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Evidence for Increased Peroxidative Activity in Muscles from Streptozotocin-Diabetic Rats[1] (41837)

CAROL J. LAMMI-KEEFE,[2] P. B. SWAN, AND P. V. J. HEGARTY

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108

Abstract. The ability of cardiac and skeletal muscles from diabetic rats to metabolize superoxide and hydrogen peroxide was determined by the activities of superoxide dismutase (SOD) and catalase, respectively. Male and female Sprague–Dawley rats, 43 days old, were made diabetic with a single intravenous injection of streptozotocin (70 mg/kg body weight). On the 80th day after injection the blood glucose concentration of these rats was increased fourfold, and the plasma insulin concentration was decreased four- to fivefold compared to controls. Body weights of male diabetic rats were 61% and those of female diabetic rats were 66% of their *ad libitum*-fed controls. The seven different skeletal muscles examined weighed less in the diabetic rats than in controls of the same age and body weight. The hearts of the diabetic rats weighed more than those of controls of the same age and body weight. Comparison to the body weight controls allowed the distinction of specific effects due to lack of insulin from effects due to retardation in muscle growth. Increased catalase activity in all muscles examined from diabetic rats (plantaris, gastrocnemius, and heart) suggested a response in catalase activity similar to that of starved rats. SOD activity was not altered in the diabetic rat skeletal muscles and erythrocytes, but was somewhat decreased in the heart.

The importance of superoxide dismutase (SOD) and catalase in controlling tissue levels of potentially toxic superoxide radicals and hydrogen peroxide has been emphasized (1, 2). The presence of highly reactive forms of oxygen in cells can lead to disruption of cellular functions and damage to membranes. SOD and catalase have been studied extensively in red blood cells (3–5), lung (6), and liver (7–9). These enzymes appear to be central to the defense of the cell against oxidative damage. Their role in skeletal muscle is less well studied. However, both enzymes are present in muscle (10) and it is assumed that their role in this tissue is similar to that demonstrated in other tissues. Catalase is located primarily in peroxisomes in liver (11) and appears to be located in microperoxisomes in both cardiac (12) and skeletal muscles (13). SOD is located in mitochondria and in the cytosol in liver (14).

The metabolism and morphology of skeletal muscles in diabetic and starved animals is similar in many respects. Decreased utilization of glucose (15, 16), increased utilization of fatty acids and ketones (15, 17, 18), lighter muscle weights, or fewer muscle fibers (19–21) have been observed in both conditions. There is increased catalase activity in muscles from starved animals (10, 22), but catalase activity in muscles of diabetic animals has not been reported previously. In a short-term experiment, Horie *et al.* (23) have reported an increase in the peroxisomal β-oxidation of fatty acids in livers from alloxan-diabetic rats, but the catalase activity in these livers was unaltered 7 days after injection of alloxan. Certain tissues from streptozotocin-treated rats have been reported to have decreased SOD activity compared with *ad libitum*-fed controls (24, 25). This is in contrast to the unchanged SOD activity in muscles of starved rats (10).

The similarity in muscle metabolism during the starved and diabetic states coupled with the observation that muscle catalase activity increases during starvation suggested that catalase activity might be increased in diabetes. If so, this finding could add to an understanding of the changes occurring in muscles in response to a lack of insulin. The experiment

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[2] Present address: Department of Nutritional Sciences, University of Connecticut, Storrs, Conn. 06268.

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reported here was designed to test the hypothesis that catalase activity would be increased in the diabetic state and that this increase was specific to the lack of insulin and not simply due to the retardation in growth observed in the young diabetic rats.

Materials and Methods. Male and female Sprague–Dawley rats born in our laboratory were divided into four groups of six males and six females each. A baseline control group was killed at the beginning of the experiment when the rats were 43 days old, and muscles and organs were weighed. The other groups were: nondiabetic rats fed *ad libitum,* diabetic animals fed *ad libitum,* and weight-paired control animals whose food intake was restricted so that their body weights matched those of diabetic animals. All rats were housed individually and fed a commercial cereal based diet (Ralston Purina, St. Louis, Mo.). The room lighting cycle consisted of 12 hr of light and 12 hr of dark. Lights were on from 1900 hr until 0700 hr.

Rats were made diabetic at the start of the experiment (43 days of age, 188 g body weight) with a single intravenous injection of streptozotocin (70 mg/kg body weight) (Sigma Chemicals, St. Louis, Mo.). Polydypsia, polyuria, and polyphagia, as well as blood glucose and insulin levels (Table I), were diagnostic indicators of diabetes. Control animals were sham-injected with a solution of 5% dextrose in 0.9% saline. Eighty days after streptozotocin injections, blood was collected from nonfasted rats at 0800 to 1000 hr by heart puncture under ether anesthesia, and the animals were killed by carbon dioxide. Seven skeletal muscles (biceps brachii, sternomastoideus, soleus, plantaris, gastrocnemius, extensor digitorum longus, and tibialis anterior) and the heart were dissected, weighed, and stored in ice until dissections were completed. These seven skeletal muscles were chosen so that there was at least one muscle from the forelimb, hindlimb, and trunk. Thus, an indication of the effect of diabetes on muscles throughout the body was obtained. The gastrointestinal tracts were removed and weighed to determine empty body weights to correct for the substantial intestinal contents of polyphagic, diabetic animals. Blood glucose estimations were made from frozen plasma samples by the glucose 6-phosphate dehydrogenase method (26), and insulin levels were measured by the radioimmunoassay method of Hales and Randle (27).

Enzyme activities were determined im-

TABLE I. BODY AND ORGAN WEIGHTS, PLASMA GLUCOSE, AND INSULIN LEVELS IN CONTROL AND DIABETIC MALE AND FEMALE RATS

	Start	Weight control		Diabetic		*Ad libitum*-fed control
Males						
Body wt (g)						
Whole	188 ± 17	279 ± 10	NS	280 ± 18	<0.01	393 ± 8
Empty	169 ± 16	259 ± 10	NS	225 ± 20	<0.01	366 ± 8
Glucose (mg%)		143 ± 11	<0.01	595 ± 21	<0.01	171 ± 19
Insulin (U/ml)		64 ± 10	<0.01	17 ± 4	<0.01	93 ± 20
Heart (g)	0.65 ± 0.04	0.87 ± 0.04	<0.05	1.12 ± 0.10	NS	1.20 ± 0.03
Plantaris (mg)	178 ± 25	306 ± 16	<0.05	223 ± 23	<0.01	421 ± 12
Gastrocnemius (mg)	966 ± 125	1649 ± 56	<0.05	1164 ± 123	<0.01	2155 ± 52
Females						
Body wt (g)						
Whole	145 ± 5	203 ± 10	NS	195 ± 12	<0.01	245 ± 5
Empty	131 ± 5	183 ± 9	NS	155 ± 11	<0.01	234 ± 2
Glucose (mg%)		124 ± 4	<0.01	629 ± 30	<0.01	149 ± 8
Insulin (U/ml)		42 ± 11	NS	13 ± 2	<0.01	74 ± 12
Heart (g)	0.56 ± 0.02	0.67 ± 0.02	<0.05	0.80 ± 0.04	NS	0.84 ± 0.02
Plantaris (mg)	135 ± 4	245 ± 18	<0.05	177 ± 17	<0.01	295 ± 8
Gastrocnemius (mg)	720 ± 33	1266 ± 35	<0.01	867 ± 77	<0.01	1509 ± 37

Note. Data are expressed as means ± SEM. Each value represents the mean of six animals. Statistical comparisons are made between the diabetic group and the weight control and *ad libitum*-fed control groups using Student's *t* test (38).

mediately following tissue dissections. Methods for tissue preparations are described elsewhere (10). Assay procedures for catalase and SOD were those of Baudhuin *et al.* (28) and Misra and Fridovich (29), respectively. Blood samples for each group of rats were pooled and centrifuged at 4°C, 1000*g* for 10 min. The packed blood cells were washed once with an equal volume of cold 0.9% saline and recentrifuged. Three milliliters of cold water was added to 3 ml of packed blood cells and the blood cells were lysed in an ice bath for 2 h with gentle stirring. Catalase activity was determined in 300 μl of this lysate. To the remaining 5.7 ml was added 2.4 ml of a mixture of chloroform:ethanol (3:5) at 0°C to precipitate the hemoglobin, followed by 0.9 ml of cold water, with mixing. This mixture was centrifuged for 10 min at 1000*g* and the supernatant removed for SOD assay (30).

Results. Body weights of the diabetic male and female rats were significantly less than the *ad libitum*-fed control group (Table I). Glucose levels were elevated and insulin levels were low in the diabetic rats when compared with either control group (Table I), thus confirming their diabetic state.

Weight of the retroperitoneal fat pad was less in the diabetic rats when compared to either control group. Weight of the heart in the diabetic rats was the same as that in the *ad libitum*-fed controls, but larger than the weight-paired controls. Each of the seven skeletal muscles from the streptozotocin-induced diabetic rats weighed less than the corresponding muscle from the weight-paired control rats despite the similarity in body weight (unpublished data). The weights of the plantaris and gastrocnemius muscles are given in Table I. The weights of the liver and kidneys were significantly greater in the diabetic rats when compared to either control group (unpublished data).

Catalase activity in muscles from diabetic rats was significantly higher than in nondiabetic animals, *ad libitum*-fed (Table II). When the comparison is made with the nondiabetic body weight control group the elevation in catalase activity due to diabetes was significant in the plantaris muscle. Catalase activity in muscles from the weight control group was not different from that in muscles from the *ad libitum* control group. Catalase activity in erythrocytes was similar in all three groups (unpublished data). This observation is in agreement with a recent study on children with insulin-dependent diabetes mellitus which showed no change in erythrocyte catalase (31).

SOD activity remained unchanged in the plantaris and gastrocnemius muscles of the diabetic rat (Table II). There was a slight decrease in SOD activity in the hearts of diabetic rats. The downward trends in SOD activity

TABLE II. CATALASE AND SUPEROXIDE DISMUTASE ACTIVITIES IN THE MUSCLES AND ERYTHROCYTES OF STREPTOZOTOCIN-DIABETIC RATS AND CONTROL RATS

Tissue	Weight control		Diabetic		*Ad libitum*-fed control
		Catalase[a,b]			
Heart	47.1 ± 1.2	NS	88.4 ± 10.9	<0.05	39.7 ± 9.0
Plantaris	6.2 ± 0.8	<0.05	17.2 ± 1.8	<0.05	4.1 ± 0.6
Gastrocnemius	6.0 ± 1.5	NS	14.6 ± 0.2	<0.05	4.2 ± 0.6
		Superoxide Dismutase[a,c]			
Heart	179.6 ± 3.6	NS	125.7 ± 5.8	<0.05	202.3 ± 6.1
Plantaris	33.1 ± 4.3	NS	34.6 ± 9.2	NS	50.4 ± 4.7
Gastrocnemius	49.6 ± 7.3	NS	46.6 ± 6.6	NS	46.3 ± 6.9

Note. Data are expressed as means ± SEM.

[a] Each determination represents a pool of two to three muscles or pooled blood from two to three rats. There was one female group and one male group. Because enzyme activities did not differ between males and females, these were combined and the paired *t* test used to determine the statistical significance of the differences (38).

[b] μg eq/g wet tissue weight ($\times 10^{-1}$).

[c] μg eq/g wet tissue weight.

in the erythrocytes from the diabetic rats in the present study was similar to that observed in erythrocytes from children with insulin-dependent diabetes mellitus (31).

Discussion. It has been suggested that increased catalase activity in skeletal muscles reflects muscle wasting (22). However, the diabetic rats in the present study did not lose weight, they simply failed to gain at the usual rate. Yet, the catalase activity in their muscles was elevated, whereas, the catalase activity in muscles from rats similarly restricted in growth (due to food restriction) was not different from *ad libitum*-fed controls. Thus, the elevated catalase activity was not a response to muscle wasting (loss of muscle) but, as we have suggested previously (10), was more likely a reflection of altered energy metabolism in diabetic muscles (15, 16). Muscle catalase activity is elevated in response to feeding hypolipidemic drugs (32) and diets high in fat (33). Under these conditions, as well as during starvation and diabetes, relative utilization of fatty acids as a source of energy is increased in muscles.

Assuming that muscle catalase is located in microperoxisomes as has been suggested by others (12, 34), its increased activity may reflect increased activity of the hydrogen peroxide-generating fatty acyl CoA oxidase that initiates the β-oxidation of fatty acids in peroxisomes (35). Hence, our data suggest that microperoxisomes in muscle may play an important role in the adaptation of muscle to the lack of insulin.

Matkovics (24) has reported decreased SOD activity in heart, an undesignated skeletal muscle, and red blood cell hemolysates from streptozotocin-diabetic rats. In the present study there was no change in SOD activity in the gastrocnemius or the plantaris but a slight decrease in the cardiac muscle. Earlier studies indicated that muscle SOD was unchanged during short-term starvation (10). Thus, the effects of diabetes and starvation on SOD activity in muscles are less than the effects on catalase activity. However, the SOD activity in muscle should probably be investigated more thoroughly, including measurements of the total activity in mitochondria versus that in the sarcoplasm. For example, should there be an alteration in the activity of mitochondrial SOD, this may be difficult to observe unless the muscle is carefully fractionated to quantitatively recover the mitochondria in the fraction being assayed for enzyme activity and to separate this fraction from the sarcoplasmic fraction that also contains SOD (14).

Crouch *et al.* (36) reported a direct effect of streptozotocin on SOD activity. It is unlikely that the lack of response in SOD activity in this experiment was due to such a direct effect because streptozotocin has a short half-life in tissues (37). The tissues in the present experiment were assayed 80 days after the injection of streptozotocin. The lack of a change in SOD activity in skeletal muscles of diabetic rats is in keeping with the observations in starved rats. In 12-month-old rats, which were starved until they lost 39% of their body weight, SOD activity was unchanged in both heart and skeletal muscles (10).

We favor the idea that an increase in muscle catalase activity is reflecting a change in fatty acid metabolism due to a lack of insulin. This may be due to changes in the availability of fatty acids to muscles in the diabetic animal, or to alterations in the pathways for fatty acid oxidation.

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