Aa	9.15± 4.03 Cc	32.57± 15.27 Bb	11.22± 0.76 Bb
Control G.	1.05± 0.30 Aa	5.58± 1.59 Aa	3.91± 0.26 Aa	1.05± 0.19 Aa	5.33± 1.50 Aa	4.02± 0.26 Aa	1.00± 0.24 Aa	6.50± 2.12 Aa	3.99± 0.3 Aa

- Result represent Mean ± SD at P<0.05 level.
- The capital letter refers to the significant difference at level (P<0.05) between the values of the same group.
- The small letter refers to the significant difference at level (P<0.05) between the values the different groups.

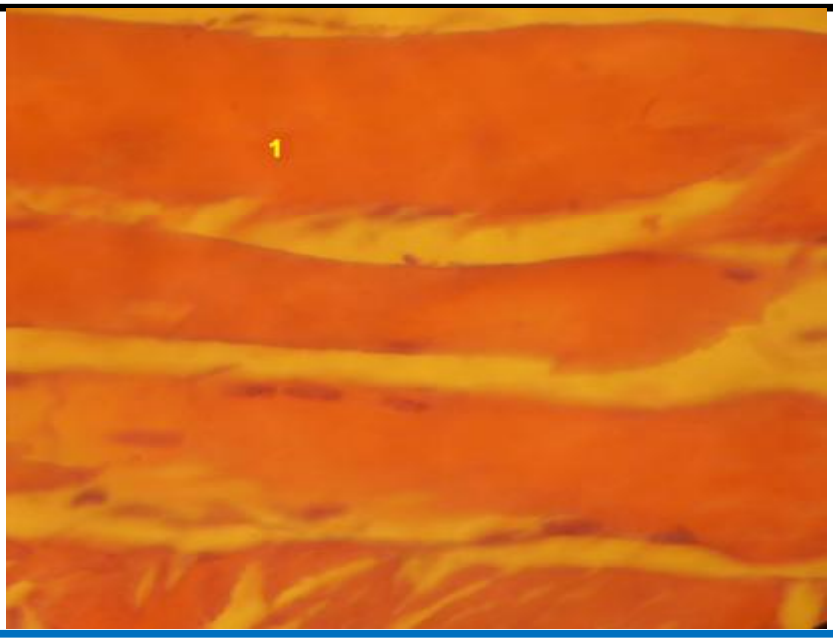

Fig (4)The fibers of the muscle in this field are swollen and have lost their striations and their nuclei (1). (400X H&E stain)

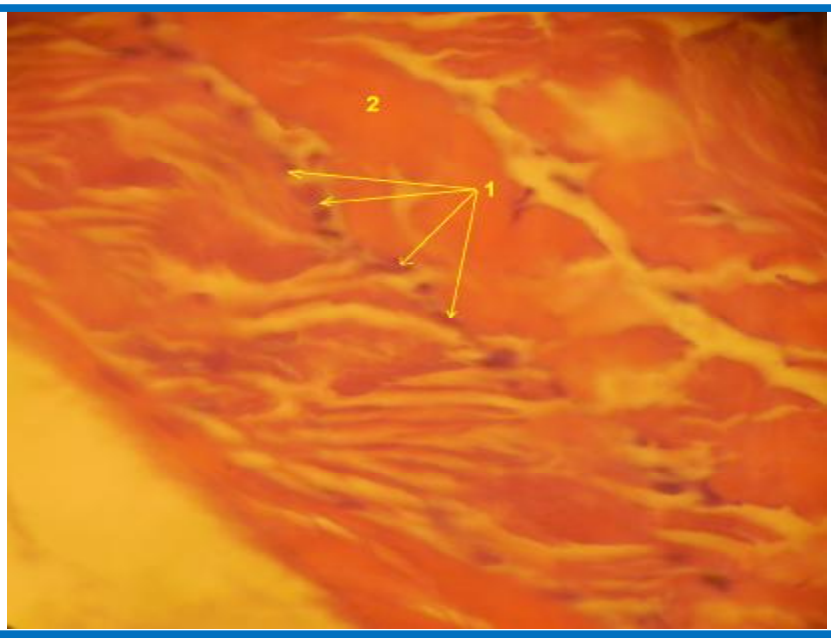

Fig. (5) Several muscle fibers. One of the fibers has lost its eosinophilic myofibrils however and is now a slender basophilic fiber that is attempting to regenerate (1). Other fibers are swollen and have lost their striations and their nuclei (2). (400X H&E stain).

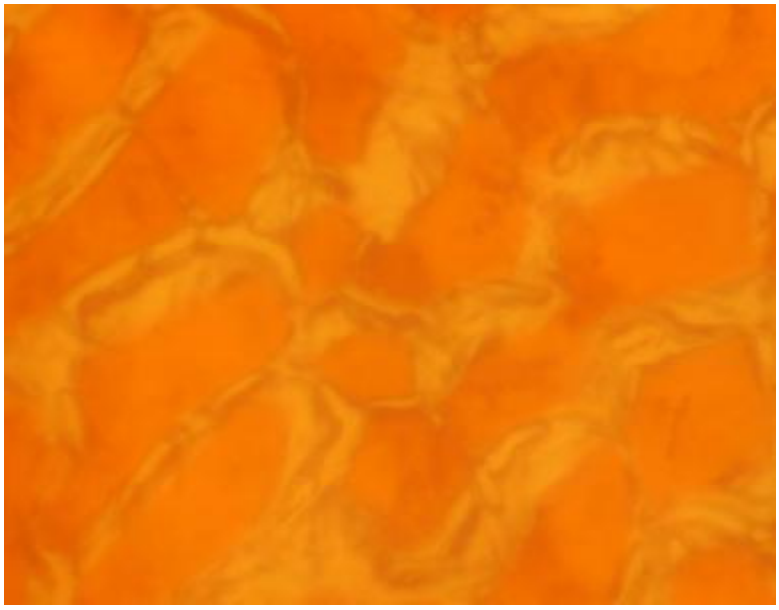

Fig.(6) The muscle fibers are rounded and vary considerably in size, most relatively large and several much smaller. (400X H&E stain)

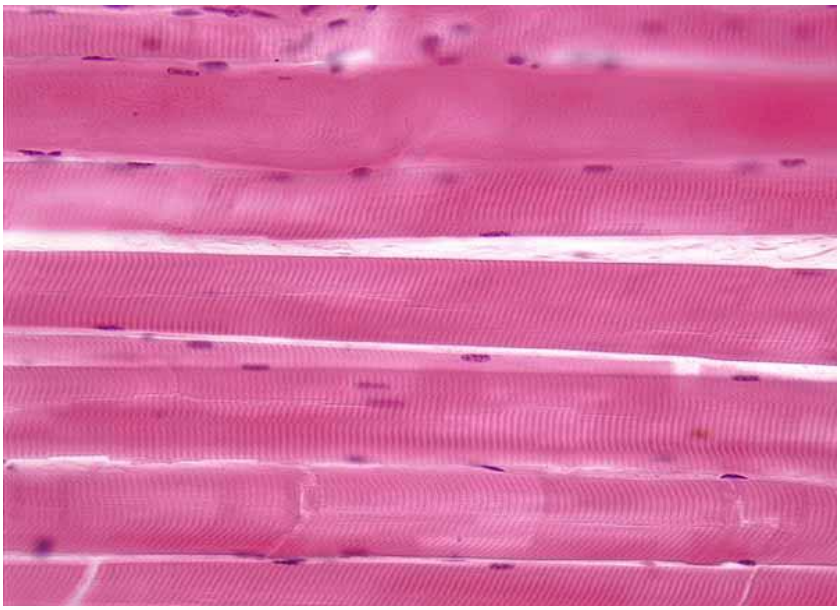

Fig. (7) Normal muscle, nuclei are usually located along the margin of the fibers. Sarcomere length (distance from one striation to the next) varies with state of contraction. (400X H&E stain)

Discussion

The action of corticosteroids is mediated by glucocorticoid receptors (GR). Two functionally distinct receptor forms αand ß have been demonstrated in human tissues (13). The ß GR form is considered as a dominant negative inhibitor of the αform (14). Several studies have shown that the αGR is down regulated in lung cells and peripheral lymphocytes after exposure to corticosteroids (15). However, little is known about the autoregulation of the ß form. In rats, no information is available about the existence of the ß GR in skeletal muscles. Both the αand ß glucocorticoids receptor mRNA were found to be down regulated in the muscle even after corticosteroid inhalation, suggesting a systemic effect in the peripheral skeletal muscle after this local corticosteroid administration. It is to be expected that similar effects could be found after systemic administration, but further research is needed to study GR mRNA expression under the Knutsson se conditions (16). Bissonnette et al., (17), observed increased glycogen levels after cortisone treatment. However, our study found increased glycogen stores in the peripheral skeletal muscle after *triamcinolone*. This increased muscle glycogen concentration did not seem to be related to alterations in muscle function. An increased glucose influx was observed in the hindquarter muscles after *triamcinolone* treatment. This increased glucose muscle influx is very surprisingly, since other studies have demonstrated a decreased glucose uptake by the muscle after dexamethasone treatment (18). However, the increased glucose muscle influx after *triamcinolone* administration could very well contribute to the increased muscle performance of the peripheral skeletal muscle. Our Histopathological study of skeletal muscle reveal pronounced fiber size variation associated with atrophy and hypertrophy, endomysial and perimysial fibrosis, internalization of nuclei, marked hypercontraction, and segmental necrosis of muscle fibers with phagocytosis and regeneration (basophilic fibers), this is agreed with, (19, 20). Muscle derived enzymes (CK, AST, ALP) are elevated representing damage to the muscle either through reduction in vascular supply or by direct immunogenic cytotoxicity. The CK (creatine kinase) elevation is usually in the range of several hundred, versus the much higher elevations in muscular dystrophies (21). A muscle biopsy is beneficial in establishing the definitive diagnosis. It may not provide a specific diagnosis, if the muscle biopsied is not significantly affected by the disease process or if it not obtained appropriately (22). The quadriceps or biceps are typically biopsied in accessing proximal muscle weakness. The muscle histopathology shows infiltration with inflammatory cells, perifascicular atrophy, and capillary necrosis (23)**.** Many etiologies can cause the breakdown and necrosis of muscle, leading to the "spilling" of muscle enzymes into the circulation, including CK and myoglobin. Possible etiologies of the patient's presenting symptoms include trauma/compression, exercise, metabolic myopathies including glycogen or lipid storage diseases, certain drugs or toxins, infection, endocrine disorders, and inflammatory myopathies including dermatomyositis and polymyositis (24) as sources of rhabdomyolysis; amyotrophic lateral sclerosis, muscular dystrophy, metabolic myopathy, and inflammatory myopathy as a source of proximal muscle weakness (25). Serum muscle enzymes, including CK, LDH, AST, and ALT, are elevated, usually all at once. As I alluded to before, each presents with symmetric proximal muscle weakness (26). Braund et al., (12) reported a degenerative myopathy in two female Bouvier des Flandres dogs and a Clinical signs were observed when dogs were about 2 years of age. Signs included regurgitation, exercise intolerance, generalized muscle atrophy, weakness, and a

peculiar paddling gait characterized by overextension of the paws when walking. Cranial nerve function, postural reaction testing, and segmental spinal reflexes were normal. Contrast studies reveal megaesophagus. Serum creatine kinase (CK) levels were markedly elevated. Other hematological and blood chemistry values were normal. Muscle changes were characterized by moderate to pronounced fiber size variation associated with atrophic and hypertrophic fibers of histochemical types (types 1 and 2), occasional giant-sized fibers with a whorled internal architecture and clefts, numerous internalized nuclei, multifocal necrosis, variable phagocytosis, basophilia, and marked increase in perimysial and endomysial fibrosis. These changes were seen in both limb and esophageal muscle samples. Peripheral and intramuscular nerves were normal. Muscle biopsy samples taken from two clinically normal related dogs showed similar but less severe histopathological changes. Prognosis is guarded to poor since the disease appears to progress rapidly. Corticosteroids given to one dog had no clinical effect. The clinical signs, elevated CK (creatine kinas) levels, and muscle pathology are similar to those seen in some dog withmmuscular dystrophy (27).

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