The effects of diet and duration of diabetes on hypermethioninemia in streptozotocin-diabetic rats

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There are conflicting reports concerning the existence of severe hypermethioninemia in rats made diabetic with the pancreotoxin, streptozotocin. To determine whether this discrepancy is due to experimental differences in the severity of diabetes or the diet fed to the animals, streptozotocin-diabetic and control rats were fed either a casein-based semipurified diet or laboratory chow for 2 or 5 weeks. Plasma methionine concentrations were elevated six- to nine-fold after 2 weeks in the casein-fed diabetics compared with both their own controls and the chow-fed diabetics, respectively. Circulating methionine levels had declined sharply by 5 weeks in the casein-fed diabetics but were still more than twice those of the casein-fed control and chow-fed diabetic levels. Since methionine intakes were only 30% greater in the casein-fed diabetics than in the chow-fed diabetics, it is unlikely that this is the sole cause of the large differences in plasma methionine levels. The reason for the difference in circulating Met levels could not be explained on the basis of overall amino acid availability, since growth, nitrogen balance, and plasma large neutral amino acid profiles (excluding Met) were similar within control and diabetic groups fed the two diets.

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Il existe des rapports discordants quant à l'existence d'une hyperméthionémie sévère chez les rats rendus diabétiques par la pancréotoxine, streptozotocine. Pour déterminer si ce désaccord est dû à des différences expérimentales dans la sévérité du diabète ou à la diète donnée aux animaux, les rats rendus diabétiques par streptozotocine et les rats témoins ont été nourris avec une diète semipurifiée à base de caséine ou avec une nourriture de laboratoire, pendant 2 ou 5 semaines. Les concentrations de méthionine plasmatique se sont élevées d'un facteur six à neuf après 2 semaines chez les diabétiques nourris avec la caséine comparées à celles de leurs propres témoins ou aux diabétiques nourris avec la nourriture de laboratoire, respectivement. Les taux de méthionine circulante ont diminué abruptement après 5 semaines chez les diabétiques nourris avec la caséine, mais ils étaient toujours au moins deux fois plus élevés que ceux des témoins nourris à base de caséine et des diabétiques nourris avec la nourriture de laboratoire. Étant donné que les absorptions de méthionine n'ont été que de 30% supérieures, chez les diabétiques nourris à base de caséine, à celles des diabétiques nourris avec la nourriture de laboratoire, il est improbable que cela ait été l'unique cause des grandes différences dans les taux de méthionine plasmatique. La raison de la différence dans les taux de MET circulante ne pourrait s'expliquer à partir de la disponibilité générale des acides aminés, puisque les profils de croissance, de bilan azoté et des principaux acides aminés neutres plasmatiques (excluant la MET) étaient similaires chez les groupes témoins et diabétiques nourris avec les deux diètes.

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Several investigators have reported plasma amino acid concentrations in rats made diabetic with the pancreotoxin, streptozotocin (SZ) (Bloxam 1972; Brosnan et al. 1983; Crandall and Fernstrom 1983), with one group finding that these animals were severely hypermethioninemic (Glanville and Anderson 1984, 1985). While hypermethioninemia was associated with SZ-induced diabetes, it was absent in genetically diabetic BB Wistar rats, which spontaneously develop diabetes, strongly indicating that the hypermethioninemia was a side effect of the SZ rather than a general effect of diabetes per se. It was suggested (Glanville and Anderson 1984) that the hypermethioninemia was due to an impairment in these rats' ability to clear methionine (Met) caused by a nonpancreatic effect of SZ, and that other investigators had not observed it because they had used more severely diabetic rats that were not sufficiently hyperphagic to exceed their Met clearance capacity.

Alternatively, the discrepancies in plasma Met concentrations reported between laboratories may have resulted from the different diets fed to the animals. That is, hypermethioninemia (Glanville and Anderson 1984, 1985) was only observed when rats were fed a semipurified casein-based diet, but not when animals were fed standard laboratory chow (Bloxam 1972; Brosnan et al. 1983; Crandall and Fernstrom 1983). Thus, to determine whether the degree of hyperphagia or diet composition was the important variable for this discrepancy, nitrogen balance and plasma large neutral amino acid profiles were examined in rats with a similar severity of diabetes and fed either a semipurified casein-based diet or laboratory chow. The results of this study indicate that diet influences hypermethioninemia and that the duration of diabetes ameliorates this hypermethioninemia.

Materials and methods

Animals and diets

Male Wistar rats (Charles River Breeding Labs, Ottawa, Ont.) initially weighing 100–140 g were individually housed in wire-meshed, hanging stainless steel cages in a room kept at 23 ± 1°C and lit daily for 12 h (07:00–19:00 h). Animals were allowed to acclimate to the housing facilities for 3–6 days prior to the inception of diabetes. During this period they had free access to both water and No. 5001 rodent laboratory chow (Ralston-Purina Co, St. Louis, MO). After confirmation of diabetes, half the animals continued to consume the chow diet while the other half were fed a semipurified, casein-based diet (Table 1). The amino acid profile of both diets is provided in Table 2. Body weights and food intakes were monitored throughout the experimental period.

Production and maintenance of diabetes

To produce diabetes, a single i.p. dose of SZ (80 mg/kg body weight; Sigma Chemical Co., St. Louis, MO) was individually weighed for each animal, dissolved in normal saline (pH 4.5 with 0.1 M citrate), and injected immediately. Control rats received an equal volume of the vehicle. All injections were made between 16:00 and 18:00 h to rats fasted for 16 h. To test for the presence of diabetes, a

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TABLE 1. Macronutrient composition of diets

	Chow[a]		Casein[b]	
	%	Origin	%	Origin
Carbohydrate	53.0	Mixed plant	63.5	Cornstarch and lactose
Fat	>4.5	Animal and plant	10.0	Corn oil
Protein	>23.0	Animal and plant	20.0	Casein
Fibre	20.4	Mixed plant	<1.0	Cellulose in vitamin mix

[a]Rodent laboratory chow No. 5001 (Ralston-Purina Co.).
[b]Casein diet made in laboratory from semipurified ingredients as described in Musten et al. (1974). Vitamin mixture (2.5%, Teklad Test Diets No. 67233) and Bernhart–Tomarelli (1966) mineral mixture (4.0%, Teklad Test Diets) were added during mixing.

TABLE 2. Amino acid content of diets

Amino acid (mg/kcal diet)	Diet	
	Casein	Chow
Asp	2.94	6.07
Thr	1.81	2.44
Ser	2.25	2.27
Glu	9.03	8.64
Pro	3.70	2.49
Gly	0.98	2.42
Ala	1.39	2.61
Cys	0.14	0.36
Val	2.55	2.35
Met	1.05	0.82
Ile	1.99	1.97
Leu	3.74	3.64
Tyr	1.89	1.61
Phe	2.05	2.23
Lys	3.15	2.96
His	1.17	1.27
Arg	1.62	3.13

NOTE: Amino acid composition of diets was measured in acid hydrolysates prepared from mixed diets. Trp values are unavailable due to its destruction during the hydrolysis procedure.

24-h urine sample was collected on the 3rd day postinjection, urinary glucose was measured (Clinitest Reagent, Ames Division, Miles Laboratories, Rexdale, Ont.), and those SZ-injected rats with urinary glucose concentrations less than 2 g/dL were dropped from the study. The maintenance of diabetes was verified at the end of the experimental period by a second urinary glucose analysis. The confirmation of diabetes marked the beginning of the experimental period, with the rats being kept in a frank diabetic state throughout the period.

Experimental protocol

The purpose of this experiment was to examine the effect of diet on the expression of hypermethioninemia in SZ-diabetic rats. Control and diabetic rats ($n = 8$ per group) were fed either chow or the casein diet for 2 or 5 weeks. On the last 2 days of the experimental period, rats were transferred to metabolic cages and urine and feces were collected for glucose and nitrogen analysis. Animals were allowed free access to food up until 2 h before the time of sacrifice. Animals were killed by decapitation (09:00–10:00 h), and blood was collected from the cervical stump into heparinized tubes and kept on ice. Plasma was separated by centrifugation and frozen at −70°C until analyzed for glucose and amino acids.

TABLE 3. Plasma glucose concentrations and body weight gain in control and SZ-diabetic rats

Time	Diet	Treatment	Plasma glucose (mg/dL)	Body weight gain (g)
2 weeks	Chow	Control	161 ± 3	115 ± 4
		Diabetic	483 ± 21	52 ± 9
	Casein	Control	163 ± 3	122 ± 1
		Diabetic	622 ± 48	72 ± 7
5 weeks	Chow	Control	162 ± 4	218 ± 12
		Diabetic	579 ± 78	134 ± 13
	Casein	Control	175 ± 4	226 ± 10
		Diabetic	546 ± 16	110 ± 5

Sources of variation	*p* value	
Time	NS	0.001
Diabetes	0.001	0.001
Time × diabetes	NS	0.001

NOTE: Rats made diabetic with SZ (80 mg/kg body weight) were kept in a frank diabetic state and fed either a casein-based or chow diet for 2 or 5 weeks. Each value is the mean ± SEM for eight rats. Statistical analysis was by ANOVA; NS, nonsignificant.

Biochemical analyses

Plasma glucose concentration was assessed using a glucose oxidase method (kit No. 510, Sigma Chemical Co.).

Plasma amino acid concentrations were determined in samples pooled from pairs of rats. Plasma was deproteinized using equal volumes of 15% sulfosalicyclic acid (containing 200 μM norleucine as an internal standard), centrifuged, and a 200 or 300 μL aliquot of the supernatant was injected into a Beckman 116/119 amino acid analyzer (Palo Alto, CA). The amino acids eluted off a UR-30 resin column with lithium citrate buffers as previously described (Zlotkin et al. 1981). Plasma tryptophan was measured fluorometrically by the method of Dencla and Dewey (1967), using the Bloxam and Warren (1974) modification.

A diet amino acid profile was determined in acid hydrolysates (Blackburn 1978) prepared from the premixed diets, and analysed on the Beckman 116/119 amino acid analyzer as above, using buffers appropriate for acid hydrolysates. No correction for possible amino acid loss during the hydrolysis procedure was made and it was assumed that the losses would be similar for both diets. Total nitrogen content of diets was verified by micro-Kjeldahl analysis (Association of Official Analytical Chemists 1970). Metabolizable energy content of the casein-based diet (4.25 kcal/g) was calculated using Atwater values of 4, 4, and 9 kcal/g for protein, carbohydrate, and fat, respectively, while that of the chow diet (3.6 kcal/g) was provided by the manufacturer.

Feces and urine were pooled over the 2-day collection period from the same pairs of rats used for the plasma amino acid analysis. Total nitrogen was measured by micro-Kjeldahl nitrogen analysis (Association of Official Analytical Chemists 1970). To further characterize fecal nitrogen into fecal microbial nitrogen and neutral detergent fibre nitrogen, a particulate fraction was isolated by the following modification of the method of Mason (1981). A 300-mg sample of feces was diluted with 10 mL of water, vortexed for 60 s, and then centrifuged at 20 000 *g* for 60 min. The pellet was collected for nitrogen analysis. Neutral detergent fibre (NDF) and NDF-nitrogen content of the feces were measured by the methods of Robertson and Van Soest (1982). Since the fecal particulate fraction contains nitrogen from both insoluble dietary fibre and microbial material whereas NDF-nitrogen measures only the undigested fibre nitrogen, the difference in fecal particulate nitrogen and NDF-nitrogen was used as a measure of fecal microbial nitrogen (Mason 1981).

Nitrogen balance was determined by comparing nitrogen intake and

TABLE 4. Daily energy, nitrogen and methionine intakes in control and SZ-diabetic rats

Time	Diet	Treatment	Energy intake (kcal/(day·100 g BW))	Nitrogen intake (g/(day·100 g BW))	Methionine intake (mg/(day·100 g BW))
2 weeks	Chow	Control	35.9±0.9	0.38±0.01	29.5±0.7
		Diabetic	62.6±1.8	0.63±0.02	51.3±1.5
	Casein	Control	34.5±0.8	0.27±0.01	36.2±0.9
		Diabetic	65.6±2.7	0.51±0.03	68.9±2.9
5 weeks	Chow	Control	25.1±0.8	0.27±0.01	20.6±0.7
		Diabetic	47.7±1.9	0.50±0.03	39.1±1.6
	Casein	Control	23.9±1.0	0.19±0.01	25.1±1.1
		Diabetic	45.5±2.5	0.34±0.02	47.7±2.7
Sources of variation				*p* value	
Time			0.001	0.001	0.001
Diet			NS	0.001	0.001
Diabetes			0.001	0.001	0.001
Time × diet			NS	NS	0.003
Time × diabetes			0.006	0.044	0.012
Diet × diabetes			NS	NS	0.027
Time × diet × diabetes			NS	NS	NS

NOTE: Rats made diabetic with SZ (80 mg/kg body weight) were kept in a frank diabetic state for 2 or 5 weeks and fed either a casein-based or chow diet. Twenty-four hour intakes were measured during the last 2 days prior to sacrifice and the average of the 2-day intake was calculated. Each value is the mean ± SEM for eight rats. Statistical analysis was by ANOVA; NS, nonsignificant.

nitrogen excretion (fecal + urinary nitrogen). Corrections were not made for additional nitrogen losses, such as hair loss.

Statistical analysis

Statistical analysis was done on an enhanced IBM PC computer using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) by analysis of variance for a 2 × 2 × 2 factorial design. For both the fecal and urinary nitrogen and plasma amino acid data, analysis of covariance was also performed, using the previous day's intake of either nitrogen or the specific amino acid, respectively, as the covariate. Data provided in tables give probability values for statistically significant ($p<0.05$) sources of variation.

Results

Diabetic parameters

All diabetic rats exhibited hyperglycemia (Table 3), glucosuria (>2 g/dL), polyuria, hyperphagia (Table 4), and polydipsia. The SZ-diabetic groups gained less weight than their respective control groups (Table 3). The different diets fed to the rats, however, had no effect on either total weight gain during the experimental period or glycemic control, measured 2 h postprandially.

Food consumption

Diet did not affect the energy intakes of either the control or diabetic groups (Table 4). Although the energy density of chow (3.6 kcal/g) was less than that of the casein diet (4.25 kcal/g), the chow-fed animals consumed more food on a gram per day basis and had energy intakes per unit of body weight similar to those of the casein-fed animals. As expected, on both diets energy intake per unit body weight was very significantly increased by diabetes and decreased by aging.

Total nitrogen intake (Table 4) reflected both the diabetic hyperphagia (Table 3) and the greater nitrogen content of chow in comparison to the casein-based diet (Table 1). That is, on both diets, nitrogen intake was increased by diabetes and chow-fed animals consumed more nitrogen than casein-fed rats within the control and diabetic groups, respectively.

Methionine intakes were moderately greater in both the casein-fed control and diabetic animals than in their chow-fed counterparts (Table 4). The casein-fed controls consumed approximately 23% more Met than the chow-fed controls at both 2 and 5 weeks, while the casein-fed diabetics consumed 34 and 22% more Met than the chow-fed diabetics at 2 and 5 weeks, respectively. This was due to the 27% greater Met content of the casein diet (Table 2).

Nitrogen balance

Total fecal nitrogen output was increased sixfold in both control and diabetic animals consuming the chow diet compared with the casein-fed animals (Table 5). In addition, diabetic animals had increased fecal nitrogen output when compared with their respective controls, independent of the diet fed to the rats. The significant effect of diabetes on total fecal nitrogen output was observed when total nitrogen intake was used as a covariate, suggesting that diabetic hyperphagia alone could not be responsible for the increased nitrogen output.

Similar results were observed when microbial nitrogen excretion was examined (Table 5). As anticipated, the consumption of higher levels of fermentable carbohydrate in the form of dietary fibre increased microbial nitrogen excretion 3.6-fold in the control and diabetic chow-fed animals. Microbial nitrogen excretion accounted for 46–65% of total fecal nitrogen output in the chow-fed animals, and 86–94% in the casein-fed groups.

Urinary nitrogen excretion was approximately 50% greater in control animals consuming the chow diet compared with those fed the casein-based diet. A similar but less dramatic effect of diet on urinary nitrogen excretion was observed in the diabetic animals (Table 5). Diabetes also had a marked effect on urinary nitrogen losses, with the diabetic animals excreting approximately 2 times more urinary nitrogen on a daily basis. This pattern of urinary nitrogen excretion with both diet and diabetes was not affected by the duration of the experimental period.

TABLE 5. Nitrogen balance in control and SZ-diabetic rats

Time	Diet	Treatment	Nitrogen intake (g/day)	NE fecal (g/day)		NE urinary (g/day)	Nitrogen balance (g/day)
				Total	Microbial		
2 weeks	Chow	Control	1.05±0.17	0.26±0.09	0.12±0.10	0.44±0.02	0.35±0.03
		Diabetic	1.28±0.73	0.33±0.32	0.21±0.23	0.69±0.02	0.26±0.04
	Casein	Control	0.74±0.23	0.04±0.04	0.04±0.04	0.28±0.02	0.41±0.02
		Diabetic	1.08±0.67	0.06±0.10	0.06±0.10	0.64±0.06	0.38±0.02
5 weeks	Chow	Control	1.04±0.63	0.26±0.13	0.17±0.04	0.43±0.05	0.35±0.06
		Diabetic	1.37±0.43	0.41±0.09	0.27±0.21	0.78±0.03	0.18±0.02
	Casein	Control	0.70±0.49	0.04±0.03	0.04±0.02	0.30±0.02	0.35±0.06
		Diabetic	0.86±0.57	0.09±0.11	0.08±0.15	0.66±0.14	0.12±0.12
Sources of variation				p value			
Time			NS	0.004	0.001	NS	0.015
Diet			0.001	0.001	0.001	0.008	NS
Diabetes			0.001	0.007	0.038	0.001	0.004
Time × diabetes			NS	0.007	NS	NS	NS
Diet × diabetes			NS	0.001	0.002	NS	NS

NOTE: NE, nitrogen excretion. Rats made diabetic with SZ (80 mg/kg body weight) were kept in a frank diabetic state and fed either a casein-based or chow diet for 2 or 5 weeks. Feces and urine were collected on the 2 days prior to sacrifice. Nitrogen balance was calculated on the basis of total nitrogen intake minus (fecal + urinary) nitrogen excretion. Each value is the mean ± SEM for four pools of two animals each. Statistical analysis of fecal nitrogen excretion is by analysis of covariance; the previous day's nitrogen intake was used as the covariate. NS, nonsignificant.

TABLE 6. Plasma large neutral amino acids in SZ-diabetic rats

Time	Diet	Treatment	Plasma amino acids (nmol/mL)						
			Met	Val	Leu	Ile	Tyr	Phe	Trp
2 weeks	Chow	Control	41	250	160	86	69	120	117
		Diabetic	31	707	423	238	73	126	92
	Casein	Control	45	408	197	110	111	144	138
		Diabetic	274	889	438	248	92	149	116
5 weeks	Chow	Control	53	225	184	100	77	122	119
		Diabetic	48	669	512	286	90	127	101
	Casein	Control	69	242	160	89	128	121	117
		Diabetic	100	527	380	221	96	113	83
(Pooled SD)			92	261	145	84	24	14	19
Sources of variation			p values						
Time			NS	0.004	NS	NS	NS	0.002	0.005
Diet			NS	NS	NS	NS	0.001	0.001	NS
Diabetes			NS	0.001	0.001	0.001	NS	NS	0.001
Time × diet			NS	0.008	0.016	0.025	NS	0.001	0.001
Diet × diabetes			0.015	NS	NS	NS	0.011	NS	NS

NOTE: Rats made diabetic with SZ (80 mg/kg body weight) were kept in a frank diabetic state and fed either a casein-based or chow diet for either 2 or 5 weeks. Animals were killed 2 h after removal of food. Each value is the mean of four pools of two animals each. Statistical analysis was by analysis of covariance; the previous day's intake of the respective amino acid was used as the covariate. Analysis of Trp was by simple ANOVA, owing to the unavailability of dietary Trp levels. NS, nonsignificant.

Despite the differences in nitrogen consumption and excretion, nitrogen balance was unaffected by diet in both the control and diabetic animals (Table 5). Nitrogen balance was positive in all animals, reflecting their growth phase. Not surprisingly, diabetic animals had lower nitrogen balances compared with control animals, and the degree of positive nitrogen balance declined with time as the animals became more metabolically compromised.

Plasma large neutral amino acid concentrations

Hypermethioninemia in the diabetic rats at 2 weeks was promoted by the casein diet, but not chow; however, the effect of the casein diet had greatly diminished by 5 weeks (Table 6). Plasma Met concentrations of the casein-fed diabetics were 6 times greater than those of their controls and 9 times greater than those of the chow-fed diabetics. After 5 weeks, the plasma Met concentrations were only 1.5 and 2 times greater than those of

their controls and the chow-fed diabetics, respectively. In the chow-fed diabetics, plasma Met levels at both 2 and 5 weeks were somewhat reduced compared with chow-fed control levels. Comparison of the control groups fed the two diets revealed that the plasma Met concentrations were greater in the casein-fed groups than in the chow-fed groups, probably owing to the higher Met intakes on the casein diet ($p > 0.001$, statistical analysis not shown).

To determine whether the effect of diet on circulating Met levels was due to the increased Met content of the casein diet, or a consequence of some other attribute of the diet, data were analyzed using analysis of covariance, and the average Met intake (g/day) consumed on the 2 days prior to sacrifice was used as the covariate. While the main effects of diet and diabetes were no longer significant, the interaction between diet × diabetes still remained statistically significant (Table 6), indicating that the differences observed in circulating Met levels in the diabetic animals fed the two diets could not be explained simply on the basis of Met consumption alone.

As anticipated, diabetes resulted in increased circulating concentrations of the branch chain amino acids (BCAA) valine, leucine, and isoleucine. The diet fed to the rats did not influence the level of circulating BCAA in either the control or diabetic animals. On the other hand, while diabetes did not influence plasma levels of the aromatic amino acids, tyrosine and phenylalanine, circulating levels of both these amino acids were higher in the casein-fed animals. This effect of diet on aromatic amino acids was observed both when the intake of the amino acid was used as a covariate (Table 6) and when it was not ($p = 0.001$ for both Tyr and Phe).

Discussion

Inconsistencies exist in the literature regarding plasma Met levels in SZ-diabetic rats, with most investigators reporting normal Met levels (Bloxam 1972; Brosnan et al. 1983; Crandall and Fernstrom 1983), but others demonstrating severe hypermethioninemia (Glanville and Anderson 1984, 1985). This study has confirmed the presence of hypermethioninemia in the SZ-diabetic rat, and in addition, has shown that the hypermethioninemia is greatly affected by diet and duration of diabetes. A severalfold increase in plasma Met levels after 2 weeks of SZ-induced diabetes is present when the rats are fed a casein-based diet, but is absent when they are fed chow, and the severity of this hypermethioninemia declines sharply by 5 weeks to less than twice the control values (Table 6). This effect of diet on plasma Met concentrations in the SZ-diabetic rat appears to be the reason why other investigators using the SZ model (Bloxam 1972; Brosnan et al. 1983; Crandall and Fernstrom 1983) have not observed the hypermethioninemia reported by Glanville and Anderson (1984, 1985), since only Glanville and Anderson fed rats a semipurified casein-based diet.

The reason for the diet's effect on plasma Met levels in the diabetic rats is not clear from these studies. While these diets differ markedly in composition (Table 1), overall metabolic handling of the diets is similar, based on comparable growth, blood glucose levels, and nitrogen balance within the control and diabetic groups (Tables 3 and 5). Met content of the diet differs both on a dry weight basis and as a proportion of total calories (Tables 1 and 2) such that the casein-fed diabetics consumed 34% more Met than their chow-fed counterparts in the 2 days prior to sacrifice (Table 4). However, this seems hardly sufficient to have caused the observed ninefold difference in plasma Met concentrations. Furthermore, when the plasma Met data were analyzed using Met intake as a covariate (Table 6), only the diet × diabetes interaction was statistically significant, suggesting that the effect of the casein-based diet on circulating Met levels in the diabetic animals was independent of actual Met intake.

Diet components, other than amino acid composition, may influence bioavailability and utilization of protein. The higher fibre content of chow may have reduced amino acid availability. Furthermore, since the protein in chow originates from both cereal and animal products, it is possible that the protein digestibility of chow is less than that of the casein diet. A number of factors, however, do not support these two alternatives. First, free Met is added to chow by the manufacturers during its formulation and this should minimize any differences in Met availability for absorption. Second, if overall protein digestibility was a major contributing factor to the hypermethioninemia caused by the casein-based diet, differences in circulating levels of the other large neutral amino acids should have been observed; however, dietary protein source had no effect on plasma BCAA concentrations (Table 6). Since plasma BCAA levels are directly proportional to dietary content (assuming complete protein digestibility; Johnson and Anderson 1982), these results indicate that amino acid availability did not differ between the two diets. Finally, urinary nitrogen excretion was much greater in rats consuming the chow diet, indicating that nitrogen absorption was not limiting.

Despite the fact that the underlying mechanism(s) responsible for the hypermethioninemia is not apparent from these data, the present study illustrates that diet plays an important role in its expression. The implications of this observation are twofold. First, since Met has a variety of toxic effects, it has been suggested that results from SZ studies should be interpreted carefully to avoid the possible confusion of the effects of Met toxicity with those of diabetes (Glanville and Anderson 1984). The present study illustrates that choice of diet (i.e., semipurified versus chow) fed to diabetic animals has a major impact on circulating Met levels. Second, while the plasma Met response varies with the diet fed, the biochemical defects that is the cause of the hypermethioninemia is probably present in all SZ-diabetic rats and may be having unknown effects. For example, we previously reported that the handling of methyl groups from Met is different in the SZ-diabetic rat's brain compared with control animals and that this effect of SZ diabetes is observed independently of the diet fed to the rats and hence their plasma Met concentrations (Dyer and Greenwood 1988). Thus, while diet appears to influence plasma clearance of Met, metabolic abnormalities in Met utilization may be apparent in all animals.

In summary, this study has confirmed the existence of hypermethioninemia in SZ-diabetic rats reported by Glanville and Anderson (1984, 1985), and has shown that the hypermethioninemia is diet and time dependent. Hypermethioninemia is present in SZ-diabetic rats fed a casein-based diet, but is absent in those fed laboratory chow, and the hypermethioninemia's severity decreases markedly with the duration of diabetes. The dietary effect on hypermethioninemia explains the differences in plasma Met levels reported by investigators using SZ-diabetic rats fed different diets.

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