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The Effects of a High Fat Diet on Chronic Streptozotocin-Diabetic Rats

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Summary. The effects of a high fat diet (30% (w/w) corn oil) on chronic streptozotocin-diabetic rats were investigated at the whole body level and at the enzyme level. The diet caused significant decreases in the extent of polydipsia (66% decrease), polyphagia (49%), polyuria (67%) and glycosuria (70%). The activities of selected hepatic enzymes from the glycolytic, gluconeogenic, ureogenic and lipogenic clusters were determined. The fat diet caused significant decreases (range: 47 to 54%) in the activity of the ureogenic enzymes carbamyl phosphate synthetase, ornithine transcarbamylase and arginase; had no effect on the glycolytic enzymes glucokinase, hexokinase and pyruvate kinase; partially decreased the diabetes-induced elevated activities of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (63% decrease), serine dehydratase (90%), alanine aminotransferase (31%) and aspartate aminotransferase (65%), and partially reversed the activity of one lipogenic enzyme, ATP citrate lyase.

Key words: Fat diet, streptozotocin, urinary parameters, food consumption, caloric intake, enzymes, glycolysis, gluconeogenesis, ureogenesis, lipogenesis, diabetic rats.

Previous studies in this laboratory using the dietary regime of Patterson et al. [1] demonstrated that a high fat diet containing 30% (w/w) corn oil produced a number of beneficial effects, including a reduction in the mortality of diabetic rats, a marked reduction in cataract formation, and an increase in body weight; furthermore a limited study demonstrated a decrease in water intake and decreases in urinary excretion of glucose and urea in diabetic animals maintained on the diet for 120 days [2]. In addition, a very restricted study of the activities of a few hepatic enzymes of diabetic rats fed the corn oil diet for 106 days suggested that there were changes in the activities of some hepatic enzymes consequent to the feeding of the fat diet [2].

The aim of this work was to evaluate the effects of the corn oil diet on hepatic enzymes and on urinary parameters in diabetic rats. The dietary conditions were those of Patterson et al. [2] i. e. a 30% corn oil diet fed ad lib, in view of the beneficial effects ensuing from this particular dietary regime [1]. No attempt was made to use other dietary conditions such as pair feeding or isocaloric diets since such conditions would distort the real effects demonstrated by Patterson et al. [1] and subsequently confirmed by Hutton et al. [2].

Materials and Methods

Diets

A fat diet was prepared which was similar in nutrient composition to that used by Patterson et al. [1]. Dog meal (K9 Kennel Meal; Carnation Pty. Ltd., Sydney, Australia) was pulverised and mixed with corn oil (Vegetable Oils Pty. Ltd., Sydney, Australia) and an aqueous solution (at 60 °C) of gelatin (Davis Gelatin Pty. Ltd., Sydney, Australia). The mixture was cut into pieces (approximately 8 cm cubes) and stored at −20 °C. The proportions of the ingredients in the diet (dry weight basis) were 67% dog meal, 30% corn oil and 3% gelatin. The nutrient composition is shown in Table 1. The standard diet was Allied Rat and Mouse Kubes (Allied Feeds, Sydney, Australia). Both the dog meal and the rat cubes contained salts and vitamins (A, D and E); neither manufacturer would supply precise information on the composition of the salt and vitamin components.

Experimental Procedures

Wistar rats (initial body weight (200–220 g) were obtained from the Animal Breeding Unit, Prince Henry Hospital, Sydney, Australia. Diabetes was induced in the animals with a single

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intraperitoneal injection of streptozotocin (Upjohn & Co., Kalamazoo, U. S. A.) (40 mg/ml in 0.1 mol/l phosphate/0.4 mol/l citrate buffer, pH 6.5) at a dose of 65 mg/kg body wt. one month before starting the dietary treatment. The animals had free access to water at all times and were fed ad lib. Diagnosis of diabetes was established 48 h after the streptozotocin injection by determination of the tail vein blood glucose concentration [3], and by testing freshly voided urine samples from each animal for glucose (Labstix; Ames & Co., Mulgrave, Victoria, Australia). Any streptozotocin-treated animal which at this time had a 4 to 6h-fasting blood glucose concentration of less than 14.0 mmol/l, or in which glycosuria was not present, was eliminated from the study.

Table 1. Composition of the diets

	Standard diet (g/100 g)	Fat diet (g/100 g)
Carbohydrate	65.2	45.2
Protein	23.0	16.4
Fat	5.0	34.4
Crude fibre	6.0	3.3
Salt	0.8	0.7

For the determination of urinary excretion of glucose and urea, animals were individually housed in metabolism cages and their urine was collected for a 24 h period on 3 consecutive days. The volume of urine voided by each animal was recorded, and samples were taken for the determination of glucose and urea. Separate groups of animals were used for the urinary parameters, for the consumption studies, and for the enzyme assays.

For the determination of daily food consumption and increase in body weight per animal, measurements were taken at 3 day intervals. It was imperative to measure body weight of the animals at the same time of the day (14.00 h), since the weight of the

Table 2. Effect of the standard diet and high fat diet on water intake and urinary parameters in streptozotocin-diabetic rats

	Standard diet	High fat diet		Standard diet		
Duration of diet		2 weeks	3 weeks	1 week	2 weeks	
Water intake (ml/24 h)	117 ± 32^b	50 ± 17^a	41 ± 20^a	112 ± 31^b	132 ± 45^c	$F_{4,94} = 93^{**}$
Urine volume (ml/24 h)	92 ± 29^b	36 ± 14^a	31 ± 16^a	91 ± 27^b	114 ± 40^c	$F_{4,94} = 103^{**}$
Glucose excretion (g/24 h)	5.4 ± 1.4^b	2.0 ± 0.6^a	1.5 ± 0.5^a	6.0 ± 1.5^b	10.0 ± 2.2^c	$F_{4,94} = 184^{**}$
Urea excretion (g/24 h)	0.63 ± 0.26^b	0.27 ± 0.06^a	0.33 ± 0.08^a	0.88 ± 0.20^c	1.77 ± 0.32^d	$F_{4,94} = 195^{**}$
Blood glucose (mmol/l)	17.0 ± 1.3	15.6 ± 3.0				n. s.

The animals were maintained on the standard diet for 8 weeks to obtain basal values. The animals were then fed the fat diet, and water intake and urinary parameters determined after 2 weeks and 3 weeks on the fat diet. The fat diet was then replaced by the standard diet, and the parameters redetermined at weekly intervals for a further 2 weeks. In all cases, the animals were fed ad lib. In each case, the value is the mean value determined over a 3 day period of daily testing. The values are expressed as mean ± SD (n = 7). For the F test, the F ratio was highly significant (** = $P < 0.01$; n. s. = not significant). The values with different superscripts are significantly different ($P < 0.05$) using Duncan's Multiple Range Test [17]

The blood glucose values (4 to 6 h fasting values) were determined after 6 weeks on the initial standard diet, and after 2 weeks on the fat diet. At the completion of the feeding trial, lenticular opacities of varying severity were present in all animals

Table 3. The effect of the standard diet and high fat diet on food consumption in normal and streptozotocin-diabetic rats

	Normal		Diabetic		
	Standard diet (n = 38)	High fat diet (n = 9)	Standard diet (n = 39)	High fat diet (n = 12)	
Food consumption (g/24 h)	22.0 ± 1.2^b	15.5 ± 0.3^a	41.6 ± 1.8^c	21.1 ± 1.2^b	$F_{3,26} = 635^{**}$
Body weight (g)	241 ± 22^a	247 ± 19^a	227 ± 42^a	245 ± 54^a	n. s.
$\frac{\text{Food consumption (g/24 h)}}{\text{Body weight (g)}} \times 100\%$	10.8 ± 0.9^c	6.7 ± 0.2^a	18.2 ± 0.5^d	8.9 ± 0.7^b	$F_{3,26} = 526^{**}$
Carbohydrate intake (g/24 h)	14.4 ± 0.8^c	7.0 ± 0.1^a	27.2 ± 1.1^d	9.6 ± 0.6^b	$F_{3,26} = 947^{**}$
Protein intake (g/24 h)	5.1 ± 0.3^c	2.5 ± 0.1^a	9.6 ± 0.4^d	3.5 ± 0.2^b	$F_{3,26} = 923^{**}$
Fat intake (g/24 h)	1.1 ± 0.1^a	5.3 ± 0.1^c	2.1 ± 0.1^b	7.3 ± 0.4^d	$F_{3,26} = 1568^{**}$
Food consumption (kcal/24 h)	87.7 ± 4.9^a	86.1 ± 1.7^a	165.7 ± 7.0^c	117.4 ± 6.8^b	$F_{3,26} = 387^{**}$
Carbohydrate intake (kcal/24 h)	57.4 ± 3.2^c	28.0 ± 0.6^a	108.6 ± 4.6^d	38.2 ± 2.2^b	$F_{3,26} = 947^{**}$
Protein intake (kcal/24 h)	20.3 ± 1.1^c	10.2 ± 0.2^a	38.3 ± 1.6^d	13.9 ± 0.8^b	$F_{3,26} = 923^{**}$
Fat intake (kcal/24 h)	9.9 ± 0.6^a	47.9 ± 1.0^c	18.7 ± 0.8^b	65.4 ± 3.8^d	$F_{3,26} = 1568^{**}$

Normal animals were maintained on the high fat diet for 30 days. Diabetic animals were maintained on the standard diet for 50 days. For a separate subgroup of the same diabetic animals, the high fat diet was introduced 28 days after the induction of diabtes, and maintained for 30 days. The food consumption was determined every third day for the last 30 days of dietary treatment in each group. Values are expressed as mean ± SD. For the F test, the F ratio was highly significant (** = $P < 0.01$; n. s. = not significant). Those values with different superscripts are significantly different ($P < 0.05$) using Duncan's Multiple Range Test [17]

diabetic animals fed the standard diet fluctuated approximately 10 g every 24 h due to their polydipsia. This precaution was found to be unnecessary in the measurement of daily food consumption.

Analytical Procedures

Glucose in urine samples was determined by the glucose-oxidase method [3] and urea by the method of Beale & Croft [4]. The rates of excretion of glucose and urea were calculated from these determinations.

For the determination of enzyme activities the animals were sacrificed at approximately 10.00 h and the liver immediately removed. A portion of the liver was weighed (approximately 1 g), and then for all enzymes other than the urea cycle enzymes homogenized (Potter-Elvehjem homogeniser) in 9 volumes of an ice-cold buffered salt solution (0.15 mol/l KCl, 0.05 mol/l $KHCO_3$, 0.006 mol/l disodium ethylenediamine tetraacetate, pH 7.4). Samples of the homogenate were retained for the determination of protein [5]. For the assay of the urea cycle enzymes, the homogenizing medium was 9 volumes of 1 g/l cetyltrimethylammonium bromide, added at room temperature. The remaining homogenate was subjected to centrifugation (30000 g for 10 min at 0 °C) and samples of the supernatant fraction were used for the determination of the activities of all enzymes, except glucose 6-phosphatase (EC 3.1.3.9) (1000 g for 10 min at 0 °C), aspartate aminotransferase (EC 2.6.1.1) (liver frozen at −20 °C for 2.5 days and the homogenate then centrifuged at 1000 g for 10 min at 0 °C) and the urea cycle enzymes (centrifuged at 10000 g for 15 min at 0 °C).

Cytoplasmic alanine aminotransferase (EC 2.6.1.2) and total aspartate aminotransferase (EC 2.6.1.1) were estimated by the method of Hatch and Shaio-Lim Mau [6]. ATP citrate lyase (EC 4.1.3.8) was estimated by a modified version of the method of Srere [7]: the assay medium contained 80 mmol/l triethanolamine, pH 7.5; 4 mmol/l $MgCl_2$; 4 mmol/l ATP, pH 7.5; 0.8 mmol/l mercaptoethanol; 0.1 mmol/l CoA; 0.25 mmol/l NADH; 9U of malate dehydrogenase; 10 mmol/l potassium-citrate, pH 7.5; and 0.1 ml supernatant, in a total volume of 2.5 ml. The reaction was initiated by the addition of citrate.

Fructose 1, 6-bisphosphatase (EC 3.1.3.11) was estimated by the method of Zalitis [8]. Glucokinase (EC 2.7.1.2) and hexokinase (EC 2.7.1.1) were estimated by the method of Sharma et al. [9]. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were estimated by a modified version of the method of Glock and McLean [10]: the assay medium contained 100 mmol/l Tris HCl, pH 7.4; 10 mmol/l $MgCl_2$; 1 mmol/l NADP and either 5 mmol/l glucose 6-phosphate (G6P) or 5 mmol/l 6-phosphogluconate (6PG); and 0.1 ml supernatant, in a total volume of 1.0 ml. The reaction was initiated by the addition of G6P or 6PG. Glucose 6-phosphatase (EC 3.1.3.9) was estimated by the method of Yeung et al. [11]. Malate dehydrogenase (decarboxylating) (NADP) (EC 1.1.1.40) was estimated by the method of Ochoa [12]. PEP carboxykinase (EC 4.1.1.32) was estimated by the method of Ballard and Hanson [13]. Pyruvate kinase (EC 2.7.1.40) was estimated by a modified version of the method of Irving and Williams [14]: the assay medium contained 100 mmol/l Tris HCl, pH 7.4; 10 mmol/l $MgCl_2$; 3 mmol/l ADP; 0.3 mmol/l NADH; 2.6 U of lactate dehydrogenase; 4 mmol/l phosphoenolpyruvate (PEP); and 0.01 ml supernatant, in a total volume of 1.0 ml. The reaction was initiated by the addition of PEP. Serine dehydratase (EC 4.2.1.13) was estimated by a modified version of the method of Freedland and Avery [15]: the assay medium contained 20 mmol/l potassium phosphate buffer, pH 8.0; 0.2 mmol/l pyridoxal phosphate; 0.15 mmol/l NADH; 1.3 U of lactate dehydrogenase; 80 mmol/l L-serine, pH 8.0; and 0.2 ml supernatant, in a total volume of 1.0 ml. The reaction was initiated by the addition of L-serine. Carbamyl phosphate synthetase (EC 2.7.2.5), ornithine transcarbamylase (EC 2.1.3.3) and arginase (EC 3.5.3.10) were estimated by the method of Brown and Cohen [16]. All determinations were carried out at 30 °C, except for glucose 6-phosphatase, PEP carboxykinase and the urea cycle enzymes (37 °C).

Statistics

All statistical analyses of results were performed by analysis of variance. A one-way analysis of variance was used in all cases except the time course experiment, which involved the measurement of the water intake and some urinary excretion parameters of a group of diabetic animals fed the standard diet first, then the fat diet, and then returned to the standard diet. Since the same 7 animals were used throughout this experiment, it is feasible that any one of these animals could cause a systematic change capable of distorting the result. Thus, to eliminate any non-random rat effect, a two-way analysis of variance was performed. It was assumed that no one animal would be at an advantage or disadvantage when on a particular dietary regimen. An F test was used to determine whether one or more of the differences among means was significant, and if the F ratio was significant, the Duncan's Multiple Range Test was used to decide which of the differences were significant, at the 5% level of probability [17].

Results

Whole Body Parameters

Animals (initial weight of approximately 210 g) which were maintained in an untreated diabetic state on the standard diet showed signs of dehydration, polyuria and wasting, with the fat deposits severely depleted within 3 weeks. After transferral to the fat diet, the general condition of the animals improved, to such an extent that within 3 weeks the increase in body weight (approximately 6–7 g/week) was in the same range as that of normal animals on either the standard diet or the fat diet (approximately 7–8 g/week). There was no significant difference in the 4 to 6 hour-fasted blood glucose concentrations of the diabetic animals on either the standard diet before the introduction of the fat diet, or on the high fat diet, the respective values being 17.0 ± 1.3 mmol/l and 15.6 ± 3.0 mmol/l. Other studies in this laboratory have consistently shown that the blood glucose level of diabetic animals fed the fat diet is always 8 to 13% less than that for matched groups of diabetic animals on the standard diet, the range of levels being 13–18 mmol/l and 17–21 mmol/l respectively (unpublished results). After 3 weeks on the corn oil diet, both the daily water intake and the urine volume of the diabetic animals decreased by 66% compared to the values obtained previously for the same animals on the standard diet (Table 2). Within 2 weeks of reverting to the standard diet, the diabetic animals consumed even more water than previously

Table 4. The effect of the standard diet and high fat diet on the activities (IU/g) of some urea cycle enzymes in the liver of normal and streptozotocin-diabetic rats

	Normal		Diabetic		
	Standard diet (n = 3)	High fat diet (n = 3)	Standard diet (n = 6)	High fat diet (n = 6)	
Carbamyl phosphate synthetase	4.1 ± 2.3[a]	4.4 ± 0.9[a]	10.1 ± 1.8[b]	5.4 ± 1.1[a]	$F_{3,14} = 16.05^{**}$
Ornithine transcarbamylase	294 ± 62[b]	170 ± 21[a]	299 ± 30[b]	139 ± 36[a]	$F_{3,16} = 21.46^{**}$
Arginase	344 ± 43[a]	249 ± 58[a]	729 ± 220[b]	388 ± 72[a]	$F_{3,14} = 10.65^{**}$
Liver weight (g)	9.2 ± 1.1[a]	8.7 ± 1.2[a]	12.4 ± 2.5[b]	10.1 ± 0.9[a]	$F_{3,17} = 5.64^{*}$

Normal animals were maintained on the high fat diet for 4 weeks. Diabetic animals were maintained on the standard diet for 8 weeks. The high fat diet was introduced to a separate subgroup of the same group of diabetic animals 28 days after the induction of diabetes, and the high fat diet maintained for 5 weeks. Those values with different superscripts are significantly different (*$P < 0.05$) using Duncan's Multiple Range Test [17]. The values are expressed as mean ± SD, and the statistical analysis is the same as described in Tables 2 and 3

Table 5. The effect of the standard diet and high fat diet on the activities (IU/g) of some key hepatic enzymes in normal and streptozotocin-diabetic rats

	Normal		Diabetic		
	Standard diet (n = 6)	High fat diet (n = 6)	Standard diet (n = 5)	High fat diet (n = 5)	
Alanine aminotransferase	19.6±4.8[a]	16.9±3.8[a]	39.6±7.6[c]	27.9±3.0[b]	$F_{3,16} = 17.91^{**}$
Aspartate aminotransferase	90.5±19.6[b]	59.4±15.1[ab]	154.3±47.9[c]	55.7±9.2[a]	$F_{3,19} = 16.57^{**}$
Fructose 1,6-biphosphatase	5.3±1.2[a]	6.3±0.57[a]	6.0±0.28[a]	6.5±0.21[a]	n. s.
Glucose 6-phosphatase	11.0±1.6[a]	11.2±1.9[a]	17.0±5.2[b]	22.8±2.5[c]	$F_{3,17} = 12.37^{**}$
Phosphoenolpyruvate carboxykinase	1.51±0.41[a]	0.51±0.14[a]	4.80±1.53[b]	1.81±0.31[a]	$F_{3,20} = 25.05^{**}$
Serine dehydratase	1.58±0.96[a]	0.48±0.23[a]	17.83±9.13[b]	1.63±1.56[a]	$F_{3,26} = 33.24^{**}$
ATP citrate lyase	1.04±0.07[c]	0.43±0.08[b]	0.27±0.05[a]	0.39±0.09[b]	$F_{3,18} = 90.41^{**}$
Malate dehydrogenase (decarboxylating NADP)	0.98±0.37[b]	1.69±0.51[c]	0.14±0.02[a]	0.50±0.08[ab]	$F_{3,12} = 13.47^{**}$
Glucose 6-phosphate dehydrogenase	1.44±0.55[b]	1.52±0.59[b]	0.75±0.18[a]	1.09±0.28[ab]	$F_{3,23} = 4.01^{*}$
6-Phosphogluconate dehydrogenase	0.90±0.24[b]	1.07±0.51[b]	0.47±0.13[a]	0.53±0.09[a]	$F_{3,19} = 6.09^{**}$
Hexokinase	0.26±0.07[a]	0.32±0.04[a]	0.16±0.04[a]	0.29±0.14[a]	n. s.
Glucokinase	1.59±0.33[c]	1.14±0.30[b]	0.071±0.002[a]	0.15±0.05[a]	$F_{3,17} = 44.49^{**}$
Pyruvate kinase	33.1±12.0[b]	11.0±3.8[a]	11.8±1.1[a]	6.5±1.8[a]	$F_{3,21} = 20.18^{**}$

The experimental conditions are the same as those described in Table 4. The values are expressed as mean ± SD; values with different superscripts are significantly different ($P < 0.05$). The statistical analyses are the same as those described in Table 3; (* = $P < 0.05$) indicates a significant F ratio

on the standard diet, and a larger volume of urine was excreted.

The daily rate of excretion of glucose and urea by the diabetic animals 3 weeks after they had commenced the corn oil diet decreased by 70% and 30% respectively compared with the previous diet (Table 2). Within 2 weeks of reverting to the standard diet, the daily excretion rates of glucose and urea increased to levels well above the pretreatment values (Table 2). Values for the daily water consumption and rates of glucose and urea excretion of normal animals on either diet were in agreement with previously reported values [2].

The food consumption (g/day) of normal animals on the corn oil diet decreased by 30% compared with that on the standard diet. As a result, the daily caloric intake for normal animals on either diet was virtually the same (Table 3). Diabetic animals exhibited polyphagia, consuming twice as much of the standard diet as did the normal animals, thus receiving twice the normal total daily caloric intake. However, diabetic animals on the fat diet ate less food per day, the total daily caloric intake being only 1.3 fold greater than that of normal animals on either diet. The decreased consumption of the fat diet, compared with that of the standard diet, was not due to a taste aversion. When diabetic animals were confronted with a free choice of both standard and fat diet, the average total daily food consumption was 23 g, consisting of 14 g of fat diet and 9 g of standard diet; no conditioning period was required. Under these conditions, the total caloric intake was approximately

120 kcal/day, which is similar to that for the fat diet alone. Previous experience in this laboratory demonstrated that introduction of the high fat diet within 2 weeks of the induction of diabetes resulted in the death of all the animals within 2 to 3 days, with massive ketonuria being a characteristic feature (unpublished results). However, the introduction of the fat diet 4 weeks or more after streptozotocin treatment completely averted this problem, with chronic diabetic animals on either the standard diet or the fat diet subsequently showing negligible levels of ketonuria.

Enzyme Activities

For the urea cycle enzymes studied, in normal animals the activities of carbamyl phosphate synthetase and arginase were not affected by the fat diet while that of ornithine transcarbamylase was decreased by about 40% (Table 4). In diabetic animals on the standard diet, the activities of carbamyl phosphate synthetase and arginase were increased 2–3 fold compared to normal animals. In diabetic animals, the corn oil diet had the effect of reducing the activities of all 3 enzymes by approximately 50%.

The activities of enzymes from the glycolytic, gluconeogenic and lipogenic clusters, and some associated enzymes, were determined for both normal and diabetic animals on the standard and fat diets (Table 5).

In normal animals, the fat diet had no effect on the activities of the gluconeogenic enzymes. In the diabetic animals fed the standard diet, the activities of all gluconeogenic enzymes, except fructose 1, 6-bisphosphatase, were increased. However, in the diabetic animals fed the fat diet, the elevated activities of all the enzymes except glucose 6-phosphatase were decreased.

For the lipogenic enzymes, the fat diet resulted in a decrease in the activity of ATP citrate lyase and an increase in the activity of malate dehydrogenase (decarboxylating) in the normal animals. There was no effect on glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. In the diabetic animals fed the standard diet, all four enzymes showed decreased activities. On the fat diet, this trend was partially reversed for ATP citrate lyase; the activities of the three other enzymes were not, however, significantly changed.

Of the glycolytic enzymes, the activities of glucokinase and pyruvate kinase in the normal animals fed the fat diet were decreased. In the diabetic animals fed the standard diet, the activity of these two enzymes was decreased, and these changes were not affected by the fat diet.

Discussion

For diabetic animals maintained on the fat diet for 3 weeks, the 65% decrease in carbohydrate intake, compared with that for the standard diet, correlates well with the 70% decrease in urinary glucose excretion. Similarly, the 30% decrease in urinary urea excretion is consistent with the decrease (64%) in protein intake. The above data are in agreement with a large number of values independently obtained in this laboratory and which have consistently demonstrated that the fat diet causes a 60–65% decrease in carbohydrate intake; 60–70% decrease in glucose excretion; 50–65% decrease in protein intake and 30–50% decrease in urea excretion (unpublished results). The decreased carbohydrate intake and excretion is presumably the major cause for the concurrent decrease in polydipsia and polyuria, thus decreasing metabolic demands on renal function. The fact that the blood glucose level is only marginally reduced by the fat diet, whereas the glucose excretion is considerably decreased, suggests that the principal effect is an overall decrease in the glucose flux without a concomitant alleviation of the hyperglycaemia. In the case of protein, the decreased dietary intake would decrease the amount of α-amino nitrogen which would need to be excreted as urea. The enzyme data are consistent with this situation, insofar as the activity of the urea cycle enzymes decreased in the diabetic animals on the fat diet compared to those maintained on the standard diet.

The activities of the enzymes recorded in Table 5 for diabetic animals on the standard diet are in the same range as generally reported [18–23]. However, the activities of the enzymes for normal animals on the fat diet (Table 5) are not in complete agreement with reported values. This is not surprising as there are many inconsistencies within the literature.

In general, the experimental fat diets reported in the literature are of two types: (i) those that contain no carbohydrate, and (ii) those that contain varying amounts of carbohydrate. There is reasonable agreement that the activities of glucokinase and pyruvate kinase decrease when normal animals are given a fat diet containing no carbohydrate [24, 25, 26]. When the animals are given a fat diet containing carbohydrate the activities of these two enzymes either decrease or remain constant [27, 28].

Our results confirm the latter findings, in that in the normal animals the fat diet resulted in decreases in the activity of glucokinase and pyruvate kinase. The fat diet had no effect on the activity of these two enzymes in the diabetic animals. The continued depressed activity of glucokinase in the diabetic animals compared to that in the normal animals is con-

sistent with the fact that the insulin levels in the diabetic animals maintained on either diet remained at very low levels (unpublished results).

For the gluconeogenic enzymes, there are variations when the activities of these enzymes, particularly glucose 6-phosphatase, are considered in relation to the fat diets used [25, 26]. In normal animals, the fat diet caused a decrease in carbohydrate caloric intake from 65% of the total caloric intake on the standard diet to 33%. This decrease had no effect on the activity of the gluconeogenic enzymes. In the diabetic animals the fat diet partially reversed the diabetes-induced increases in the activity of key gluconeogenic enzymes. The tendency for increased glucose 6-phosphatase activity in diabetic animals on a fat diet is consistent with previous findings [29]. The unchanging activity of fructose 1,6-bisphosphatase in the above dietary and hormonal conditions is also consistent with previous reports [25].

Fat diets have previously been demonstrated to have a variety of effects on the activity of lipogenic enzymes in normal animals, with the extent of desaturation of the fat appearing to be an important factor; the decrease in the activity of ATP citrate lyase in normal animals on the corn oil diet is consistent with previous findings [27, 28, 30]. However, for the other lipogenic enzymes, feeding of unsaturated fat diets of various compositions have resulted in diverse changes in enzyme activities [24, 27, 30–32]. Since unsaturated fat diets, such as a corn oil diet, clearly do not result in a uniform spectrum of changes in lipogenic enzyme activities in normal animals, the effect of the diet in diabetic animals would be expected to be complex, particularly in view of the diabetes-induced decrease in fatty acid desaturase capacity [33]. In the diabetic animals on the standard diet, the activity of all the lipogenic enzymes studied was depressed. Mobilisation of fat depots during the initial ketotic stage, combined with decreased lipogenesis, resulted in complete depletion of endogenous fat. The enzymic data are consistent with these changes. Feeding of the fat diet partially reversed one of these enzyme changes. Although even after only 3 weeks on the fat diet the diabetic animals regained lipid depots, such as mesenteric fat, this increased lipid deposition is probably due to dietary fat deposition, rather than de novo lipogenesis, since fatty acid biosynthesis in these animals remained depressed (unpublished results).

Thus, in general, the effect of the fat diet in the diabetic animals is to partially reverse some of the diabetes-induced enzyme changes. These changes are manifested at the whole body level by changes in body parameters and in urinary parameters. It appears that the primary effect is dietary i. e. a reduction in dietary carbohydrate and protein, and an increase in dietary fat. This is consistent with the situation that in the absence of insulin, the caloric contribution from carbohydrate is reduced, and the increased amount of unsaturated dietary fat is more able to satisfy the energy requirements than is dietary carbohydrate. These changes in the profile of energy metabolism particularly with respect to glucose status, then provide a variety of beneficial effects, e. g. decreases in polyuria and polydipsia which presumably moderate the detrimental additional metabolic demands of the frank diabetic state. The net result of the effect of the fat diet is to allow the development of a new, albeit fragile, steady state which is characterised by the changes cited above, and by decreased mortality rates and incidence of diabetic cataractogenesis as previously reported [2].

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