Effect of environmental factors including nutrition on genetically determined diabetes of Chinese hamsters

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There is little doubt that diabetes mellitus is influenced by factors such as life style (Medalie *et al.* 1978; West, 1978), uterine environment (Gerritsen, 1975; Amendt *et al.* 1978), genetic background (Herberg & Coleman, 1977) and perhaps most important, diet (Himsworth, 1935; Burkitt *et al.* 1972; Cahill, 1977; Jenkins *et al.* 1977; Anderson & Ward, 1978; Renold *et al.* 1978; West, 1978). However, there is little agreement about the importance of specific dietary components.

Further, there is little known about the effects of diet in human prediabetics due to the difficulty in reliable identification of the human prediabetic. Results from spontaneous diabetic animal models suggest that diet plays a very important role in the aetiology and control of the disease (Dulin & Gerritsen, 1972). Normalization of energy intake of hyperphagic prediabetic Chinese hamsters retarded onset of glycosuria and ameliorated severity of the disease (Gerritsen *et al.* 1974). More recently, inbred, nonhyperphagic prediabetic animals became available and were described (Gerritsen, Blanks, Schmidt *et al.* 1976). Preliminary studies suggested that dietary restriction (2·5 g/d) was beneficial to the nonhyperphagic prediabetic Chinese hamster also (Gerritsen & Blanks, 1979). Further, a diet which contained 40 g vegetable fat/kg was shown to ameliorate ketonuria (Grodsky *et al.* 1974; Gerritsen, Blanks, Frankel *et al.* 1976). Additional studies on the effects of high- and low-fat diets and dietary restriction on ketonuric and prediabetic nonhyperphagic Chinese hamsters are presented.

Methods

Chinese hamsters used in these studies were from highly inbred sublines (F>20) which have an incidence of essentially 100% glycosuria. Control animals were from a highly inbred subline (subline M) which has produced no glycosuric animals for over fifteen generations of brother-sister mating. Hamsters of these types have been described in detail (Gerritsen *et al.* 1976). Ketonurics from inbred sublines used in these studies were similar to ketonuric diabetic Chinese hamsters described previously (Gerritsen & Dulin, 1967).

Animals were housed individually in stainless steel cages or when appropriate, in stainless steel metabolism cages. The appropriate amount of food was weighed and presented each morning. If animals were fed *ad lib.* food was weighed in and the remainder weighed out 7 d later. Food consumption was calculated by difference and mean daily food consumption determined.

Fat, protein and carbohydrate content of diets used was determined by analysis described previously (Gerritsen & Blanks, 1974). Nonfasting blood or plasma

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glucose, plasma insulin, urinary glucose and β-hydroxybutyrate excretion were measured as previously described (Gerritsen & Blanks, 1979).

In the longitudinal metabolic study on ketonic diabetic hamsters, dietary regimens were changed weekly during the course of the study. In the studies on prediabetics, half of each litter of pups was placed on one or the other dietary regimens. Statistical analysis was performed using Wilcoxon's matched-pairs signed rank test.

Results

Table 1 shows the composition of the diets used in these studies.

The analysis indicated that the major difference in the diets was in the fat content (110 g/kg and 40 g/kg respectively). Another important difference is that the high-fat diet contained a combination of animal and vegetable fat compared with vegetable fat only for the low-fat diet. When the energy contents of the diets were calculated, the high-fat diet had a slightly higher energy density than the low-fat diet (16 and 14·3 kJ/g (3·8 and 3·4 kcal/g) respectively).

The high- and low-fat content diets described in Table 1 were fed to a group of ketonuric Chinese hamsters. The diet regimen was changed weekly as shown in Table 2. Animals were maintained on the regular high-fat diet used to maintain the Upjohn Chinese hamster colony (Purina #5015 Ralston Purina Co. St. Louis, Mo.). Animals were acclimatized for one week before measurements were taken. During the second week (week 1) baseline results were collected. Food consumption was very high, approximately 20% of their body-weight (6 g/30 g body-weight per d). This represents consumption of almost 3·4 kJ (0·8 kcal)/g body-weight per d. Despite the enormous energy intake, body-weight was normal but urinary glucose and β-hydroxybutyrate excretion were significantly elevated. When the animals were shifted to the low-fat, lower-energy-density diet (week 1) their food consumption increased so that total energy consumption remained the same. Despite similar energy intake, urinary glucose and β-hydroxybutyrate excretion were significantly reduced. β-hydroxybutyrate excretion was reduced to a level which was normal for the nondiabetic Chinese hamster. A concentration of 2·5 μmol/ml was not detectable by the Ketostix (Ames Laboratories, Elkhart, Indiana) test. When animals were limited to 3·0 g/d low-fat diet (week 2), energy intake was restricted to less than 50% of normal and glycosuria was significantly reduced even further (from 1126 to 346 mg/d). This energy restriction was sufficient to cause a significant loss in body-weight but surprisingly β-hydroxybutyrate excretion did

Table 1. *Composition (g/kg)* of diets fed to Chinese hamsters

Diet	Fat	Protein	Carbohydrate	Energy (kJ(kcal)/g)
High animal and vegetable fat	110	170	530	16 (3·8)
Low vegetable fat	40	230	540	14·3 (3·4)

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Table 2. *Effect of low-fat (LF) v. high-fat (HF) diets on ketonuric Chinese hamsters*

(Values are means with their standard errors for nine animals)

Week	Diet regimen	Food consumption (g/d) Mean	Food consumption (g/d) SEM	Energy intake (kJ(kcal)/d)	Energy from fat (kJ(kcal)/d)	Body-weight (g) Mean	Body-weight (g) SEM	Glycosuria (mg/d) Mean	Glycosuria (mg/d) SEM	Urinary β-hydroxybutyrate (μmol/ml) Mean	Urinary β-hydroxybutyrate (μmol/ml) SEM
−1	HF (*ad lib.*)	6·1	0·2	97·4 (23·2)	25·4 (6·04)	30	1·2	1506	62	5·7	0·7
1	LF (*ad lib.*)	6·9	0·2	98·7 (23·5)	10·4 (2·48)	30	1·3	1126*	52	2·5*	0·4
2	LF (restricted)	3·0		42·8 (10·2)	4·5 (1·08)	25*	1·3	346*	16	2·2*	0·3
3	LF (*ad lib.*)	7·1	0·4	101·2 (24·1)	10·7 (2·56)	29	1·5	1067*	49	1·9*	0·3
4	HF (*ad lib.*)	6·2	0·4	98·7 (23·5)	25·8 (6·14)	31	1·4	1499	86	5·4	0·8
5	HF (restricted)	3·0		47·9 (11·4)	12·5 (2·97)	26*	1·2	928*	43	6·2	1·0
6	HF (*ad lib.*)	5·7	0·4	87·8 (20·9)	22·9 (5·45)	30	1·2	1421	66	9·9	1·5

*$P<0·01$.

not change (week 1, 2·5 *v.* 2·2 for week 2). After diet limitation, the hamsters were switched back to *ad lib.* low-fat feeding (week 3). All endpoints measured, body-weight included, were the same as the prerestriction period (week 1). When the diet was changed to high-fat *ad lib.* (week 4), food consumption dropped but energy consumption was similar to other *ad lib.* feeding periods (weeks −1, 1 and 3). Other endpoints were similar to the pretreatment baseline values obtained during week −1. When animals were limited to 3·0 g/d of high-fat diet (week 5), a significant loss in body-weight and a modest but significant reduction in glycosuria was observed. It is of considerable interest that no reduction in urinary β-hydroxybutyrate excretion was observed despite the fact that energy consumption was low (47·9 kJ (11·4 kcal)/d) during week 5. Further, despite the fact that energy consumption was similar during the two periods of diet restriction (weeks 2 and 5), urine glucose excretion was significantly different (346 mg/d during week 2 on 3·0 g low-fat/d compared with 928 mg/d during week 5 on 3·0 g high-fat diet/d). A similar situation occurred with β-hydroxybutyrate excretion. It was normalized when animals were fed on either low-fat diet *ad lib.* or restricted but was not decreased when the hamsters were given 3·0 g high-fat diet/d. The animals lost similar amounts of weight on either diet restriction regimen (weeks 2 and 5). It is important to note that energy available from dietary fat was similar during week 5 of high-fat diet restriction (12·5 kJ (2·97 kcal)/d) compared with week 3 of low-fat diet fed *ad lib.* (10·9 kJ (2·56 kcal)/d). During weeks 3 and 5 when total fat consumption was quantitatively similar, glycosuria was similar (1067 *v.* 928 mg/d) but β-hydroxybutyrate excretion was very different (1·9 *v.* 6·2 μmol/ml). Further, despite the fact that energy intake from fat was similar, total energy consumption was very different (week 3, 101·2 kJ (24·1 kcal)/d *v.* week 5, 47·9 kJ (11·4 kcal)/d). After the diet restriction to 3·0 g high-fat diet/d, the hamsters were returned to the *ad lib.* high-fat diet regimen. All endpoints measured returned to the pre-experimental basal levels (week −1).

Prediabetic Chinese hamsters were compared with nondiabetics matched for age, sex and litter size (Table 3). Although food consumption could not be measured before weaning, body-weight measurements suggest that food consumption was similar. Body-weights did not differ significantly during the 10 weeks of observation although there was a slight tendency for the prediabetics to weigh more than matched nondiabetics. The same was true for daily food consumption. Plasma glucose and insulin levels did not differ significantly during the first 6 weeks. However, by week 10, the 'prediabetics' were significantly hyperglycaemic as well as hyperinsulinaemic.

It is interesting to note that plasma insulin levels appear to be high in young nondiabetic and prediabetic animals compared with adult nondiabetics (range 10–30 μU/ml). The value for the young nondiabetics appeared to peak around 3 weeks of age and then declined toward normal as adult body-weights were approached by 10 weeks. However, this trend does not appear to be true for prediabetic hamsters. Their plasma insulin levels tend to increase rather than decrease (Table 3).

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Table 3. *Comparison of nondiabetic with prediabetic Chinese hamsters* ad lib. *fed on the standard high-fat diet*†

(Values are means with their standard errors for ten nondiabetics compared with ten prediabetics)

Genotype	Age (weeks)	Food consumption (g/d) Mean	SEM	Body-weight (g) Mean	SEM	Plasma glucose (mg/l) Mean	SEM	Plasma insulin (μU/ml) Mean	SEM
Nondiabetic	2	—		8·8	0·2	850	50	78	19·0
Prediabetic	2	—		9·2	0·3	920	40	117	12·0
Nondiabetic	3	—		13·8	0·3	970	50	93	12·6
Prediabetic	3	—		14·2	0·4	1040	180	98	15·0
Nondiabetic	6	3·5	0·3	20·7	0·7	870	91	65	11·4
Prediabetic	6	4·0	0·1	20·5	0·7	1190	15	123	28·6
Nondiabetic	10	3·4	0·2	24·8	1·3	1000	67	44	9·8
Prediabetic	10	3·8	0·1	27·0	1·7	2290*	256	154*	27·9

*$P<0.05$.
†110 g fat/kg diet.

Table 4. *Effect of diet limitation on prediabetic Chinese hamsters*

(Values are means with their standard errors for six prediabetics fed on high-fat diet 110 g fat/kg diet *ad lib.* compared with six matched prediabetics fed on the same diet at 2·5 g/d)

Age (weeks)	Food consumption (g/d) Mean	SEM	Body-weight (g) Mean	SEM	Plasma glucose (mg/l) Mean	SEM	Plasma insulin (μU/ml) Mean	SEM
6	4·0	0·1	22	1·3	1190	15	91	3·6
6	2·5		21	0·4	1150	12	66	15·1
8	4·0	0·2	25	1·8	1700	215	123	23·6
8	2·5		20*	0·7	890*	34	16*	3·3
10	3·8	0·1	27	1·6	2290*	226	154	17·9
10	2·5		20*	0·6	840*	31	11*	4·1
16	3·4	0·1	28	3·0	2500	310	145	22·0
16	2·5		20*	0·6	900*	55	28*	6·5

*$P<0.01$.

Prediabetic Chinese hamsters were limited to 2·5 g standard high-fat diet/d while their siblings were allowed the same diet *ad lib.* (Table 4).

At the start of the experiment (age 5 weeks), there were no significant differences between the two groups and after one week of dietary restriction, they were still similar (age 6 weeks). However, by 8 weeks of age, animals fed *ad lib.* were hyperglycaemic and nonfasting plasma glucose increased during the course of the experiment. Hamsters limited to 2·5 g/d did not become hyperglycaemic and plasma insulin levels were low, similar to levels observed in adult nondiabetic

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hamsters. In this experiment, diet restriction prevented weight gain in these prediabetics.

In order to evaluate the effect of dietary fat in prediabetics, Chinese hamster litters were weaned at 3 weeks of age and half the hamsters in each litter were fed on the high-fat diet and the other half were given the low-fat diet. All animals were allowed food *ad lib.*

As shown in Table 5, there was no difference in total energy consumption by animals on either diet. No significant difference in body-weight was observed but there was a tendency for hamsters eating the high-fat diet to weigh more. Despite similar energy consumption, after 3 weeks on the high-fat diet (age 6 weeks) prediabetics had significant elevation of nonfasting blood glucose. By contrast, prediabetic siblings fed low-fat diet *ad lib.* had significantly lower nonfasting blood glucose levels.

Discussion

The results suggest that diabetes mellitus of the Chinese hamster can be ameliorated by several dietary manipulations. Reduction of energy intake without alteration of dietary composition reduced glycosuria as shown in Table 2 and reduced blood glucose (Dulin & Gerritsen, 1972). Reduction in fat from 110 to 40 g/kg diet without alteration in total energy intake resulted in a reduction in glycosuria (Table 2). An unexpected observation was that this reduction in dietary fat intake eliminated ketonuria (Table 2) (Grodsky *et al.* 1974).

Table 5. *Effect of low-fat (LF) v. high-fat (HF) diets fed* ad lib. *on Chinese hamsters*

(Values are means with their standard errors for seven prediabetics fed on low-fat diet compared with seven prediabetics fed on high-fat diet)

Age (weeks)	Diet	Food consumption (g/d) Mean	SEM	Energy intake (kJ(kcal/d) Mean	SEM	Body-weight (g) Mean	SEM	Blood glucose (mg/l) Mean	SEM
4	LF	4·0	0·2	57·1 (13·6)	3·4 (0·8)	15	0·3	1160	73
4	HF	3·8	0·1	60·5 (14·4)	0·8 (0·2)	15	0·6	1170	27
6	LF	3·8	0·2	54·2 (12·9)	2·1 (0·5)	17	1·2	970	67
6	HF	3·4	0·1	54·2 (12·9)	0·8 (0·2)	22	2·0	1560*	116
8	LF	3·8	0·1	54·2 (12·9)	1·7 (0·4)	20	0·6	1000	58
8	HF	3·5	0·3	55·9 (13·3)	3·0 (0·7)	23	0·9	1720*	117
10	LF	4·3	0·2	61·3 (14·6)	2·1 (0·5)	22	0·8	1100	88
10	HF	3·6	0·2	57·5 (13·7)	3·0 (0·7)	25	1·2	1680*	161
16	LF	4·4	0·1	62·6 (14·9)	1·7 (0·4)	24	1·0	1240	156
16	HF	4·1	0·3	63·4 (15·8)	5·0 (1·2)	26	1·5	2500*	328

*$P<0·5$.

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It is of considerable interest that restriction of the high-fat diet (110 g fat/kg) did not reduce ketonuria. One possible explanation might be that the hamsters lost weight on this dietary regimen and some of the metabolized body fat was excreted as β-hydroxybutyric acid. However, it should be pointed out that the same animals did not excrete β-hydroxybutyric acid during the restriction period of low-fat diet (40 g fat/kg) when total energy intake and weight loss were similar. This suggests that other explanations such as improved pancreatic performance might be more plausible. At the end of the experiment with the ketonuric, diabetic Chinese hamsters, plasma insulin measurements were attempted but levels were below the sensitivity of the assay. However, in another experiment, the same low-fat diet was fed *ad lib.* to similar ketonuric hamsters for 17 d. It was observed that β-cell granulation improved (Gerritsen & Blanks, 1975). The possibility existed that feeding the low-fat diet with lower energy density resulted in less demand for insulin and less ketogenesis.

The results presented in Table 2 suggest that the type of fat consumed may influence ketogenesis of the diabetic Chinese hamster also. As pointed out previously, the low-fat diet contained vegetable fat only but the high-fat diet contained both animal and vegetable fat. During weeks 3 and 5, fat consumption was similar but β-hydroxybutyrate excretion was high on the animal-vegetable combination and eliminated on vegetable fat only. It has been reported that different chain length triglycerides have different ketogenic effects (Zeh & Zee, 1976).

Further, it is possible that something in animal fat may promote ketogenesis in the Chinese hamster, perhaps by inhibition of fatty acid synthesis from acetyl CoA or prevention of acetyl CoA carbon from entering the Krebs cycle.

Another complication might be alteration in absorption of specific nutrients since severe pathological changes in the gut of diabetic Chinese hamsters have been reported (Diani *et al.* 1976). It is conceivable that lesions of the small intestine in some way favoured absorption of animal fat compared with vegetable fat.

The prediabetics used in these studies were not hyperphagic since food consumption and body-weights were not significantly different from matched nondiabetic controls (Table 3). It appears that plasma insulin levels are high from 2 weeks of age until the approach of maturity (10 weeks). This appears to be true for both prediabetic and nondiabetic hamsters. It is possible that the high insulin levels were related to rapid growth during this stage of development. However, it should be noted that plasma insulin levels of nondiabetics tended to decrease with age. By contrast, plasma insulin levels of prediabetics tended to increase with age.

The results suggest that something happened to the prediabetics between 6 and 10 weeks of age (Table 3). At 10 weeks of age, the animals were hyperglycaemic and hyperinsulinaemic. Also, there was a trend toward higher food consumption and body-weight. It is difficult to determine cause and effect relationships and important questions become evident. Did the hyperglycaemia stimulate hyperinsulinaemia? Did the slight increase in body-weight cause insulin

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insensitivity which resulted in hyperinsulinism and hyperglycaemia or did the hyperinsulinism exacerbate diabetic symptoms by increasing body-weight as suggested by Loten *et al.* (1976)? Some insight into these complex questions comes from the work of Frankel & Grodsky (1979). Continuous infusion of insulin into prediabetic Chinese hamsters suggested exacerbation of onset and severity of the disease (Frankel & Grodsky, 1979). They point out that the insulin infusion might result in increased fat deposition and insulin resistance since overly fat cells tend to be insulin resistant (Salans *et al.* 1968).

Despite the fact that prediabetic Chinese hamsters used in these studies were not hyperphagic, diet limitation had a dramatic effect (Table 4) similar to the effect reported previously for hyperphagic prediabetics (Gerritsen *et al.* 1974). It must be pointed out that diet limitation in these animals prevented weight gain but did not cause weight loss. Although not measured, it seems reasonable to expect that animals fed *ad lib.* had more body fat. It is not clear if energy restriction or body-weight or a combination of these factors resulted in prevention of hyperglycaemia and hyperinsulinism. However, it is very clear that simple energy restriction completely normalized plasma glucose and insulin levels.

Although energy restriction and probably body-weight are very important in the development of diabetes in this animal, composition of the diet appears to play a role also. As shown in Table 5, when prediabetics were fed on either high-fat diet containing animal and vegetable fat or a low-fat diet containing only vegetable fat *ad lib.* total energy consumption was similar but animals on the high-fat diet became hyperglycaemic. In this study, there was no significant difference in body-weight but there was a trend toward higher body-weight for animals fed on the high-fat diet.

In conclusion, it appears that total energy consumption, amount and type of dietary fat and body-weight influence the severity of established diabetes in the Chinese hamster and contribute to the onset of clinical symptoms in prediabetic animals. Unfortunately, the role of these factors and probable interactions between them appear to be complex and further studies are necessary to define the specific aetiology of diabetes mellitus in this animal model.

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