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Glucocorticoid-Induced Glycogen Increases in Extensor Digitorum Longus and Soleus Grafts in the Rat (42602)

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Abstract. The purpose of the present study was to compare dexamethasone-induced glycogen increases in normal EDL and SOL muscles with that in free muscle grafts. Glycogen in mature EDL and SOL grafts in the rat equalled control concentrations irrespective of whether the graft was a nerve-intact (NI), nerve-crushed (NC), reimplanted, or cross-transplanted graft. The grafts also possessed the glycogen-regulatory mechanisms to respond to the glucocorticoid dexamethasone (DEX), which increases muscle glycogen. The increase in glycogen induced by DEX in the EDL and SOL grafts resembled that of the EDL and SOL muscles, respectively, whether the grafted muscle was originally an EDL or SOL. DEX induced an approximate twofold increase in glycogen concentration in control muscles and nerve-intact SOL grafts, and a smaller but significant increase in all other free grafts. Nerve crushing prior to grafting resulted in no significant change in muscle weight, glycogen concentration, or DEX-induced glycogen increase in these grafts. The data suggest that skeletal muscle grafts are qualitatively similar to normal muscles in terms of metabolic responsiveness to hormones. Leaving the nerve intact during grafting quantitatively enhances the graft's hormonal sensitivity but the technique of nerve crushing prior to grafting has no such effect.

Substantial research has been done on mammalian skeletal muscle transplantation and regeneration (1–6), and the ability of mammalian skeletal muscle to regenerate in free grafts is well established (7). Much of this research examined histological changes. Relatively little work, however, has focused on biochemical characteristics, such as glycogen metabolism, of regenerated muscle grafts. Glycogen is a substrate for energy during contraction in all types of muscle fibers (8) and changes in glycogen during degeneration and regeneration would reflect the sensitivity of glycogen to the various physiological conditions of muscle grafts. Muscle glycogen decreases much earlier than the loss in weight or strength (9). Conversely, the onset of functional reinnervation is associated with a rapid and almost complete recovery of muscle glycogen which occurs sooner than the recovery of mass and strength (9, 10). In free muscle grafts of rats, glycogen usually decreases to a minimum on the fifth postoperative day, when degeneration of muscle fibers is maximal, and only 3 or 4% of the original fibers in the periphery of the graft survive (3, 10). Muscle glycogen increases as the graft regenerates. In rats, glycogen concentration is restored to its final value in approximately 3 weeks in SOL grafts (cross-transplanted EDL) and in approximately 6 weeks in EDL grafts (cross-transplanted SOL) (10). Glycogen concentrations of mature (60-day) EDL and SOL grafts were found to be near normal (86–89% of control values) (11). This suggests that glycogen metabolism has returned to normal in mature free grafts. Monitoring glycogen concentration in muscle grafts, following the administration of hormones or muscle contraction, provides an indication of the extent of regeneration that has occurred in the grafts' metabolic integrity.

Glucocorticoids are hormones that can affect glycogen levels. Glucocorticoids induce an early rise in blood sugar, followed by increases in liver, heart, and muscle glycogen (12–15). The precise mechanisms by which glucocorticoids induce increases in muscle glycogen are unclear. Muscle glycogen in EDL and SOL muscles of rats peaks about 16 hr after being injected with dexamethasone (DEX), a synthetic glucocorticoid (15). The effect of DEX on regenerated muscle grafts, however, has not been investigated. The purpose of the current project was to compare DEX-induced glycogen increases in normal EDL and SOL muscles with that in free muscle grafts, including

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nerve-intact grafts (NI) and grafts of muscles whose nerves were previously crushed (NC) to facilitate reinnervation.

Predenervation was originally thought to be the key to the success of free muscle grafts (4). It reduces the muscle to the "plastic state" (16) and increases the number of satellite cells which are presumably the myogenic cells (3, 17). Predenervation by sectioning or crushing the nerve reduces the size of muscle fibers, and increases the number of surviving fibers from approximately 3% to approximately 30% in muscle grafts (3). There is a more rapid breakdown and removal of muscle fibers (about a day earlier) in the early stages of regeneration (1–3, 18, 19). It has also been shown that crushing the sciatic nerve 2 weeks before excision resulted in a 27% increase in the axon elongation rate (20). It appears that nerve crushing primes both nerve and muscle fibers for regeneration. Starting at about 10 days after grafting, however, a difference in the rate of regeneration between predenervated and normal free grafts, as studied histologically, is not evident (3, 19). Predenervation does not improve the ultimate size and contractile properties of muscle grafts (2, 3). However, the effects of predenervation on the metabolic capabilities of muscle grafts have not been investigated.

Since successful restoration of muscle grafts depends on both revascularization and reinnervation, the relative contribution of each factor must be evaluated. Comparing NI grafts with free grafts allows examination of the contribution of innervation to graft regeneration. In NI grafts, the terminal parts of the nerve fibers and over 70% of the muscle fibers degenerate because of ischemia (3). Neuromuscular connections are restored within 7 or 8 days in the rat (3). The weight and contractile properties of NI grafts are restored to 90% or more of control values (21) whereas they are only about 50% of normal values in free grafts. This indicates that revascularization is not a great impeding factor in small muscle grafts (4). It is therefore suspected that NI grafts might show a better biochemical integrity than standard free grafts.

Materials and Methods. Seventy-two male Sprague–Dawley rats (Charles River Co.) (150–200 g) were housed two per cage, fed *ad libitum,* and exposed to a 12-hr lighting cycle. They were assigned to five groups: reimplantation (R), cross-transplantation (XT), nerve-crushed reimplantation (NCR), nerve-crushed cross-transplantation (NCXT), and nerve-intact SOL reimplantation (NIR). The rats were anesthetized with sodium pentobarbital (1 mg/100 g body wt) during all surgical procedures. Reimplantation results in grafts exposed during regeneration to the same muscle bed and neural influences as the original muscle. With cross-transplantation the regenerating grafts are reinnervated by nerves with a different pattern of activity than the original muscles had experienced (19). Nerve crushing was used to see if this procedure, typically used to prime nerve and muscle fibers for grafting, would influence the metabolic machinery of grafts as reflected in glycogen changes. Nerve-intact grafts were included to see if the grafts' biochemical integrity would be enhanced by this procedure as are the contractile aspects of the muscle (21).

The right sciatic nerve of the NCR and NCXT groups was crushed 14 days prior to grafting. The sciatic nerve was lifted up gently, and then carefully crushed (with moderate pressure) with forceps (1 mm in diameter) for 5 sec. For grafting, the proximal and distal tendons of the EDL and SOL muscles were transected. All vessels and nerves entering the muscle were severed as close to the muscle as possible. EDL and SOL muscles were reimplanted into their own beds in the R and NCR groups, and cross-transplanted into each other's beds in the XT and NCXT groups. The grafts were sutured to the tendon stumps left *in situ.* No attempt was made to reestablish neural or vascular connections between the graft and the host. Though it may be difficult for spontaneous reinnervation to occur in soleus transplants because of possible nerve retraction (22), the procedures used in the present study resulted in both SOL and EDL grafts being effectively innervated. This is evidenced by decreases in glycogen in such SOL and EDL grafts during running, indicating their innervation and active participation in exercise (23). Schmalbruch (24) also observed reinnervation in 60-day-old soleus grafts with no special care taken to ensure contact between nerve and graft. In the NIR group the right SOL muscle was rotated 180° clockwise and reimplanted into its own bed with its innervation left intact.

The EDL in this group was not used because of the difficulty of leaving its nerve intact during rotation and reimplantation. Muscles in the left leg were left intact and served as normal controls in all rats.

Sixty days after grafting, about half of the rats from each group were randomly chosen to be injected intraperitoneally with 400 μg (0.1 ml) of DEX (dexamethasone sodium phosphate, Kodak Co.) at approximately 3:00 to 5:00 PM. The rest of the rats received 0.1 ml of control solution, which had the same composition as the DEX solution except for the exclusion of the dexamethasone sodium phosphate. The rats were sacrificed 17 hr later (between 8:00 and 10:00 AM). Rats were anesthetized with pentobarbital during the excision of muscles. The EDL and SOL grafts together with their contralateral counterparts were removed, blotted dry, and then quickly frozen between slabs of dry ice wrapped with aluminum foil. Each muscle was then weighed and its glycogen concentration was determined by the anthrone method (25).

Mean values and standard errors for muscle weight and glycogen concentration were determined by the method of least squares. Differences between means were compared using modified t tests. Values were considered to be significantly different if $P < 0.05$. The statistical analysis of data took into consideration the degree of pairing that existed when comparing experimental and control muscles in the same rat and the degree of nonpairing that occurred when comparing different treatments (e.g., free vs NI grafts) done on different groups of rats.

Results. Values for EDL and SOL muscle weights and graft weights are shown in Table I. The average weight of the grafts was approximately 40% of the control values except with the NI SOL grafts which weighed approximately 66% of the control values.

The EDL muscle, being composed primarily of fast-twitch glycolytic (FG) and fast-twitch oxidative glycolytic (FOG) fibers, has a significantly ($P < 0.05$) higher glycogen concentration than the SOL muscle which is composed primarily of slow twitch fibers (Figs. 1 and 2). Glycogen concentrations (μg/mg muscle wt) in all EDL and SOL grafts returned to control values 60 days after transplantation, irrespective of whether the grafts were freely reimplanted or cross-transplanted, nerve-intact or nerve-crushed (Figs. 1 and 2).

Muscle glycogen increased approximately twofold in normal EDL and SOL muscles after DEX injection (Figs. 1 and 2). The DEX responses in the EDL and SOL grafts resembled those of the EDL and SOL muscles, respectively. The response, however, was generally not as high as in normal muscles, except in the NIR SOL group which has a glycogen increase similar to that of normal SOL muscle (Fig. 2). Thus the DEX response in the SOL muscle, when cross-transplanted into the EDL bed, was transformed to that of the EDL and vice versa. There was no significant difference in the glycogen content of the EDL and SOL grafts between the NC and non-NC groups. This implies that nerve crushing did not improve the DEX response.

Discussion. Previous studies have shown that glycogen concentration in mature cross-

TABLE I. MEAN WEIGHTS (g) OF EDL AND SOL MUSCLES AND GRAFTS HAVING RECEIVED (DEX) OR NOT RECEIVED (NO DEX) DEXAMETHASONE

	EDL		SOL	
	DEX	No DEX	DEX	No DEX
C	204.3 ± 4.4	204.8 ± 5.4	162.5 ± 4.4	162.5 ± 5.4
R	55.6 ± 19.3	103.6 ± 16.0	50.8 ± 19.3	68.3 ± 19.5
XT	102.9 ± 16.1	98.6 ± 15.1	60.6 ± 17.0	81.8 ± 15.1
NCR	62.2 ± 19.3	64.1 ± 19.5	62.5 ± 19.3	79.1 ± 21.3
NCXT	71.2 ± 19.3	86.0 ± 18.0	55.6 ± 19.3	75.1 ± 19.5
NIR			123.8 ± 20.9	93.3 ± 21.3

Note. All values are means ± SE. Muscle weights were significantly different for each transplanted group, except for the NIR (DEX) group, as compared to the control group ($P < 0.05$). C, control group; R, reimplantation group; XT, cross-transplantation group; NCR, nerve-crushed reimplantation group; NCXT, nerve-crushed cross-transplantation group; NIR, nerve-intact SOL reimplantation group.

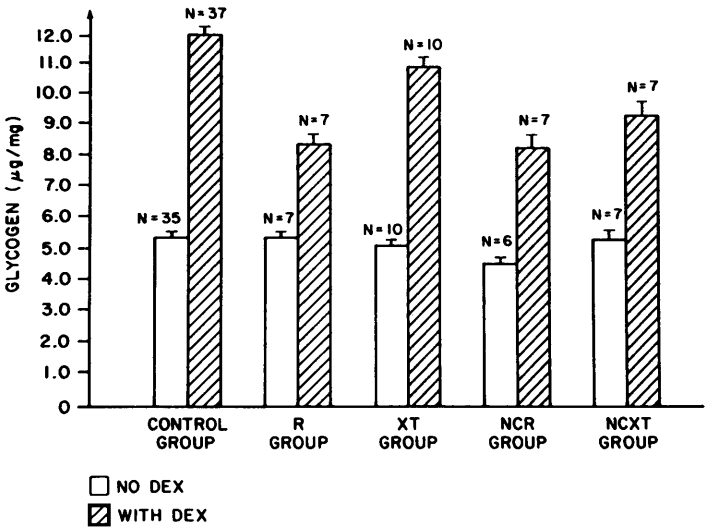

FIG. 1. Groups were normal EDL muscles (Control), EDL reimplanted into its own bed (R), SOL cross-transplanted into the EDL bed (XT), EDL reimplanted into its own bed 14 days after sciatic nerve crushing (NCR), and SOL cross-transplanted into the EDL bed 14 days after sciatic nerve crushing (NCXT).

transplanted EDL and SOL grafts in rats is restored to near normal values (10, 11). Many studies have demonstrated a conversion in the contractile, histochemical, and biochemical properties in cross-transplanted muscles (7, 10, 26, 27). SOL muscles grafted into the bed of the EDL demonstrated a virtually complete conversion in contractile properties, whereas the transformation is less marked in the cross-transplanted EDL (SOL graft) (26, 27). In spite of the incomplete conversion of muscles in cross-transplanted grafts, results from the present study show that the glycogen concentrations of all muscles grafted into the EDL and SOL beds are not significantly different from the normal values of 5.5 ± 0.15 μg/mg

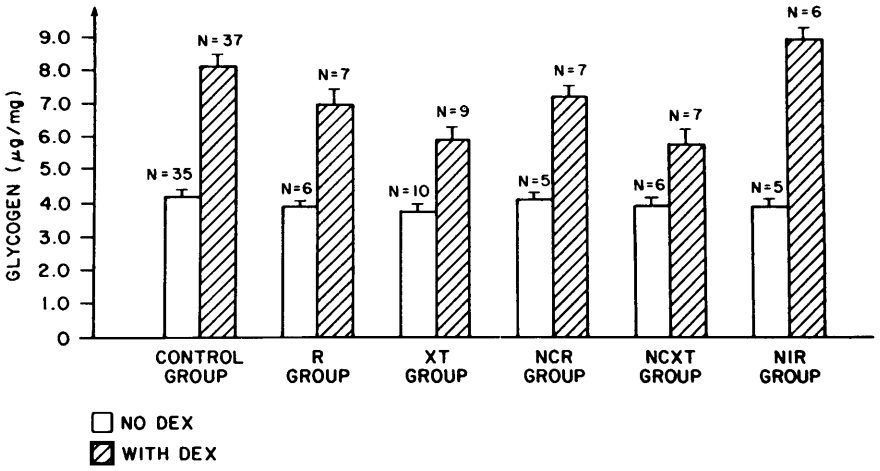

FIG. 2. Groups were normal SOL muscles (Control), SOL reimplanted into its own bed (R), EDL cross-transplanted into the SOL bed (XT), SOL reimplanted into its own bed 14 days after sciatic nerve crushing (NCR), EDL cross-transplanted into the SOL bed 14 days after sciatic nerve crushing (NCXT), and SOL reimplanted into its own bed with the nerve left intact (NIR).

EDL muscle weight and 4.3 ± 0.15 μg/mg SOL muscle weight, respectively. This is true regardless of whether the grafted tissue was originally an EDL or SOL. As far as muscle glycogen is concerned, these results exhibit a full restoration in the EDL and SOL autografts and a complete conversion in the EDL and SOL cross-transplants.

It is known that glucocorticoids such as DEX induce glycogen increases in skeletal muscles (25). Results from the present study demonstrate that DEX induces a glycogen increase in NI, NC, and free muscle grafts as well as in normal skeletal muscle. The response of EDL and SOL grafts resembles that of normal EDL and SOL muscles, respectively. NI grafts showed an approximately twofold increase in glycogen, similar to that in normal muscle. Standard free grafts or cross-transplants also responded to DEX, although the glycogen increase was generally lower than that in control muscles. These data indicate that mature grafts have regenerated the metabolic capacity to respond to DEX, resulting in increases in glycogen. The lower response in free grafts may be associated with reduced reinnervation and revascularization. It may also be due to reduced activity of the key enzymes involved in the DEX response. NI grafts have been shown to be nearly normal in their weight and contractile properties when compared with the less well-reinnervated free grafts (3). It was shown in the present study that NI grafts also respond normally to DEX.

It has been reported that predenervation does not result in long-term improvement in the size or functional properties of muscle grafts weighing less than 6 g (3, 4). The DEX response in NC grafts in the present study is likewise not significantly different from normal free grafts. It is apparent that predenervation is unnecessary in small muscle grafts and is contraindicated since it requires an extra operation.

The precise mechanisms by which glucocorticoids induce glycogen increase in skeletal muscle is not known (28, 29). Glucocorticoids may act directly on the rate-limiting enzymes, glycogen synthetase and phosphorylase, or on other systems involved in the regulation of these enzymes (13, 30–32). Glucocorticoids may induce the activation of other hormones such as insulin which in turn act on the glycogen regulatory system (33, 34). Glucocorticoids cause massive protein degradation in skeletal muscle and increase free fatty acid oxidation, thus decreasing glucose utilization. This may lead to the subsequent sparing of muscle glycogen (35, 36). Regardless of which mechanisms are involved, mature muscle grafts have regenerated the necessary components in the glycogen regulatory pathways with respect to the DEX response. The response or sensitivity of muscle grafts to other substances such as epinephrine has also been studied (10). Results showed that cross-transplanted EDL and SOL grafts were able to respond to the glycogenolytic effect of epinephrine as early as 10 days after transplantation (10). As far as the glucocorticoid response is concerned, exactly when muscle grafts lose the response, if they ever do, or when they start to regain the response has not been investigated.

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