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Studies on Experimental Diabetes in the Wellesley Hybrid Mouse

III. Dietary Effects and Similar Changes in a Commercial Swiss-Hauschka Strain*

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Summary. Hybrid mice that develop mild diabetes and pancreatic hyperplasia were fed chow rations at two caloric concentrations. In addition, a strain of commercial laboratory mice were fed these diets in both powdered and pellet form. In all animals, hyperglycemia, increased weight gain, and elevated levels of immunoreactive insulin in serum were correlated with each other and resulted from increased caloric intake. These studies emphasize the critical role of diet in the study of experimental diabetes.

Etudes du syndrome diabétique des souris hybrides de Wellesley. III. Effets du régime et altérations similaires observées chez une souche de souris «Suisses-Hauschka» disponibles dans le commerce.

Résumé. Des souris hybrides produites par le croisement des souches C3Hf et I ont été soumises à une alimentation standard de laboratoire, à deux niveaux de concentration calorique. De plus, des souris d'une souche ordinaire ont reçu leur nourriture sous forme de poudre ou de conglomérés. Dans tous ces cas, nous avons observé une corrélation entre l'hyperglycémie, l'accroissement de poids et les taux sériques élevés d'insuline immunoréactive résultant de l'apport augmenté de calories. Ces observations soulignent l'importance extrême du régime dans l'étude du diabète expérimental.

Der experimentelle Diabetes der hybriden Wellesley-Maus. III. Auswirkungen der Diät und ähnliche Veränderungen bei einem im Handel erhältlichen Stamm von "Swiss-Hauschka" Mäusen.

Zusammenfassung. Mäuse, die aus der Kreuzung C3Hf × I hervorgingen, und die zu einer milden Form von Diabetes und Hyperplasie der Bauchspeicheldrüse neigen, erhielten zwei Sorten Laboratoriumskeks von unterschiedlichem Kaloriengehalt. Einem Stamm gewöhnlicher Labormäuse wurde außerdem das Futter als Pulver oder in Keks-Form verabreicht. Hyperglykämie, vermehrte Gewichtszunahme und erhöhte Spiegel an immunreaktivem Insulin verhielten sich untereinander parallel und waren das Resultat vermehrter Kalorienaufnahme. Diese Untersuchungen heben die kritische Rolle der Diät bei Studien des experimentellen Diabetes hervor.

Key-words: Spontaneous Diabetes, Wellesley hybrid mouse, Hybrid mouse diabetes, Genotype: C3Hf × I—F1, Strains of mice: C3Hf and I, Mice: C3Hf and I, Swiss-Hauschka mice, Hauschka Swriss mice, Nutrition and diabetes, Diet and diabetes, Beta cell hyperplasia, Insulin in Serum, Obesity, Caloric Intake.

In the previously reported study (Cahill et al., 1967), there appeared to be a dietary effect on the occurrence and severity of experimental diabetes in the Wellesley Hybrid mouse. This paper describes the results of an additional study of dietary influences on the development of hyperglycemia in these mice and a subsequent experiment utilizing a commercial mouse strain.

Material and Methods

The parentage of the Wellesley Hybrid mouse (C3Hf × I) has been previously reported (Jones, 1964). The commercial mouse strain[1] used in this study is random-bred from a cross between the Hauschka and Mirand-Roswell Park Memorial Institute inbred lines. This strain is designated HaU/ICR and is referred to by the commercial breeder as CD^{R}-1.

The initial study involved 12 adult male Wellesley mice obtained from Dr. Elizabeth E. Jones of the Children's Cancer Research Foundation, Boston, Massachusetts. The follow-up study utilized 100 weanling males from the commercial mouse strain. All animals were maintained under standard laboratory conditions. Food and water were available *ad libitum.*

Two commercial laboratory animal diets were utilized in both pellet and powdered forms. The analyses of the diets are as follows:

	Diet A[2] %	Diet B[3] %
Protein	19	23
Fat	11	4
Fibre	2	5
Nitrogen Free Extract (NFE)	52	51

The 12 adult Wellesley mice had been maintained on Diet A pellets (11% fat) from weaning, a period of approximately 10 to 12 months. The animals were weighed, and blood was taken four times during a seven-week period before making any dietary change. Then the animals were placed on Diet B pellets (4% fat) for a five-week period after which they were returned to Diet A pellets and were fed this for an additional 14 weeks. Body weights and blood samples were collected periodically during the Diet B pellet period and during the subsequent Diet A pellet period. The 100 commercial strain mice were separated

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Paper II of this series is Cahill et al. 1967.

[1] Obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts.

[2] Old Guilford Breeder Pellets, Guilford, Connecticut.

[3] Purina Laboratory Chow, St. Louis, Missouri.

at random into four equal groups and were treated as follows: Group I, Diet A pellets; Group II, Diet A powder; Group III, Diet B pellets; Group IV, Diet B powder. Body weights and blood samples were collected twice a month for six months postweaning and monthly thereafter. An additional sampling was made after six and a half months on the diets following an overnight fast. Blood samples were obtained from the tail at each sampling; 0.05 ml for glucose determination was rapidly transferred to a cup containing 0.95 ml of 0.9% sodium chloride, and was frozen. Blood glucose was determined by Auto-Analyser (Technicon Instruments Corporation, Chauncey, New York) using Technicon method N-9a. Serum immunoreactive insulin (IRI) was determined using a double antibody method (SOELDNER and SLONE, 1965)[4]. At those intervals when serum IRI levels were desired, an additional 0.2 ml of tail blood was collected directly in a small polyethylene test tube, allowed to clot overnight at 4°C, then centrifuged, the serum separated and frozen until assay.

Results

Mean body weights and glucose levels for 12 adult male Wellesley Hybrid mice are summarized in Table 1. From these data, the wide variation and the changes in glucose levels following dietary change are apparent.

Table 1. *The effect of diet on body weight and blood glucose levels in Wellesley Hybrid mice (n = 12)*

No. of weeks	Diet	Body weight (g) (Mean ± S.E.M.)	Blood glucose (mg%) (Mean ± S.E.M.)
0	A	45.3 ± 1.2	154 ± 24.4
1	(11% fat)	—	134 ± 5.2
2		45.9 ± 1.1	156 ± 3.7
7		49.9 ± 0.8	201 ± 11.5
1	B	49.1 ± 1.1	146 ± 9.7
2	(4% fat)	48.8 ± 1.0	140 ± 2.4
3		48.8 ± 1.0	140 ± 9.6
5		48.9 ± 1.0	145 ± 20.6
1	A	49.9 ± 1.0	180 ± 18.8
2	(11% fat)	50.5 ± 1.2	220 ± 36.2
3		50.1 ± 1.0	141 ± 8.5
4		50.8 ± 1.1	185 ± 20.0
5		51.1 ± 1.2	234 ± 27.3
6		52.6 ± 1.4	281 ± 34.5
7		52.9 ± 1.3	303 ± 30.1
11		53.0 ± 1.1	264 ± 26.3
13		52.8 ± 1.4	233 ± 29.3
14		54.1 ± 1.5	267 ± 30.4

At the last sampling prior to changing the animals to Diet B pellets (4% fat), seven of the 12 animals had glucose levels greater than 200 mg/100 ml. Within one week after Diet B pellets had been initiated, only one animal in the group remained above the 200 mg/100 ml level. At the end of the second week all animals were well below 200 mg/100 ml, the highest level being 157 mg/100 ml. By the fifth week on Diet B, however, one mouse, which had previously shown levels of 172 mg/100 ml or less for the last three test periods on Diet A pellets (11% fat), had a value of 368 mg/100 ml and remained over 200 for the remainder of the study period. After five weeks on Diet B the animals were again returned to Diet A pellets. One-half of the group (6) had glucose levels over 200 mg/100 ml after five weeks on this diet. This number increased to nine by the seventh week, but declined to seven after 14 weeks.

Following the completion of 14 weeks on Diet A pellets, the 12 Wellesley mice were divided into two equal groups, one including three and the other four of the animals with glucose levels greater than 200 mg%. Both groups were fed powdered Diet A, one group having their diet supplemented with an oral hypoglycemic agent (tolbutamide, 1 gram/100 gram of chow) and the other serving as a control group. After two weeks on the regimen the animals in both groups were normoglycemic with glucose levels of 133.8 ± 16.4 and 130.3 ± 11.5 mg/100 ml, respectively.

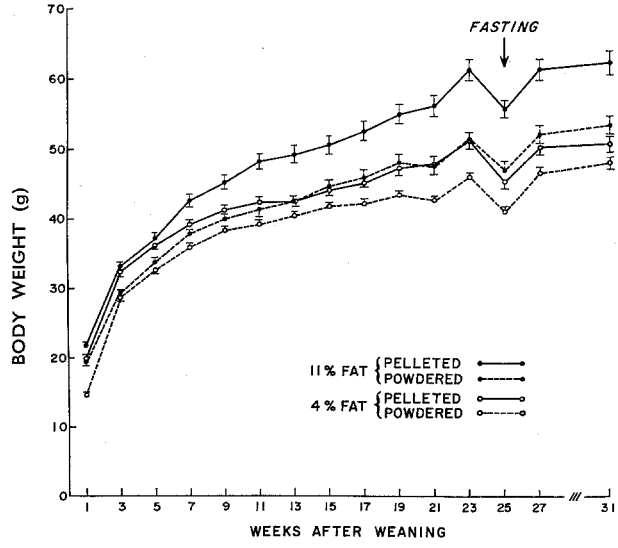

Fig. 1. Growth rates of a commercial strain of mouse on high and low fat diets presented as either pellets or as a powder. Twenty-five animals in each group. One weighing followed an overnight period of fasting, explaining the fall in weight at 25 weeks

A follow-up study was initiated to determine if a dietary effect similar to that described above in the Wellesley Hybrid mouse was apparent in a random-bred commercial mouse strain maintained on different diets from weaning. The mean body weights and glucose levels are shown in Fig. 1 and 2, respectively. Comparison of the two figures suggests that, in general, blood glucose concentration is related to body weight. Diet A pellets (11% fat) produced the most rapid growth rate, and that group also showed highest glucose levels. Similarly, animals fed on Diet B powder (4% fat) showed the slowest rate of growth and also had the lowest glucose levels.

[4] Rat insulin used as standards was a gift from Dr. J. SCHLICHTKRULL, Novo Research Institute, Copenhagen, Denmark.

Covariance analysis was used to test the hypothesis of no differences among diet means for glucose levels adjusted for the regression of glucose level on body weight. These data were analyzed after one week, four, six, six and one-half, seven, and eight months on the dietary regimens. At each of the six time periods, analysis of variance showed highly significant differences between the four diet groups with regard to both body weight and glucose levels (P is less than 0.01). When glucose levels were adjusted for the regression of glucose on body weight, the group differences became nonsignificant in all but the initial period (one week post-weaning). The reason for the early significant differences (P is less than 0.01) in glucose levels, which appear to be related to diet but not to body weight, is not known.

Table 2. *Relationship of diet regimen to body weight (B.Wt., g.), blood glucose (B.G., mg/100 ml), and immunoreactive insulin (IRI, μU/ml) at various intervals post-weaning*

Time Post-Weaning		Diet A (11% Fat)		Diet B (4% Fat)	
		Pellet	Powder	Pellet	Powder
1 week	B.Wt.	21.8 ± 0.51	19.4 ± 0.4	19.9 ± 0.5	14.6 ± 0.4
	B.G.	137.0 ± 3.0	132.0 ± 3.0	148.0 ± 3.0	123.0 ± 3.4
	IRI	—	—	—	—
2 months	B.Wt.	45.2 ± 1.1	40.0 ± 0.7	41.2 ± 0.7	38.4 ± 0.5
	B.G.	162.0 ± 7.8	151.0 ± 4.5	128.0 ± 3.8	128.0 ± 3.9
	IRI	34.0 ± 15.0	24.0 ± 3.4	9.0 ± 1.4	10.0 ± 1.7
4 months	B.Wt.	54.9 ± 1.4	48.0 ± 1.2	47.2 ± 1.0	43.4 ± 0.6
	B.G.	172.0 ± 12.0	148.0 ± 3.8	139.0 ± 3.3	130.0 ± 3.2
	IRI	192.0 ± 42.7	63.0 ± 9.4	44.0 ± 7.3	62.0 ± 10.9
$6^1/_2$ months (Fasting)	B.Wt.	55.5 ± 1.2	46.8 ± 1.3	45.1 ± 0.9	40.9 ± 0.7
	B.G.	102.0 ± 5.7	74.0 ± 3.1	76.0 ± 2.7	66.0 ± 1.8
	IRI	23.0 ± 5.6	12.0 ± 2.1	8.0 ± 1.1	47.0 ± 20.0

[1] (Mean ± S.E.M.).

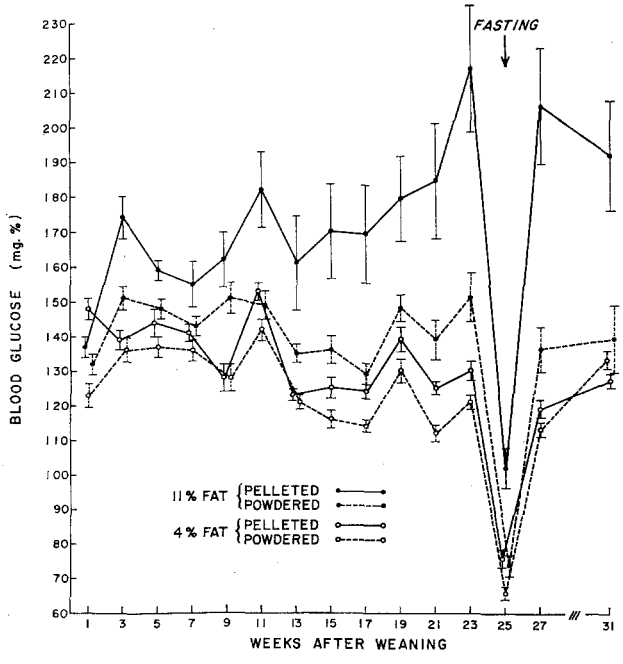

Fig. 2. Mean levels of blood glucose of the animals whose weights are presented in Fig. 1. The profound fall in glucose concentration at 25 weeks is following an overnight fast. The animals on the 11% fat pellets (Diet A) still have significantly higher levels of blood glucose

Table 3. *Simple correlation of data obtained after four months on different dietary regimens*

	Correlation coefficient
Diet A (11% fat) pellets	
Blood glucose vs. IRI	0.6094[2]
Blood glucose vs. body wt.	0.7921[2]
IRI vs. body wt.	0.6547[2]
Diet A (11% fat) powder	
Blood glucose vs. IRI	0.5130[1]
Blood glucose vs. body wt.	0.6329[2]
IRI vs. body wt.	0.6620[2]
Diet B (4% fat) pellets	
Blood glucose vs. IRI	0.6007[2]
Blood glucose vs. body wt.	0.5375[2]
IRI vs. body wt.	0.5085[2]
Diet B (4% fat) powder	
Blood glucose vs. IRI	0.2443
Blood glucose vs. body wt.	0.0874
IRI vs. body wt.	0.1194

[1] $P < 0.05$ (approaching the 0.01 level).
[2] $P < 0.01$.

Immunoreactive insulin (IRI) was measured four times during the study, at one week, two, four, and six and one-half months post-weaning. The mean IRI levels, glucose levels, and body weights are summarized in Table 2. Analysis of the IRI data obtained after four months on the dietary regimens indicated that differences between the four groups were highly significant (P is less than 0.01). When adjustment was made for the regression of IRI on body weight, however, the differences became non-significant.

Simple correlations were performed between glucose levels and IRI, glucose levels and body weights, and IRI and body weights after four months on the dietary regimes. The results are summarized in Table 3. With

one exception, all correlation coefficients obtained from both Diet A groups and from the Diet B pellet group were highly significant (P is less than 0.01). The correlation of glucose levels and IRI in the Diet A powder group was significant at the 0.05 level and approached significance at the 0.01 level. In the Diet B powder group, however, none of the correlation coefficients were statistically significant. The reason for this lack of relationship is not apparent.

Discussion

From these studies it appears that diet influences the incidence of experimental diabetes in the Wellesley Hybrid mouse, and that similar effects occur in a random-bred commercial mouse strain. In the latter strain, however, these changes are closely related to the rate of growth. It is of interest that some mice remain normoglycemic regardless of the diet they receive, whereas other individuals readily become hyperglycemic, and maintain high levels of blood glucose for prolonged periods of time. The proportion of this dietary effect on experimental diabetes in these two strains of mice that can be attributed to genetic factors has yet to be determined.

It is also of interest that the form in which the diet is presented can have such profound metabolic influences. For instance, grinding the pellets into a powder results in a smaller weight gain and no diabetic syndrome, emphasizing the variability of the animal's appetite and satiety mechanisms. This manoeuvre also enforces that it is the amount of diet consumed that is the provocative agent. These studies also emphasize the importance of diet in studying parameters relative to carbohydrate metabolism in experimental animals.

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