Studies on the utilization and mobilization of lipid in skeletal muscles from streptozotocin-diabetic and control rats

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Abstract Several aspects of lipid metabolism in the soleus and diaphragm muscles of streptozotocin-diabetic and control rats were investigated. The triglyceride content of both muscles was elevated in the diabetic state and the presence of increased intracellular lipid was confirmed by electron microscopy. In vitro glucose and palmitate oxidation studies showed that both types of muscle from the diabetic animals metabolized more fat than did the soleus and diaphragm from control rats. While isoproterenol alone produced a significant lipolytic response in both the soleus and diaphragm from control and diabetic animals, there was no difference in the percent increase in fatty acids released from muscles of diabetic rats compared to controls. However, the absolute difference was greater when the diaphragms were compared. Muscles from experimental and control animals showed a marked reduction in the amount of free fatty acids released in response to insulin. In addition, in the presence of the hormone, both the absolute and percent isoproterenol-stimulated increases in fatty acids were significantly greater for both diaphragm and soleus muscles from diabetic rats. The effects of insulin, isoproterenol, and the combination of these two hormones on the amount of glycerol released into the incubation medium were similar to those found on free fatty acid release. The results of these experiments show that there is an apparent increase in fat utilization in skeletal muscle of diabetic rats. Furthermore, measurements of triglyceride concentration and the enhanced response to isoproterenol stimulation in the muscles from these animals suggests that they may have an increased capacity for mobilization of intracellular lipids. Finally, in the diabetic state, both the soleus and diaphragm appear to demonstrate an increased response to the antilipolytic effect of insulin as measured by the decreased amount of fatty acid released into the incubation medium, the percent change also being significant for the soleus muscle.—**Stearns, S. B., H. M. Tepperman, and J. Tepperman.** Studies on the utilization and mobilization of lipid in skeletal muscles from streptozotocin-diabetic and control rats. *J. Lipid Res.* 1979. **20:** 654–662.

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It is well recognized that diabetes is associated with elevated plasma triglyceride and free fatty acid levels. In addition, there is an increase in the total fat content of the liver and accompanying alterations in hepatic lipid metabolism. However, the metabolic events surrounding the accumulation of excess lipid in skeletal muscle of diabetics have received little attention. In conjunction with their studies of the abnormalities associated with carbohydrate metabolism in heart and diaphragm of alloxan diabetic rats, Garland and Randle (1) found that both muscle types contained and released more free fatty acids than normal tissue. In addition, these workers found that there was an increased concentration of long-chain fatty acyl CoA and an elevated acetyl CoA/CoA ratio present in these two muscles, which they suggested could be the result of increased hydrolysis of endogenous glycerides (1). More recently, using the perfused hindlimb preparation, Goodman, Berger, and Ruderman (2) noted an increase in the acetyl CoA/CoA ratio in skeletal muscle of diabetic animals which could result from a high rate of fatty acid oxidation within the tissue.

Since skeletal muscles in rats constitute about 45% of total body weight (3) and depend upon lipid as an important source of fuel, any change in the uptake or release of fat could contribute not only to hyperlipemia, but also to alterations in the quantity or in the utilization of endogenous substrates that supply energy to the muscle cell. In the present report we have characterized several aspects of fat metabolism in the soleus and in the diaphragm of streptozotocin-diabetic and control rats. These two muscles, comprised almost entirely of red fibers (4, 5), utilize fat as the primary source of fuel (6) and were therefore particularly suited to our study. Using these two muscles we have *a*) quantitated the amount of lipid stored as triglyceride; *b*) determined the relative amounts of carbohydrate and fat metabolized in an

Abbreviations: FFA, free fatty acid.

in vitro situation; *c*) assessed insulin responsiveness in terms of its antilipolytic effect; *d*) measured the lipolytic response to the beta adrenergic agonist, isoproterenol; and *e*) studied the morphology of individual cells at the ultrastructural level. The results of these studies show that there is an increased utilization and mobilization of the already elevated amount of fat in skeletal muscle of diabetic animals which occurs together with an exaggerated response to isoproterenol. Furthermore, muscles from the diabetic rats appear to demonstrate an increased sensitivity to insulin when compared to control tissue.

MATERIALS AND METHODS

Animals

Sprague-Dawley male rats weighing 50–60 g were purchased from Taconic Farms, Germantown, NY. Rats were fasted overnight and then injected intraperitoneally with streptozotocin (100 mg/kg body weight) dissolved in citrate buffer, pH 4.5, one week prior to the time of the experiment. The criterion for successful establishment of the diabetic state was a 2- to 3-fold elevation in plasma glucose over control values, 48 hr after administration of the drug. The animals were maintained in stainless steel cages with access to food (Purina Rat Chow) and water ad lib. Control rats gained an average of 55 g during the 7-day period while the mean weight gain for animals injected with streptozotocin was 15 g. On the day of the experiment the animals were killed by decapitation between 8 and 10 AM, thus minimizing any diurnal variation in nutritional state which could have affected the fat content of the muscle. Blood was collected in chilled tubes which were placed immediately on ice; plasma obtained after centrifugation was frozen at −20°C until it was assayed for glucose, free fatty acids, and triglycerides.

Preparation of tissue

The cut or the intact diaphragm preparation was used, depending upon the experimental protocol. The cut diaphragm was prepared by removing the muscle from its insertions along the rib cage and from the central tendon. The muscle was then cut along its midline, one half serving as the control while the other was used for the experimental procedure. This preparation was used for the oxidation studies described below.

The intact diaphragm was prepared according to the procedure described by Kipnis and Cori (7). This preparation is considered to be more physiological than the cut diaphragm as each hemidiaphragm maintains its insertions to the spine, central tendon, ribs, and xiphoid process. The entire diaphragm was removed, hemisected, and the extraneous bone, muscle, and fat were removed prior to incubation.

The soleus muscle was exposed and dissected free from the hindlimb musculature by cutting the tendons at their points of insertion.

Glucose and palmitate oxidation studies

Oxidation of D-[U-^{14}C]glucose and of [1-^{14}C]palmitic acid was estimated by collecting the $^{14}CO_2$ produced from these substrates by both muscles. The D-[U-^{14}C]glucose and [1-^{14}C]palmitic acid were obtained from New England Nuclear, Boston, MA. The radiopurity of these substances was initially 99% and they were used very shortly after purchase. The animals were decapitated and their muscles were quickly excised, rinsed in saline, blotted on moist filter paper, and weighed. The incubations were carried out in Krebs bicarbonate buffer (with one half of the recommended calcium concentration), pH 7.4, containing 3% albumin, 5.6 mM glucose, 1.0 mM palmitic acid, and either 0.4 μCi/ml [1-^{14}C]palmitic acid or 0.6 μCi/ml D-[U-^{14}C]glucose, depending on the substrate being measured. The tissue and appropriate medium were placed in plastic incubation vessels and sealed with rubber caps with plastic well inserts, each well containing a rolled 2 × 8 cm strip of Whatman #1 filter paper. The vessels were gassed for 2 min with 95% O_2–5% CO_2 and were incubated for 60 min in a Dubnoff metabolic shaker at 37°C with shaking speed of 110 oscillations per min. At the end of 1 hr, by means of a syringe and needle, the strip of paper in the center well was moistened with 0.2 ml of NCS tissue solubilizer (Amersham/Searle) and the incubation medium was acidified with 0.2 ml of 4.0 N HCl. The vessels were again placed in the Dubnoff shaker and the $^{14}CO_2$ was collected over the next 45 min. At the end of this time, the filter paper wicks were carefully removed and placed in scintillation vials containing a mixture of 100% ethyl alcohol and OCS (Organic Counting Scintillation Solution. Amersham/Searle) (1:10, v/v) and the radioactivity was measured. The results are expressed as nmol substrate converted to CO_2/100 mg tissue per 45 min.

Lipolysis studies

Animals were killed by decapitation and the muscles were excised, rinsed in saline, and prepared for incubation. Intact hemidiaphragms and soleus muscles were incubated in Krebs bicarbonate buffer containing 3% albumin (bovine albumin, fraction V, fatty acid-poor, Pentex, Miles Laboratories, Kankakee, IL).

Lipolysis was initiated by the addition of 2 μg/ml of isoproterenol (Isuprel, Winthrop Laboratories) or inhibited by the addition of 12.5 mU/ml of insulin. The vessels were sealed with rubber stoppers and gassed for 1 min with 95% O_2–5% CO_2. The incubation was carried out in a Dubnoff water bath shaker for 1 hr at 37°C with a shaking speed of 110 oscillations per min. At the end of this time, the vessels were transferred to an ice bath and aliquots of medium were removed and stored at −20°C for subsequent free fatty acid and glycerol determinations. The diaphragm was dissected free from the attached ribs and spinal column, blotted on filter paper, and weighed. Soleus muscles were also blotted and weighed.

Chemical determinations

Portions of soleus and diaphragm were rapidly excised, weighed, and homogenized in chloroform–methanol 2:1 (8). The following day, aliquots of the chloroform layer were evaporated to dryness under a stream of nitrogen. The residue was dissolved in isopropyl alcohol and the triglycerides were extracted and assayed according to the Tri-Chol procedure (Oxford Reagent Set. Oxford Laboratories, Foster City, CA). Plasma triglycerides were extracted and determined in a similar manner. Plasma free fatty acids and the free fatty acid content of aliquots of incubation medium were measured according to the method of Falholt, Lund and Falholt (9). Glycerol was determined according to the procedure described by Lambert and Nersh (10) as modified by Korn (11). Plasma glucose was measured by a glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Inc., Fullerton, CA).

Statistical analysis

Values obtained from biochemical determinations are presented as mean ± SEM. Mean values were compared by Student's t test. Differences with P values < 0.05 were considered significant. Paired t tests were used to evaluate responses produced by insulin and/or isoproterenol in each experiment on both types of muscle.

TABLE 1. Plasma glucose, plasma free fatty acid, and plasma triglyceride concentrations in control and streptozotocin-diabetic rats

	Total Animals	Plasma Glucose	Plasma Free Fatty Acids	Plasma Triglycerides
		mg/dl	*mmol/l*	*mg/dl*
Control	(10)	153 ± 7	216 ± 19	116 ± 15
Diabetic	(10)	596 ± 27[a]	492 ± 51[a]	550 ± 21[a]

Values are presented as mean ± SEM of number of determinations.
[a] $P < 0.005$ when compared with control animals.

TABLE 2. Triglyceride concentrations in soleus muscle and in diaphragm from control and streptozotocin-diabetic rats

	Total Animals	Triglycerides	
		Soleus	Diaphragm
		mg/g tissue	
Control	(8)	5.4 ± 0.4	16.8 ± 0.3
Diabetic	(8)	8.4 ± 0.8[a]	31.1 ± 3.5[b]

Values are presented as mean ± SEM of number of determinations.
[a] $P < 0.005$ when compared with control animals.
[b] $P < 0.01$ when compared with control animals.

Electron microscopy

Portions of the soleus and diaphragm were removed from control and experimental animals and were immediately cut into small pieces and placed in a solution of 1% osmium tetroxide buffered at pH 7.4 with veronal acetate for 2 hr. Following fixation, the tissues were dehydrated with increasing concentrations of ethanol and embedded in Araldite 502 by standard procedures (12). The resin blocks were cut with glass knives on a Sorvall Porter-Blum MT-2B ultramicrotome. The sections were stained with uranyl acetate followed by lead citrate and examined with an RCA EMU-3F electron microscope.

RESULTS

Plasma glucose, free fatty acid, and triglyceride concentrations

In order to assess the severity of diabetes, blood samples were obtained from animals selected at random throughout the course of the study at the time of killing and were analyzed for sugar and fat content. Rats injected with streptozotocin were shown to be severely diabetic. Their plasma glucose levels were increased approximately 6-fold over those found in control animals (**Table 1**). Likewise, plasma free fatty acid (FFA) and triglyceride concentrations were increased in the diabetic rats.

Muscle triglyceride content

The triglyceride content of the soleus was greater in the streptozotocin-injected rats (**Table 2**). The diaphragm from the diabetic animals contained 31.1 ± 3.5 mg triglyceride per g of tissue while control diaphragm contained 16.8 ± 0.3 mg triglyceride per g of muscle.

TABLE 3. Oxidation of D-[U-^{14}C]glucose and [1-^{14}C]palmitic acid by muscles isolated from streptozotocin and control rats

	[^{14}C]Glucose to $^{14}CO_2$	*P*	[^{14}C]Palmitic Acid to $^{14}CO_2$	*P*
	nmol/100 mg tissue		*nmol/100 mg tissue*	
Hemidiaphragm				
Control (8)	278.7 ± 38.2[a]	<0.005	14.5 ± 1.2	<0.005
Diabetic (8)	125.8 ± 16.6		21.4 ± 1.6	
Soleus				
Control (8)	210.0 ± 22.6	NS	14.2 ± 0.4	<0.025
Diabetic (8)	236.3 ± 30.6		18.0 ± 1.5	

The number of animals is shown in parentheses.
[a] Values are presented as the mean ± SEM.

Oxidation of D-[U-^{14}C]glucose and [1-^{14}C]palmitic acid

These studies were carried out in an attempt to determine whether or not there is a difference in the amount of carbohydrate or lipid used by muscles from diabetic animals when compared with the quantity of these two substrates oxidized by control tissue. The hemidiaphragm from the streptozotocin-injected rat oxidized less glucose than control tissue (**Table 3**). However, the amount of [1-^{14}C]palmitic acid oxidized to $^{14}CO_2$ was greater in the diaphragm from experimental animals than in control muscle. Similarly, there was an increase in the amount of lipid utilized by the soleus muscle isolated from streptozotocin-diabetic animals (**Table 4**), while the quantities of [^{14}C]glucose oxidized by this muscle from both experimental and control rats were comparable.

Effect of insulin and isoproterenol on the release of free fatty acid

The addition of insulin (12.5 mU/ml) to the incubation medium resulted in a reduction in the amount of free fatty acids liberated from both muscle types in both control and diabetic animals (statistics not shown) (**Fig. 1**). The absolute change in the amount of free fatty acids released in response to insulin was greater in both soleus and diaphragm from diabetic rats. When these values were calculated as percent change relative to baseline levels, the decrease in free fatty acids released in response to insulin was 22% greater in the soleus of the diabetic animal when compared to control rats. Insulin produced a 24% decrease in FFA released from the diaphragm of the diabetic rats and a 16% decrease from the same muscle of control animals. In addition, it can be seen that the amount of free fatty acids released into the incubation medium under basal conditions was significantly greater in both types of muscle obtained from diabetic rats.

When isoproterenol (2 μg/ml) was included in the incubation medium there was a significant lipolytic response in both control and diabetic rat tissue (statistics not shown) (**Fig. 2**). The absolute change in the amount of free fatty acid release produced by isoproterenol was greater in the diaphragm from diabetic animals when compared with the same muscle from control rats. Likewise, the absolute change in the amount of isoproterenol-induced lipolysis tended to

TABLE 4. Glycerol released into incubation medium

Experimental Conditions	Soleus		Diaphragm	
	Control	Diabetic	Control	Diabetic
	μmol/g tissue		*μmol/g tissue*	
Basal	2.75 ± 0.12	4.74 ± 0.51[b]	4.37 ± 0.21	6.38 ± 0.38[a]
Insulin	2.80 ± 0.04	4.64 ± 0.53[b]	3.93 ± 0.19	5.67 ± 0.40[b]
Basal	4.35 ± 0.29	6.84 ± 0.49[b]	5.71 ± 0.22	6.51 ± 0.82
Isoproterenol	4.76 ± 0.23	7.07 ± 0.35[a]	7.13 ± 0.29	10.40 ± 0.86[b]
Basal	3.37 ± 0.11	6.27 ± 0.33[a]	8.53 ± 0.72	10.70 ± 0.40[c]
Insulin + Isoproterenol	3.53 ± 0.09	6.92 ± 0.47[a]	9.80 ± 0.67	13.73 ± 0.51[a]

The means of three sets of six experiments are presented with their standard errors. Where indicated, insulin (12.5 mU/ml) and/or isoproterenol (2 μg/ml) were added to the incubation medium. When values from muscle of diabetic animals are compared to those from control rats, significance is obtained at the following levels: *a*, $P < 0.001$; *b*, $P < 0.01$; *c*, $P < 0.05$.

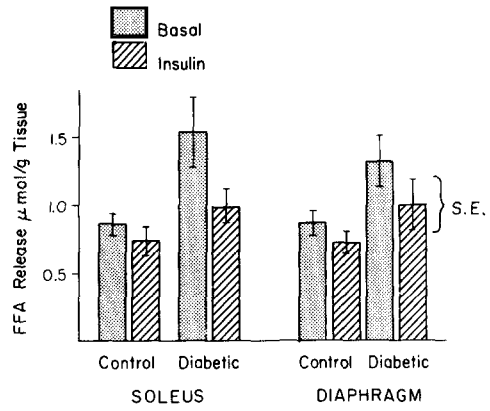


Fig. 1. Effect of insulin (12.5 mU/ml) on the in vitro release of free fatty acids from the soleus and the intact hemidiaphragm of control and streptozotocin-diabetic rats. The means (of six experiments) are represented with their standard errors. The table below gives the levels of significance between control and diabetic values in basal and experimental conditions. The absolute Δ and the % Δ are measures of the changes produced by insulin and the *P* value represents a comparison of these changes between control and diabetic animals. Values obtained from biochemical determinations in all figures are presented as mean ± SEM. Mean values were compared by Student's *t* test. Differences in the *P* values <0.05 were considered significant.

Tissue	Basal	Insulin	Absolute Δ	% Δ
Soleus	$P < 0.005$	NS	$P < 0.005$	$P < 0.005$
Diaphragm	$P < 0.05$	NS	$P < 0.05$	NS

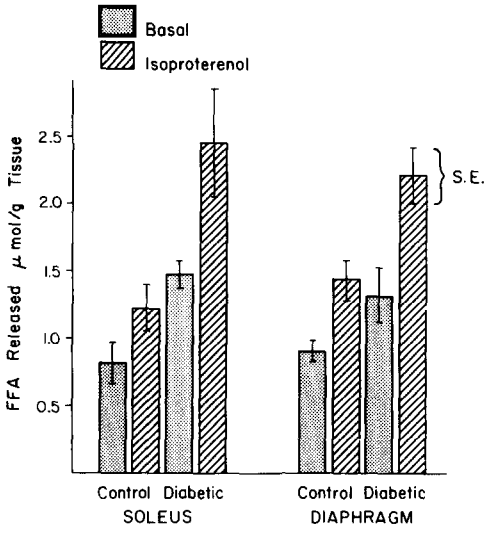


Fig. 2. Effect of isoproterenol (2 μg/ml) on the in vitro release of free fatty acids from the soleus and the intact hemidiaphragm of control and streptozotocin-diabetic rats. The means (of six experiments) are represented with their standard errors. The table below gives the levels of significance between control and diabetic values in basal and experimental conditions. The absolute Δ and the % Δ are measures of the changes produced by isoproterenol and the *P* value represents a comparison of these changes between control and diabetic animals.

Tissue	Basal	Isoproterenol	Absolute Δ	% Δ
Soleus	$P < 0.005$	$P < 0.025$	$P < 0.1$	NS
Diaphragm	$P < 0.05$	$P < 0.01$	$P < 0.025$	NS

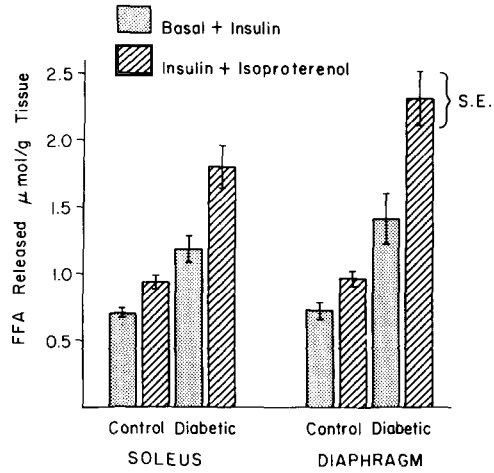


Fig. 3. Effect of isoproterenol (2 μg/ml) on the in vitro release of free fatty acids from the soleus and the intact hemidiaphragm of control and streptozotocin-diabetic rats. Insulin (12.5 mU/ml) is included in the incubation medium. The means (of six experiments) are represented with their standard errors. The table below gives the levels of significance between control and diabetic values in basal and experimental conditions. The absolute Δ and the % Δ are measures of the changes produced by isoproterenol and the *P* value represents a comparison of these changes between control and diabetic animals.

Tissue	Basal (Insulin Added)	Isoproterenol (Insulin Added)	Absolute Δ	% Δ
Soleus	$P < 0.005$	$P < 0.005$	$P < 0.025$	$P < 0.05$
Diaphragm	$P < 0.005$	$P < 0.005$	$P < 0.005$	$P < 0.025$

be greater in the soleus from the diabetic animal. However, when these changes were expressed as percent increase over baseline values, the amount of stimulation produced by isoproterenol was the same in both control and diabetic rat tissues. Under both basal and isoproterenol-stimulated conditons, the total free fatty acid content of the medium containing muscle of diabetic rats was always greater than that containing control tissue.

The conditions of the experiment shown in **Fig. 3** were the same as those in Fig. 2 except that insulin (12.5 mU/ml) was included in the incubation medium. In this case, both the absolute change and the percent change produced by isoproterenol were significantly greater in both the soleus and diaphragm from diabetic rats when compared to the lipolytic effect produced in normal tissue. It was also true that the amount of free fatty acids released from both muscles of the diabetic animals under basal and stimulated conditions was greater when compared with the amount released from the soleus and diaphragm of control rats.

Effect of insulin and isoproterenol on the release of glycerol

The pattern of glycerol release in response to insulin, isoproterenol, and the combination of the two

agents was similar to that seen for free fatty acids but was not as pronounced (Table 4). It can be seen that the addition of insulin (12.5 mU/ml) to the incubation medium produced a minimal response in the soleus from the diabetic animal and no response in the same muscle from the control rat. There was, however, a significant and comparable antilipolytic effect of the hormone seen in the diaphragm from both experimental and control animals. The addition of isoproterenol (2 μg/ml) produced an increase in glycerol release from both the soleus and diaphragm of diabetic and control animals. When calculated as percent increase over baseline values, the response in the diaphragm from the streptozotocin-injected rats was 25% greater than it was in the controls. Similarly, when insulin was present in the incubation medium, the percent increase over baseline levels in the amount of glycerol released in response to isoproterenol was 2-fold greater in both muscles from the diabetic rats than it was in the soleus and diaphragm of control animals. Overall, it can be seen that, with only one exception, there was significantly more glycerol released from the muscles of streptozotocin-injected rats in both basal and experimental conditions. The observation that the effects of isoproterenol and insulin on free fatty acid release were greater than those on glycerol release suggests that the hormones may have influenced the rate of triglyceride synthesis as well as that of lipolysis.

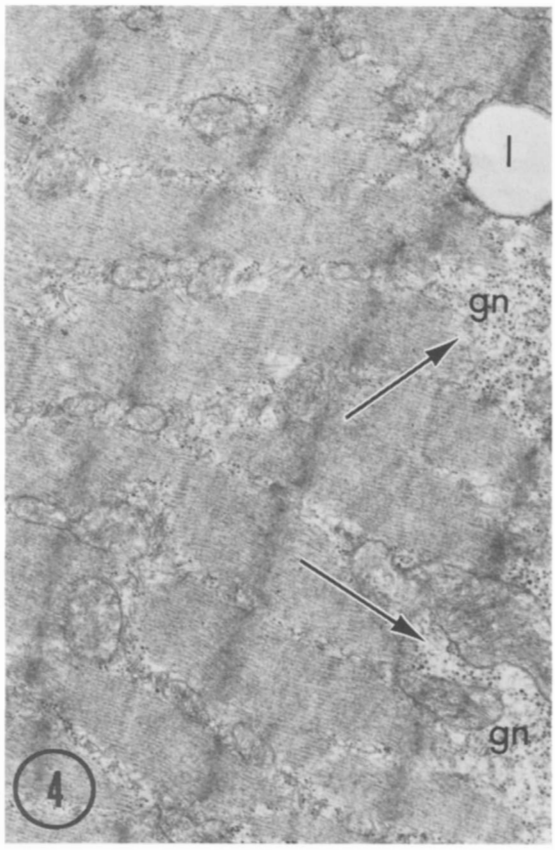
Electron microscopy

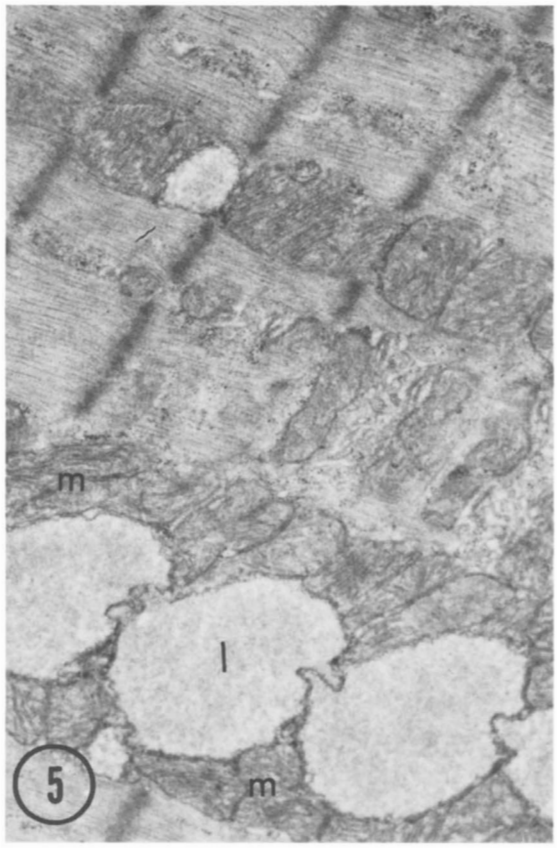
At the ultrastructural level there was evidence of marked intracellular lipid deposition in both diaphragm and soleus from diabetic animals (**Figs. 5** and **7**). There were occasional fat droplets in the interfibrillar spaces in cells from both diaphragm and soleus of control rats, together with an abundance of glycogen (**Figs. 4** and **6**). In contrast, the interfibrillar spaces of muscle cells from a comparable portion of diabetic rat tissue were frequently enlarged and contained large lipid droplets surrounded by numerous mitochondria (Fig. 5). Cells from diabetic rat's soleus also contained many fat deposits which varied in size and which were located in close proximity to mitochondria. In some areas it appeared that elements of the sarcoplasmic reticulum were infiltrated with lipid. Overall, muscle cells from both diaphragm and soleus of diabetic rats appeared to contain less glycogen than control muscle cells.

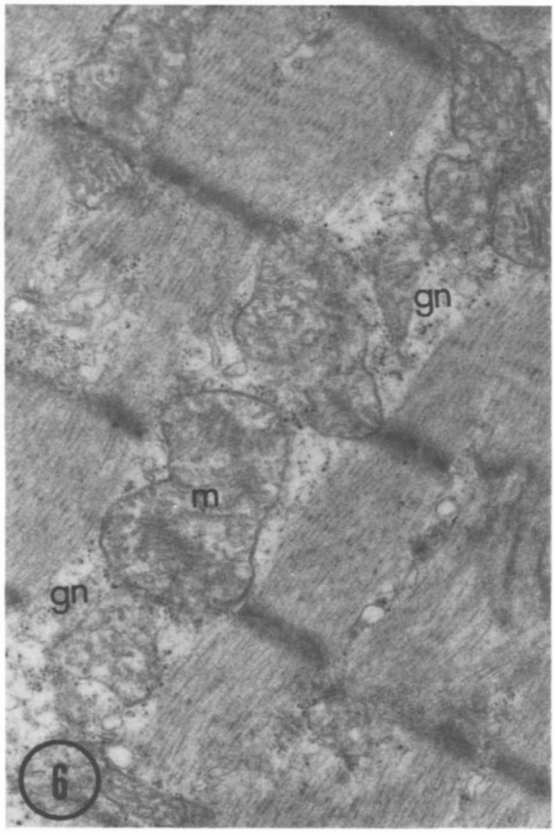
DISCUSSION

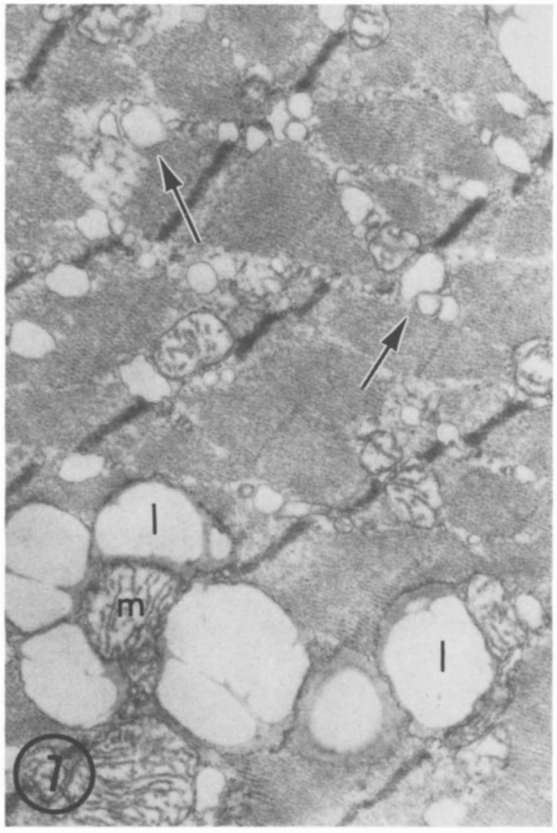
Garland and Randle (1) have stressed the interrelationship that exists between carbohydrate and fat metabolism in skeletal muscle. They were able to show in the heart and diaphragm of alloxan-diabetic rats that an increased availability and subsequent oxidation of fatty acids produced an inhibition of glucose uptake and glycolysis. Randle et al. (13) interpreted the increases in triglyceride concentration and the accelerated lipolysis and oxidation of fatty acids as evidence of insensitivity of the muscle cell to insulin, produced by a deficiency of the hormone. In experiments in which insulin was added to the incubation medium, Garland and Randle (1) were able to show a decrease in the free fatty acid content of the diaphragm from diabetic rats that was comparable to the decrease produced by the hormone in control tissues. Our data concerning the effect of insulin on the in vitro release of free fatty acids suggest that the muscle of streptozotocin-injected animals is more responsive to the hormone than control tissue. The antilipolytic effect of insulin seemed to be accentuated in the soleus where both the absolute change in the amount of free fatty acids released in response to insulin as well as the percent decrease from baseline values were highly significant. Cuendet et al. (14) measured 2-deoxyglucose uptake in response to insulin in the soleus muscle from streptozotocin-injected and lean mice and were able to demonstrate that the response to the hormone was increased in the muscle from the diabetic animals. There was also a tendency toward an increased response to insulin in the diabetic rats' diaphragms as well. We found that glycerol output was significantly increased in the diabetic rat diaphragm, an observation that is in agreement with the report of Garland and Randle (1). However, in contrast to their data and that of Reimer et al. (15), we were able to show that the addition of insulin to the incubation medium produced a decrease in glycerol release from both muscles obtained from diabetic rats. In the diaphragm, this decrease was comparable to and in the soleus was greater than the response found in control tissue. The results of this portion of our study suggest that the antilipolytic action of insulin is maintained in skeletal muscle of diabetic animals and may, in fact, be enhanced. The fact that we and others (2) found that the maximal effect of the hormone was achieved at concentrations of the hormone which were above physiologic concentrations should be considered in the interpretation of these data.

The lipolytic effect of isoproterenol on the release of free fatty acids appeared to be greater in the diabetic rat diaphragm and soleus than in control muscle. Moreover, the addition of isoproterenol evoked a 60% increase over baseline levels in the amount of glycerol released from the diaphragm of streptozotocin-injected animals in comparison to a 25% increase seen









in the tissue from controls. This increased effect on the release of both free fatty acids and glycerol from muscles of diabetic animals was particularly striking in the experiments in which insulin was included in the medium, a condition which may resemble the in vivo state more closely than the incubation studies carried out with isoproterenol alone since some insulin is present even in the diabetic rats. The increase in the amount of free fatty acids released in response to the isoproterenol stimulation was 77.7% in the soleus from the diabetic animals vs. 32.2% in the control muscle ($P < 0.05$) and 73.3% in the diaphragm of the diabetic rats compared to 25.8% in control tissue ($P < 0.025$). In addition, the absolute change as well as the percent change over baseline values in glycerol released from both the diaphragm and the soleus was significantly greater in the diabetic rats. The biochemical mechanism by which the hydrolysis of intracellular triglyceride stores occurs in the muscle cell remains to be elucidated (16). This report, together with a previous study conducted in our laboratory (17) and the work of Reimer et al. (15) show that skeletal muscle appears to respond to the presence of catecholamines. In addition, it has also been established that the blockade of beta-adrenergic receptors by propranolol effectively prevented intramuscular mobilization of triglyceride stores during exercise and decreased exercise capacity (18). Together these data suggest that catecholamines may be responsible for the breakdown of fat within the muscle cell. In this regard, it is known that plasma catecholamine levels are elevated when diabetes is accompanied by ketosis (19) as it is in the streptozotocin-induced form of the disease. It may be, therefore, that chronic catecholamine stimulation in the presence of insulin deficiency may be responsible for the consistently greater amount of basal lipolytic activity found in the skeletal muscle from the diabetic rats.

In view of the increased concentration of plasma FFA, it is perhaps not surprising that there would be an increased amount of FFA that was taken up from the circulation by skeletal muscle, esterified to form triglycerides, and eventually incorporated into lipid droplets. Electron micrographs of portions of cells of both the soleus and diaphragm obtained from diabetic animals show that there is an abundance of intracellular fat. The lipid droplets are frequently associated with mitochondria, suggesting that they may be a source of fuel for the cell. The studies in which we compared the relative amounts of [^{14}C]glucose and [^{14}C]palmitic acid oxidized by control and diabetic rat muscle show that the tissue from the diabetics utilized significantly more fat, and in the case of the diaphragm, significantly less carbohydrate. We have shown[1] that there is a decrease in the amount of glycogen in skeletal muscles from diabetic rats. This difference in the intramuscular carbohydrate pool may explain our observation that the soleus muscles from control and experimental animals appeared to oxidize comparable amounts of glucose. Felber et al. (20) measured carbohydrate and lipid oxidation in normal and juvenile diabetic subjects and found that there was an overall increase in the rate of lipid oxidation and a decrease in carbohydrate utilization. It would appear that, when it is presented with an elevation in plasma FFA levels such as that which accompanies diabetes, skeletal muscle adapts to the situation by increased utilization and storage of this substrate at the expense of carbohydrate. The decreased peripheral utilization of glucose by skeletal muscle would therefore contribute to the prevailing hyperglycemia.

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Fig. 4. Portion of a muscle cell from the diaphragm of a control rat. Glycogen deposits (*gn*) and occasional lipid droplets (*l*) are found in the interfibrillar spaces. ×14,100.

Fig. 5. Large lipid droplets (*l*) are associated with many mitochondria (*m*) in a typical muscle cell from the diaphragm of a diabetic rat. ×14,100.

Fig. 6. Portion of a cell from the soleus of a control rat showing mitochondria (*m*) and glycogen (*gn*) in interfibrillar space. ×14,100.

Fig. 7. Numerous lipid droplets (*l*) of various sizes (arrows) are seen in a portion of a muscle cell from the soleus of a diabetic rat. Lipid droplets surround mitochondrion (*m*) at lower left. ×14,100.

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