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Metabolic and Mitochondrial Disturbances in Streptozotocin-Treated Sprague–Dawley and Sherman Rats (42323)

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Abstract. This study provides explanation for conflicting evidence in the literature relating to changes in mitochondrial function and metabolic parameters during chemically induced diabetes. Diabetes of 3 days' duration (early ketosis) did not alter heart, kidney, or liver mitochondrial respiratory rates with glutamate or succinate even though serum glucose and triglycerides were elevated. Diabetes of 5 weeks' duration did not alter kidney or liver mitochondrial function in the *fed* adult rat although weight gain was depressed. The amount of kidney mitochondrial protein isolated per gram of tissue was increased by 30% in the diabetic. This increase was reversed by insulin treatment as were the other biochemical modalities measured. Superimposition of a 24-hr fast resulted in enhanced gluconeogenesis as measured by an animal weight loss of 17% within 24 hr (liver weight loss, 21%) and an elevation of serum urea nitrogen by 180% compared to fasted control. Respiratory rates of diabetic kidney mitochondria with glutamate were unaffected in the fasted animal whereas diabetic liver mitochondrial respiratory rates during succinate oxidation were reduced by 43%. Respiratory control was unchanged in the fasted diabetic rat. All the observed changes were reversed by insulin. Variation in the serum and liver metabolic indices (urea nitrogen, creatinine, glycerol, free fatty acids, free amino acids, triglycerides, and glucose) and liver mitochondrial responses to 7 weeks of chemically induced diabetes was affected by the rat strain, Sprague–Dawley versus Sherman, and rat weight, 72 g versus 222 g. Liver mitochondrial respirations in fed Sherman rats were not depressed by diabetes. Both rat strains had elevated liver free fatty acids and glutamate dehydrogenase activity in the diabetic state. Serum leucine, isoleucine, and valine were more elevated and serum lysine and arginine were more depressed in the diabetic Sprague–Dawley rat than in the Sherman rat. Conjectures on these results are presented in the text.

Review of the literature concerned with experimental diabetes produced by treatment with streptozotocin or alloxan revealed conflicting evidence for effects on mitochondrial function and metabolism. For example, Parks *et al.* (1) reported in 1955 that liver mitochondria were not affected in their uptake of inorganic phosphate or oxygen when the organelles were obtained from rats treated with alloxan. On the other hand, Hall *et al.* (2) showed in 1960 that rats treated with alloxan had liver mitochondria that were deficient in their ability to synthesize ATP. In 1972, Harano *et al.* (3) said that alloxan diabetes only lowered respiratory control in liver mitochondria when the rats were in a ketotic state and this lowering was primarily due to elevation of the State 4 rate. Conversely, Mackerer *et al.* (4) reported in 1971 that alloxan and streptozotocin stimulated respiratory control in liver mitochondria without altering P:O ratios. On the other hand, DiMarco and Hoppel (5) concluded in 1975 that streptozotocin treatment did not alter the respiratory properties of liver mitochondria or the amount of mitochondrial protein recovered per gram of liver.

We could not interpret the above results because those investigators used four different strains of rats: unknown (1), Sherman (2), Wistar (3), and Sprague–Dawley (4, 5). They varied the length of diabetic status from 3 days (3) to 6 months (1). The initial weights of the animals varied from 150 g (1) to over 400 g (5) prior to being injected intravenously or subcutaneously with the diabetogenic agent. Some rats were fed until sacrificed (1, 2, 4) whereas others were fasted at least 24 hr before sacrifice (3, 5).

Therefore we have attempted to reproduce the salient features of the preceding works in the hope that we might understand the variations in metabolism and mitochondrial function which were reported. First, the influence of ketosis and early diabetes on mitochondrial respiratory control in male Sprague–

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Dawley (350–400 g) rats was determined. The rats were sacrificed 3 and 4 days postinjection with streptozotocin when urinary ketone production was at a maximum as determined by Lab-Stix. Second, the effect of the stress of a 24-hr fast on metabolism and mitochondrial function was determined in male Sprague–Dawley (210–220 g) rats 5 weeks after streptozotocin was imposed. Finally, the metabolic and mitochondrial effects of streptozotocin on male Sprague–Dawley (67–72 g) and Sherman (54–75 g) rats growing to maturity (358 and 284 g, respectively) without a 24-hr fast were investigated. The severity of diabetes was monitored from measurements of serum urea nitrogen, creatinine, glycerol, free fatty acids, free amino acids, triglycerides, and glucose.

Materials and Methods. Sprague–Dawley and Sherman male rats were obtained from Flo Laboratories, Dublin, Virginia, and Camm Research Laboratories, Camden, New Jersey, respectively. They were individually housed, placed on a light regime of 12 hr on/12 hr off, and given water and Purina rat chow pellets *ad libitum*. They were made diabetic after a 24-hr fast by injecting intravenously in the tail vein streptozotocin (6) 70 mg/kg body wt. Three days after the injections, some of the diabetic rats who were secreting glucose in their urine (Lab-Stix glucose oxidase test, Miles Lab, Elkhart, Ind.) were normalized by daily subcutaneous injections of protamine zinc insulin (5 units of insulin per rat). At the end of the experiment, the rats were decapitated, blood was collected, and the selected organs were excised immediately, weighed, and homogenized in ice-cold 0.25 *M* sucrose–0.5 m*M* disodium ethylenediaminetetraacetate (EDTA) solution, pH 7.4.

Chemicals. Commercial sources were sodium deoxycholate, EDTA, and sucrose (Fisher Scientific Co., Pittsburgh, Pa.); mannitol (Pfanstiehl Laboratories, Waukegan, Ill.); ADP, glutamic acid, succinic acid, and crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.); streptozotocin (Ben Venue Laboratories, Bedford, Ohio); and protamine zinc insulin (Iletin, Eli Lilly Co., Indianapolis, Ind.). All other chemicals were reagent grade.

Mitochondrial respiratory control assay. Mitochondria were obtained from liver, kidney, and heart as previously described (7, 8). The mitochondrial pellet was resuspended in the sucrose–EDTA solution and used immediately. All preparative operations were at 0 to 4°C.

Respiratory velocities of mitochondria in resting and active metabolic states were determined (7, 8) by monitoring oxygen consumption (nanogram atoms per minute per milligram of mitochondrial protein) at 30°C with a biological oxygen monitor (Model 50, Yellow Springs Instrument Co., Yellow Springs, Ohio) and a Clark fixed-voltage polarographic electrode. The 3-ml reaction mixture (pH 7.4) contained 0.33 *M* mannitol, 10 m*M* $MgCl_2$, 3.5 m*M* K_2HPO_4, 3.5 m*M* KCl, 0.33 m*M* EDTA, 4 mg dialyzed crystalline bovine serum albumin, 1.4 m*M* L-glutamate or succinate, and mitochondria corresponding to 2.5 mg mitochondrial protein. Active state respiration commenced on addition of ADP (final concentration was 130 μM). Substrates linked via pyridine nucleotide (glutamate) or flavoprotein (succinate) were used so that each of the major mitochondrial respiratory chains could be examined.

The respiratory control ratio is the most sensitive measurement of mitochondrial membrane integrity and hence phosphorylation capacity and it is defined as the ratio of ADP stimulated respiratory velocity (active state, State 3) to the velocity obtaining on exhaustion of ADP (resting state, State 4) (9). Mitochondrial protein was estimated in the presence of 1% sodium deoxycholate by a biuret method (10) with crystalline bovine serum albumin as a standard.

Serum analyses. Serum urea nitrogen, creatinine, protein, glycerol, triglyceride, and glucose analyses were performed through the courtesy of Dr. Hanns-Dieter Gruemer and Dr. Gregory Miller on the SMAC (sequential multiple analysis plus computer) system (11) in the Division of Clinical Pathology at the Medical College of Virginia. Separate urea nitrogen analyses (12) in our laboratory gave data in agreement with the SMAC system.

Free fatty acids were estimated in serum and liver homogenates by a modification (13) of the method of Novak (14). Glutamate dehydrogenase was assayed in liver homogenates by the procedures of Schmidt (15).

Statistical analyses. A two-tailed Student *t* test (16) and a nonparametric rank-sum (Mann–Whitney) test (25) were used to ascertain levels of significance. Probability values of 0.05 or less were considered significant.

Results and Discussion. Adult male Sprague–Dawley rats, 400 ± 50 g, were made diabetic with streptozotocin and sacrificed 3 days post-treatment when their urine showed maximum production of ketone bodies (Lab-Stix). Controls included insulin-treated streptozotocin and saline-treated normal rats. The diabetic rats were polydypsic, polyphagic, and polyuric (17). They lost 15% of their body weight and had serum glucose values of 500 ± 29 mg/dl and triglyceride values of 1851 ± 41 mg/dl (Table I). Insulin treatment of the diabetic rat restored weight gain and reduced the glucose and triglyceride values. Mitochondria were isolated from liver, heart, and kidney tissue and they were examined for respiratory differences. No differences were observed for the recovery of mitochondrial protein (mg of protein/g of tissue) from each organ among the three groups (data not shown); nor were any respiratory differences noted for these organs in spite of the presence of a defined diabetic and ketotic state. State 4 and State 3 respiratory rates with L-glutamate as substrate were not altered for liver, heart, or kidney mitochondria. The respiratory control ratio (RCR) remained constant for each tissue. Therefore early diabetes and ketosis in the adult male Sprague–Dawley rat does not affect tissue mitochondrial respiration or the amount of mitochondrial protein recovered per gram of tissue and this is in agreement with the work of DiMarco and Hoppel (5) who stated "that during diabetic ketoacidosis there is no alteration in hepatic mitochondrial function." They also made the same statement in regard to the effects of starvation; however, when we applied a dietary stress of 24 hr of starvation to normal, streptozotocin-treated, and insulin-treated streptozotocin rats our results were different.

The metabolic and mitochondrial activities of adult male Sprague–Dawley rats after 5 weeks of streptozotocin treatment are presented in Tables II and III. The latter table represents those rats who had been stressed by a 24-hr fast prior to sacrifice. Table II shows

TABLE I. METABOLIC AND MITOCHONDRIAL ACTIVITY OF SPRAGUE–DAWLEY RATS AFTER 3 DAYS OF STREPTOZOTOCIN TREATMENT

Treatment	Control	Streptozotocin	Streptozotocin plus insulin
Number of rats	4	5	5
Initial weight	400 ± 50[a]	400 ± 50	400 ± 50
Final weight	426 ± 33	340 ± 4[b]	416 ± 29
Serum glucose (mg/dl)	—	500 ± 29[c]	73 ± 5
Serum triglycerides (mg/dl)	—	1851 ± 41[b]	163 ± 74
Glutamate respiration in liver mitochondria[d]			
State 4	11.4 ± 0.8	11.5 ± 1.4	12.3 ± 1.1
State 3	62.2 ± 8.5	69.3 ± 8.6	82.2 ± 8.4
Respiratory control ratio	5.47 ± 0.63	6.21 ± 0.77	6.73 ± 0.50
Glutamate respiration in heart mitochondria[d]			
State 4	12.0 ± 1.5	12.6 ± 0.6	11.3 ± 0.6
State 3	62.9 ± 9.9	61.7 ± 11.9	56.2 ± 5.8
Respiratory control ratio	5.19 ± 0.37	4.75 ± 0.81	5.00 ± 0.50
Glutamate respiration in kidney mitochondria[d]			
State 4	15.6 ± 1.3	18.8 ± 1.1	16.1 ± 1.1
State 3	59.5 ± 4.9	69.4 ± 5.3	68.7 ± 7.1
Respiratory control ratio	3.86 ± 0.34	3.73 ± 0.38	4.24 ± 0.19

[a] Mean ± SEM.
[b] $P < 0.05$.
[c] $P < 0.001$.
[d] Respiratory rates are nanogram atoms of oxygen per minute per milligram of mitochondrial protein.

TABLE II. METABOLIC AND MITOCHONDRIAL ACTIVITY OF SPRAGUE–DAWLEY RATS AFTER 5 WEEKS OF STREPTOZOTOCIN TREATMENT AND THEN FED FOR 24 hrs

Treatment	Control	Streptozotocin	Streptozotocin plus insulin
Number of rats	5	5	2
Initial weight (g)	226 ± 10[a]	222 ± 8	220 ± 10
5th Week weight (g)	386 ± 11	259 ± 15[b]	344 ± 18
Final weight (g)	401 ± 14	269 ± 19[b]	366 ± 7
Serum glucose (mg/dl)	132 ± 2	568 ± 33[b]	146 ± 45
Serum triglycerides (mg/dl)	247 ± 36	907 ± 384[c]	207 ± 12
Serum urea nitrogen (mg/dl)	20 ± 1	27 ± 3[c]	21 ± 1
Liver weight(g)	14.44 ± 0.54	13.12 ± 0.42	16.98 ± 2.08
Liver mitochondrial protein (mg/g of tissue)	20.4 ± 0.8	18.5 ± 2.0	23.6 ± 6.5
Succinate respiration in liver mitochondria[d]			
State 4	29.3 ± 2.3	34.8 ± 7.1	21.0 ± 1
State 3	133.6 ± 16.1	116.9 ± 11.1	103.3 ± 2.4
Respiratory control ratio	4.61 ± 0.54	3.69 ± 0.46	4.92 ± 0.09
Kidney weight (g)	2.69 ± 0.12	3.05 ± 0.22	2.54 ± 0.07
Kidney mitochondrial protein (mg/g of tissue)	13.8 ± 0.8	18.0 ± 1.3[c]	12.1 ± 1.5
Glutamate respiration in kidney mitochondria[d]			
State 4	16.7 ± 0.9	18.4 ± 5.2	19.9 ± 1.5
State 3	65.5 ± 6.3	50.2 ± 12.9	71.1 ± 11.4
Respiratory control ratio	3.97 ± 0.41	3.14 ± 0.48	3.56 ± 0.31

[a] Mean ± SEM.
[b] $P < 0.001$.
[c] $P < 0.05$.
[d] Respiratory rates are nanogram atoms of oxygen per minute per milligram of mitochondrial protein.

that 5 weeks of streptozotocin treatment reduced the fed adult male Sprague–Dawley rats' growth 23% and elevated serum glucose, serum triglyceride, and serum urea nitrogen 330, 267, and 35%, respectively. Total liver and kidney weights were not changed by streptozotocin; on the other hand, comparison of the organ weights per 100 g body wt showed that chemically induced diabetes caused a relative increase in liver and kidney, 35 and 69%, respectively. The amount of liver mitochondrial protein recovered per gram of tissue was not altered by the streptozotocin treatment and no change was noted in the mitochondrial oxidation of succinate or glutamate (not shown). However, the amount of *kidney* mitochondrial protein recovered per gram of tissue was increased 30% by streptozotocin treatment even though no significant differences were noted in the glutamate or succinate (not shown) respiratory properties of the kidney mitochondria. Insulin treatment reversed the observed effects of streptozotocin.

It is our hypothesis that the elevated kidney mitochondrial protein in the diabetic rat might be a reflection of the kidney's adaptation for its increased role as a pump in diabetes. More mitochondria would be capable of providing more ATP for pump needs and it is well known that urine output is greatly enhanced in the diabetic.

Table III shows that streptozotocin treatment again reduced the growth 28% of adult male Sprague–Dawley rats fed 5 weeks when compared to controls. Next, the stress of a 24-hr fast was superimposed upon the three treatments (normal, diabetic, and insulin-treated diabetic). Comparison of the final rat weight with that of the 5th week rat weight showed a greater proportional loss of body weight by the streptozotocin-treated rats when compared to controls, 17% versus 7%. This loss was reduced by insulin treatment. The 24-hr fast did not affect the serum glucose levels of the three treatments, whereas a reduction in serum triglyceride levels occurred, i.e., control 69%, streptozotocin 77%, streptozotocin plus insulin 68% (Table III versus Table II). Similarly a

TABLE III. METABOLIC AND MITOCHONDRIAL ACTIVITY OF SPRAGUE–DAWLEY RATS AFTER 5 WEEKS OF STREPTOZOTOCIN TREATMENT AND THEN FASTED FOR 24 hrs

Treatment	Control	Streptozotocin	Streptozotocin plus insulin
Number of rats	4	4	2
Initial weight (g)	211 ± 6[a]	216 ± 8	215 ± 6
5th Week weight (g)	392 ± 18	266 ± 3[c]	360 ± 19
Final weight (g)	364 ± 17	222 ± 4[c]	328 ± 7
Serum glucose (mg/dl)	135 ± 14	520 ± 134[e]	119 ± 24
Serum triglycerides (mg/dl)	77 ± 24	209 ± 75	66 ± 21
Serum urea nitrogen (mg/dl)	15 ± 1	42 ± 5[c]	12 ± 2
Liver weight (g)	10.01 ± 0.53	10.36 ± 0.43	8.90 ± 0.74
Liver mitochondrial protein (mg/g of tissue)	22.5 ± 1.8	24.7 ± 1.8	25.5 ± 0.5
Succinate respiration in liver mitochondria[b]			
State 4	29.2 ± 2.5	17.3 ± 2.2[d]	29.3 ± 1.1
State 3	148.1 ± 8.8	84.3 ± 13[f]	170.4 ± 8.4
Respiratory control ratio	5.13 ± 0.40	4.89 ± 0.54	5.82 ± 0.07
Kidney weight (g)	2.40 ± 0.05	2.41 ± 0.09	2.20 ± 0.19
Kidney mitochondrial protein (mg/g of tissue)	11.5 ± 1.2	17.2 ± 0.4[f]	12.2 ± 3.7
Glutamate respiration in kidney mitochondria[b]			
State 4	16.2 ± 2.1	15.3 ± 3.5	20.2 ± 2.8
State 3	56.8 ± 9.6	73.9 ± 14.2	70.2 ± 1.8
Respiratory control ratio	3.48 ± 0.41	5.01 ± 0.29	3.53 ± 0.42

[a] Mean ± SEM.
[b] Respiratory rates are nanogram atoms of oxygen per minute per milligram of mitochondrial protein.
[c] $P < 0.001$.
[d] $P < 0.02$.
[e] $P < 0.05$.
[f] $P < 0.005$.

reduction in the serum urea nitrogen was recorded for control (25%) and streptozotocin plus insulin treatment (43%) but a *rise* in the serum level of urea nitrogen was observed for the streptozotocin treatment (56%) (Table III versus Table II). No differences were observed in total liver or kidney weights among experimental groups (control, streptozotocin, and streptozotocin plus insulin) even though the 24-hr fast depleted the respective organ weights (Table III versus Table II). The 24-hr fast did not reduce the elevated (50%) kidney mitochondrial protein in the streptozotocin-treated rat nor were significant differences in State 4 and State 3 respiratory rates recorded (cf. Table II).

On the other hand, while fasting did not alter the amount of *liver* mitochondrial protein (control versus streptozotocin versus streptozotocin plus insulin) a major difference in respiratory properties was observed for liver succinate oxidation in the diabetic rat. Liver mitochondrial State 4 and State 3 succinate respiratory rates were decreased by 42%. These rates returned to normal when the streptozotocin rat was treated with insulin. The phosphorylation efficiency of the diabetic mitochondria was not affected by the 24-hr fast because the respiratory control ratio was not changed. Thus only the oxidative capacity of the streptozotocin liver mitochondria was reduced by the 24-hr fast.

We propose that the reduction in liver mitochondrial oxidative capacity in the adult male starved diabetic rat may represent a sparing adaptation for the retention of essential nutrients for gluconeogenesis while maintaining normal phosphorylation efficiency. The elevated serum urea nitrogen (180%) in the diabetic may indicate considerable protein and amino acid catabolism for gluconeogenesis, under the stress of fasting.

The metabolic and mitochondrial effects of streptozotocin on young male Sprague–Dawley and Sherman rats growing in 7 weeks to maturity without a 24-hr fast are presented in

TABLE IV. METABOLIC AND MITOCHONDRIAL ACTIVITY OF SPRAGUE–DAWLEY AND SHERMAN RATS AFTER 7 WEEKS OF STREPTOZOTOCIN TREATMENT

	Sprague–Dawley		Sherman	
Rat strain	Control	Diabetic	Control	Diabetic
Number of rats	9	8	10	10
Initial rat weight (g)	67 ± 2[a]	72 ± 3	54 ± 3	75 ± 1
Final rat weight (g)	358 ± 11	223 ± 25[d]	284 ± 16	165 ± 10[d]
Serum glucose (mg/dl)	140 ± 4	643 ± 45[d]	143 ± 3	619 ± 19[d]
Serum triglycerides (mg/dl)	146 ± 29	428 ± 92[f]	108 ± 23	740 ± 289[j]
Serum free fatty acids (μeq/dl)	30.7 ± 2.9	65.9 ± 6.4[d]	35.4 ± 2.3	57.8 ± 8.7[j]
Serum glycerol (mg/dl)	7.4 ± 0.4	18.9 ± 3.3[e]	5.0 ± 0.4	24.4 ± 2.7[d]
Serum protein (mg/ml)	72.7 ± 1.3	68.8 ± 1.6	74.0 ± 1.4	69.3 ± 3.5
Serum creatinine (mg/dl)	0.60 ± 0.04	0.87 ± 0.03[d]	0.63 ± 0.03	1.02 ± 0.05[d]
Serum urea nitrogen (mg/dl)	18.1 ± 1.3	28.8 ± 1.9[d]	22.6 ± 0.7	35.8 ± 2.8[e]
Liver weight (g)	13.88 ± 0.47	11.43 ± 0.96[j]	11.20 ± 0.54	8.51 ± 0.42[g]
Liver protein (mg/g of tissue)	296 ± 12	287 ± 9	298 ± 5	301 ± 13
Liver free fatty acids (μeq/g of tissue)	9.8 ± 0.4	16.4 ± 1.0[d]	8.7 ± 0.7	15.5 ± 1.7[f]
Liver glutamate dehydrogenase activity[b]	6.9 ± 0.5	11.3 ± 1.1[e]	4.3 ± 0.7	7.3 ± 0.7[g]
Liver mitochondrial protein (mg/g of tissue)	25.6 ± 1.4	24.3 ± 1.2	23.6 ± 1.4	26.0 ± 1.4
Glutamate respiration in liver mitochondria[c]				
State 4	8.4 ± 0.3	7.2 ± 0.5	9.2 ± 0.4	9.1 ± 0.5
State 3	54.7 ± 2.0	39.1 ± 3.8[e]	41.6 ± 1.8	39.3 ± 2.2
Respiratory control ratio	6.55 ± 0.25	5.41 ± 0.39[h]	4.55 ± 0.15	4.37 ± 0.21

[a] Mean ± SEM.
[b] Micromoles NADH consumed per minute per gram of tissue.
[c] Respiratory rates are nanogram atoms of oxygen per minute per milligram of mitochondrial protein.
[d] $P < 0.001$.
[e] $P < 0.005$.
[f] $P < 0.01$.
[g] $P < 0.02$.
[h] $P < 0.025$.
[j] $P < 0.05$.

Tables IV and V. Examination of the growth data showed that Sherman rats did not gain at the same rate as Sprague–Dawley rats. The depression of weight gain was more pronounced for the streptozotocin-treated Sherman rat (61%) than the streptozotocin-treated Sprague–Dawley rat (48%) even though both strains had comparable values for serum glucose, triglycerides, free fatty acids, protein, and creatinine. The two strains differed in serum urea nitrogen ($P < 0.005$) and serum glycerol ($P < 0.01$) values. Streptozotocin treatment elevated proportionately glucose, free fatty acids, creatinine, and urea nitrogen in sera from both strains. Serum triglyceride and glycerol differences were magnified more in the diabetic Sherman rat than in the diabetic Sprague–Dawley rat, e.g., glycerol 4.9-fold versus 2.6-fold (diabetic versus control). Contrary to the adult rat (Table II) streptozotocin treatment diminished total liver weight 20% in the growing rat regardless of rat strain. Comparison of liver weight, grams per 100 g body wt, resulted in the diabetic rat liver being 32% larger than control irrespective of the rat strain. Serum protein, total liver protein, and liver mitochondrial protein per gram of liver were not altered by streptozotocin treatment irrespective of the rat strain and were thus similar to those found in the adult rat (Table II).

Measurements of liver mitochondrial glutamate respiration showed that phosphorylation efficiency (respiratory control ratio) was

TABLE V. SERUM AMINO ACIDS OF SPRAGUE–DAWLEY AND SHERMAN RATS AFTER 7 WEEKS OF STREPTOZOTOCIN TREATMENT

	Concentration (μmole/ml)			
	Sprague–Dawley		Sherman	
Amino acid	Control	Diabetic	Control	Diabetic
Threonine	0.310	0.303 (0.98)[a]	0.227	0.220 (0.97)
Valine	0.215	0.765 (3.56)	0.218	0.608 (2.79)
Methionine	0.049	0.066 (1.35)	0.055	0.045 (0.82)
Isoleucine	0.125	0.346 (2.77)	0.113	0.258 (2.28)
Leucine	0.208	0.611 (2.94)	0.221	0.471 (2.13)
Phenylalanine	0.064	0.088 (1.38)	0.071	0.068 (0.96)
Lysine	0.855	0.320 (0.37)	0.526	0.351 (0.67)
Histidine	0.115	0.103 (0.90)	0.098	0.096 (0.98)
Arginine	0.448	0.112 (0.25)	0.230	0.080 (0.35)
Total essential amino acids	2.39	2.71	1.76	2.20
Total nonessential amino acids	1.58	1.57	1.38	1.14

[a] Value in parentheses represents ratio of diabetic to control. Amino acid analyses of pooled serums (Table IV) were performed on a Beckman 118 amino acid analyzer by Dr. William Banks, Department of Biochemistry and Massey Cancer Center. Pooled sera for each group consisted of 0.15 ml of serum from each animal within each group; thus, the analysis represents the average from either nine control or eight diabetic Sprague–Dawley rats and either 10 control or 10 diabetic Sherman rats.

impaired in the growing diabetic Sprague–Dawley rat. The depression in respiratory control could not be attributed to uncoupling even though the free fatty acid content of diabetic liver was much greater than normal because (i) the Sherman rats were not affected and they also had elevated free fatty acids in diabetic liver, and (ii) the diminution in respiratory control was the result of depressed State 3 respiration rather than elevation of State 4 respiration. Thus the observed depression in Sprague–Dawley liver mitochondrial glutamate respiration in the diabetic rat resembled "inhibition of phosphorylation" rather than uncoupling.

Viitanen *et al.* (18) have proposed that insulin enhances the binding of hexokinase to mitochondria and this enhances the phosphorylating efficiency of the mitochondria. If such is the case then the observed decrease in phosphorylation efficiency we recorded for liver mitochondria in the growing Sprague–Dawley diabetic rat might be related to an impairment in hexokinase binding to mitochondria.

Loss of muscle mass was apparent in both strains of streptozotocin-treated rats. Enhanced protein and amino acid catabolism in both strains of diabetic rats was marked by elevated activities in the liver of glutamate dehydrogenase (64%, Table IV), aspartate aminotransferase (19), and arginase (20). Individual and total nonessential serum amino acids were not significantly affected by streptozotocin treatment (Table V). On the other hand, increased levels of the branched chain amino acids valine, isoleucine, and leucine in the serum were additional indicators of protein catabolism (21) associated with gluconeogenesis (22). The magnitude of the branched chain amino acid imbalance was greater in diabetic Sprague–Dawley rats than in diabetic Sherman rats, e.g., 3.56 versus 2.79 for valine ratio of diabetic to control rats.

Streptozotocin treatment of Sprague–Dawley rats resulted in reducing the serum levels of lysine and arginine by 63 and 75%, respectively. Similar results were observed in Sherman rats. Serum histidine and ornithine (data not shown) levels were not affected by streptozotocin treatment of either rat strain.

We speculate that the decrease in serum arginine may have reflected the increased activity of the urea cycle enzymes, e.g., arginase, which have occurred in the diabetic. The decrease in serum lysine may be related to en-

hanced acetoacetyl coenzyme A production as ketone formation is increased in the diabetic. The reduction in serum lysine levels is probably not related to transport errors such as in cystinuria or dibasic amino aciduria since no decreases were observed for serum ornithine.

Finally, the elevation of branched chain amino acids in the diabetic may have occurred in the following manner. Carnitine stimulates oxidation of branched chain amino acids in muscle and liver mitochondria (23) by activation of the decarboxylase moiety of the branched chain α-ketoacid dehydrogenase complex (24). Consequently, if carnitine were completely occupied in mitochondrial translocations required by excessive fatty acid catabolism in the diabetic rat, its availability for activation of branched chain amino acid oxidation would be compromised and might account for the two- to three-fold increases of leucine, isoleucine, and valine in the serum of diabetic rats.

Conclusion. Initially our goal was to clarify past literature on mitochondrial functions in diabetes and bring some insight into the conflicting data. Unfortunately this has not been possible because of the tremendous cost such an effort now requires for the exact duplication of past experiments. Nevertheless, we have determined that the responses of metabolic and mitochondrial respiratory functions to streptozotocin-induced diabetes in the male rat are influenced by the age and strain of the rat, the duration of the diabetic state and the stress of a 24-hr fast. A 24-hr fast produces a severe metabolic hardship for the non-insulin-treated mature diabetic animal as gluconeogenesis is greatly enhanced (fasted diabetic serum urea nitrogen levels increased by 160% when compared to fasted control) to maintain a serum glucose level of 520 mg/dl. Great animal weight loss was recorded as well as a 42% diminution in the oxidative capacity of liver mitochondria to respire with succinate. We suggest that the latter may reflect a displacement of nutrients toward gluconeogenesis rather than oxidation under the stress of a 24-hr fast.

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