METABOLIC ADAPTATIONS IN SKELETAL MUSCLE OF STREPTOZOTOCIN-DIABETIC RATS FOLLOWING EXERCISE TRAINING

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Summary - Compensatory metabolic adaptations induced in streptozotocin-diabetic rat skeletal muscle by submaximal endurance training have been investigated. The gastrocnemius muscles of sedentary streptozotocin-diabetic rats were found to have a lower than normal myoglobin content, succinate dehydrogenase activity, and capacity to oxidize pyruvate and palmitate-1-[^{14}C] . The values of these parameters were significantly increased in the diabetic skeletal muscle by the training program, obtaining levels similar to those of normal sedentary animals.

The dynamic properties of the normal skeletal muscle cell have been well documented. Since the study of Holloszy (4) in 1967, knowledge of the ability of normal skeletal muscle to adapt to a metabolic overload has progressed rapidly. It is now established that the increased energy demand required for prolonged, moderately-intense exercise training can induce cellular metabolic adaptations in Krebs cycle and electron transport enzymes that double the aerobic capacity of skeletal muscle. In comparison to the rapidly increasing knowledge of how the normal muscle cell compensates to an added metabolic demand, the compensatory alterations induced in diabetic skeletal muscle remain essentially unexplored. Furthermore, clinicians consider exercise along with insulin replacement and diet control as a component of diabetic management, yet information available as to the effects of chronic exercise on diabetic skeletal muscle is nearly nonexistent. Thus, the purpose of this investigation has been to determine if certain compensatory adaptations occur in the aerobic metabolic machinery of streptozotocin-diabetic rat skeletal muscle by prolonged, moderately-intense exercise training.

METHODS AND MATERIALS

Male Sprague-Dawley albino rats with initial body weights ranging from 200-220 grams were purchased from the Charles River Laboratories. Diabetes was induced one week prior to the commencement of training by a single intravenous injection of streptozotocin (60 mg/kg of body weight) in citrate buffer pH 4.5. The animals were allocated into normal sedentary, normal trained, diabetic sedentary and diabetic trained groups. They were fed a commercial pellet diet, watered ad libitum, and maintained at normal ambient temperature.

The training program consisted of running on a motor-driven treadmill at a 5 percent incline for 4-5 days/week. Training was progressively increased by adjusting the work rate and duration in order to challenge the maximal capacity of the diabetic animals. A final workload of 26-30 m/min at a 5 percent incline for 50-60 minutes was achieved following 8-10 weeks of training.

The animals were sacrificed 24 to 48 hours following the final exercise bout by a single i.p. injection of Nembutal (50 mg/kg) followed by exsanguination. The gastrocnemius muscles were immediately excised and placed in ice cold phosphate buffer (pH 7.4). Following the removal of visible connective tissue and fat the muscles were blotted and weighed. One gastrocnemius muscle from each animal was frozen in dry ice and used within 2 days post sacrifice for the determination of myoglobin content as described by Pattengale and Holloszy (6). The only modification in this procedure was a 10 minute centrifugation at 27,000g following the addition of .467 M potassium dibasic phosphate in order to clear the often pre-existing cloudiness. This did not alter the percent recovery of myoglobin. The method for calculation was described by Poel (8). The second gastrocnemius was minced and then homogenized in a 1:10 (w/v) dilution of 87.5 mM KCl containing .05 mM EDTA. This homogenate was used for the following assays. The capacity of raw muscle homogenates to oxidize palmitate-1-$[^{14}C]$ was determined after the method of Molé *et al.*(5) with the following exceptions: a 30 minute reaction time,

555,000 dpm/cell, .078 mM cytochrome C, and a CO_2 trapping time of 32 minutes using Hyamine with a pleated filter paper wick. Pyruvate oxidation was determined manometrically as described by Holloszy (4). Succinate dehydrogenase activities (EC 1.3.99.1) were determined spectrophotometrically following the reduction of cytochrome C (Sigma type III) at 500 nm (2).

Diabetes was verified at the time of sacrifice by the determination of plasma glucose using the glucostat method (Worthington Biochemical Corp., Freehold, N.J.). The averages for the normal and streptozotocin-diabetic rats were 127 $\pm$ 15 mg% and 408 $\pm$ 35 mg%, respectively. Polydipsia, polyuria and reduced rate of growth were used as early indicators of the diabetic state.

RESULTS

The gastrocnemius muscles of sedentary streptozotocin-diabetic rats were found to have a significantly lower myoglobin content, succinate dehydrogenase activity, and capacity to oxidize pyruvate and palmitate-1-$[^{14}C]$ than normal sedentary animals. These data are summarized in Table 1. The values of these parameters were significantly higher in the trained streptozotocin-diabetic animals as compared to their respective sedentary controls. The values for the trained diabetic were similar to those of the normal sedentary animals. The myoglobin content and succinate dehydrogenase activity of the gastrocnemius muscles from trained normal animals were significantly higher than their respective controls, while the capacity for palmitate-1-$[^{14}C]$ and pyruvate oxidation were not significantly altered by this training program.

DISCUSSION

The reduced potential for aerobic metabolism in the gastrocnemius muscles of sedentary insulin-deficient rats along with a lower than normal concentration of mitochondrial protein (3) is probably a manifestation of insulin lack on protein synthesis. The reduced ability of ribosomes from diabetic skeletal muscle to carry out protein synthesis, in combination with the reduced transport capacity of certain amino acids, and an increased proteolysis may largely

TABLE 1

Metabolic Parameters of Sedentary and Trained, Normal and Diabetic Rat Gastrocnemius Muscles

Groups	Body Weight g	Myoglobin mg/g	Palmitate-1-[^{14}C] Oxidation dpm/g/min.	Pyruvate Oxidation ul O_2/g/hr	SDH Activity um/g/min.
Normal Sedentary	469 ± 15 (10)[b]	1.23 ± .07[a] (9)	27312 ± 845 (8)	1440 ± 54 (10)	2.16 ± .06 (5)
Normal Trained	434 ± 14 (8)	1.69 ± .12[c] (8)	25396 ± 2329 (7)	1589 ± 62 (8)	2.54 ± .15[c] (5)
Diabetic Sedentary	330 ± 18 (10)	0.97 ± .08 (8)	22051 ± 1787 (9)	1218 ± 52 (10)	1.65 ± .19 (7)
Diabetic Trained	314 ± 20 (11)	1.33 ± .06[c] (9)	27648 ± 774[c] (9)	1509 ± 46[c] (9)	2.34 ± .19[c] (8)

[a] Values expressed as mean ± SE.
[b] Number in parenthesis is number in each group.
[c] Sedentary vs. trained statistically significant at least at the .05 level.

explain the reason for the reduced metabolic potential that exists in diabetic skeletal muscle (1,9).

The reduced aerobic potential along with the greater dependence on fatty acid and ketone body oxidation (7) has reduced the aerobic reserve capacity of the diabetic muscle cell. Thus when challenged by the metabolic overload imposed by prolonged, submaximal exercise, compensatory adaptations in the metabolic apparatus were induced to a greater extent in diabetic than normal skeletal muscle even though the heavier body weights of the normal animals required them to do more work.

Although the mechanism of enzyme induction that occurs with exercise training remains to be elucidated, the involvement of certain hormones seems remote since metabolic adaptations have been observed in thyroidectomized, hypophysectomized, and chemically prepared diabetic animals (3). These studies suggest these hormones serve to establish and maintain a normal level of aerobic capacity but are probably not the mediators through which peripheral stimuli (i.e., submaximal endurance training) induce enzyme synthesis.

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REFERENCES

1. Cahill, G.F. (1971) Diabetes 20: 785-799.
2. Cooperstein, S.J., A. Lazarow, and N.J. Kurfess (1950) J. Biol. Chem. 186: 129-139.
3. Gollnick, P.D., and C.D. Ianuzzo (1972) Am. J. Physiol. 223: 278-282.
4. Holloszy, J.O. (1967) J. Biol. Chem. 242: 2278-2282.
5. Molé, P.A., L.B. Oscai, and J.O. Holloszy (1971) J. Clin. Invest. 50: 2323-2330.
6. Pattengale, P.K., and J.O. Holloszy (1967) Am. J. Physiol. 213:783-785.
7. Paul, P. (1971) Adv. Exptl. Med. Bio. 11: 225-247.
8. Poel, W.E. (1949) Am. J. Physiol. 156: 41-55.
9. Wool, I.G., W.S. Stirewalt, K. Kurihard, R.B. Low, P. Bailey, and D. Oyer (1968) Rec. Progr. Hor. Res. 24: 139-211.