Reversal of Diet-Induced Obesity and Diabetes in C57BL/6J Mice

Priti I. Parekh, Ann E. Petro, Jane M. Tiller, Mark N. Feinglos, and Richard S. Surwit

We have previously shown that C57BL/6J (B6) mice develop severe obesity and diabetes if weaned onto high-fat diets, whereas A/J mice tend to be obesity and diabetes-resistant. The purpose of this study was to determine if obesity and diabetes in the B6 mouse could be completely reversed by reducing dietary fat content. After 4 months, both strains consumed more calories on a high-fat diet than on a low-fat diet, and both strains showed a higher feed efficiency (FE = weight gained/calories consumed) on the high-fat diet versus the low-fat diet. However, relative to A/J mice, B6 mice demonstrated a significantly higher FE on the high-fat diet. Hyperglycemia, hyperinsulinemia, and increased adiposity were apparent in B6 mice after 4 months on the high-fat diet regardless of whether the diet was begun at weaning or 4 months later. Correlational analyses showed that adiposity was strongly related to both insulin and glucose levels in B6 mice, but only moderately related to insulin levels in A/J mice. In obese B6 mice that were switched to a low-fat diet, obesity and diabetes were completely reversed. Adiposity, fasting glucose, and fasting insulin values in these mice were equivalent to those in B6 mice of the same age that had spent 8 months on the low-fat diet. In summary, our data show that in the B6 mouse the severity of diabetes is a direct function of obesity and diabetes is completely reversible by reducing dietary fat.

CONSUMPTION OF A DIET high in fat has been associated both with obesity and with diabetes mellitus (type 2 DM). An increase in dietary fat content has been shown to produce obesity in various strains of mice[1] and rats.[2] In humans, cross-sectional studies have demonstrated that the proportion of fat in the diet positively correlates with percent body fat independent of total energy intake,[3-5] and a longitudinal study found that increases in the proportion of fat in the diet predicted subsequent increases in body mass.[6] In addition to promoting obesity, high-fat diets have been associated with an increased risk for developing type 2 DM in epidemiological studies, and notably this association has been found independent of obesity.[7-9] Since obesity has repeatedly been shown to be one of the strongest risk markers for the development of diabetes,[10-13] high-fat diets may not only directly contribute to diabetes risk, but may also do so indirectly through their potential to induce obesity.

One animal model that is particularly susceptible to the effects of dietary fat is the C57BL/6J (B6) mouse. This animal will develop severe obesity, hyperglycemia, and hyperinsulinemia when weaned onto high-fat diets, but will remain lean and euglycemic if the fat content of the diet is limited.[14-16] As a model of type 2 DM, B6 mice seem to closely resemble common forms of the human disease in that they will only manifest the disease after developing obesity.[14] Also, diet-induced obesity in the B6 mouse is characterized by selective deposition of fat in the mesentery,[16,17] an observation consistent with the finding that abdominal obesity is an independent risk factor for diabetes.[11,13] In summary, the B6 mouse represents a model of type 2 DM in which the disease results from the interaction between environmental factors and genetic predisposition.[14,15]

While there is some consensus that high-fat diets contribute to the development of obesity and diabetes in humans and animals, it is currently not known whether diabetes can be reversed by reducing dietary fat. In animals, reversal of obesity through reduction of dietary fat has been reported,[18-21] but there is also evidence of persistent obesity on a low-fat diet,[22-24] particularly in animals with obesity characterized by adipocyte hyperplasia.[23,24] In humans, ad libitum feeding of a low-fat diet has been shown to produce weight loss,[25,26] which in patients with type 2 DM has been associated with improvements in glycemic control and insulin secretory response.[27,28] Furthermore, low-fat diets acutely produce improvements in glycemic control in type 2 DM even in the absence of weight loss,[29-31] suggesting that obesity need not be reduced in order for a low-fat diet to be beneficial in diabetes. While there is one report of weight loss producing a complete remission of diabetes,[32] it is still not clear that the disease is entirely reversible. We now report that dramatically reducing the amount of fat in the diet can completely reverse diet-induced diabetes and obesity in B/6 mice.

MATERIALS AND METHODS

Animals

Seventy A/J and 70 B6 male mice were obtained at the age of 4 weeks from Jackson Laboratories (Bar Harbor, ME). The mice were housed five per cage in a temperature-controlled room and maintained on a 12-hour light/dark cycle. All mice were given ad libitum access to water and to food as described later.

Experimental Design

Ten mice from each strain were bled and killed for baseline measurements. The remaining animals were divided into the following four groups: 10 mice from each strain were weaned onto a low-fat diet and remained on this diet for 8 months (L8); 10 mice from each strain were fed a high-fat diet for 8 months (H8); 20 animals from each strain were fed the low-fat diet for 4 months, at which time 10 mice from each strain were killed and the remaining 10 mice were switched to the high-fat diet for 4 months (L4H4); and 20 animals from each strain were fed the high-fat diet for 4 months, at which time half the mice were killed and the remaining half were switched to the low-fat diet for 4 months (H4L4). The diets were manufactured by Research Diets (New Brunswick, NJ) and their composition is listed in Table 1.

The A/J mice that were switched from the high-fat diet to the low-fat diet would not eat the low-fat diet, preferentially consuming their own

From the Departments of Psychiatry and Behavioral Sciences and Medicine, Duke University Medical Center and Department of Psychology, Social and Health Sciences, Duke University, Durham, NC.

Submitted September 30, 1997; accepted March 18, 1998.

Address reprint requests to Richard S. Surwit, Duke University Medical Center, Box 3842, Durham, NC 27710.

0026-0495/98/4709-0011$03.00/0

Table 1. Composition of the Diets

Variable	Low-Fat Diet	High-Fat Diet
kcal/g	4.07	5.55
Macronutrients (as % of total calories)		
Fat	11%	58%
Carbohydrate	73%	26%
Protein	16%	16%
Ingredients (g/kg)		
Casein	167.0	167.0
DL-Methionine	1.5	1.5
Maltodextrin 10	124.0	124.0
Corn starch	611.0	0.0
Sucrose	0.0	128.0
Soybean oil	18.3	18.3
Coconut oil, hydrogenated	29.3	244.0
Salt mix	29.3	29.3
Sodium bicarbonate, 27.4% Na	7.7	7.7
Potassium citrate, $1H_2O$, 36.2% K	2.9	2.9
Vitamin mix	7.3	7.3
Choline bitartrate	1.5	1.5
Yellow dye FD&C no. 5	0.07	0.00
Blue dye FD&C no. 1	0.00	0.00
Red dye FD&C no. 40	0.00	0.07

Abbreviation: FD&C, Food, Drug and Cosmetic.

feces and the cage bedding to keep from starving, so after 4 weeks, the low-fat diet was replaced with Purina rodent chow (Purina Mills, St Louis, MO). Chow is also low in fat, although it contains a greater percentage of indigestible fiber than the experimental low-fat diet. The caloric content of chow is 3.30 kcal/g and it derives 4.5% of its calories from fat and 23% from protein.

Body Weight, Food Intake, and Feed Efficiency

The body weight of each animal was measured weekly throughout the study. Food intake was assessed by weighing the food in each cage's dispenser daily to determine how much had been eaten. Food that spilled onto the floor of the cage was not measured, but since the diets were pelleted, spillage was minimal. Food intake was measured on a per cage basis rather than for each animal, to avoid the stress of individual housing. Feed efficiency (FE) was calculated as follows: grams of body weight gain per cage/kilocalories of food consumed per cage.

Fat-Pad Weights

The mice were killed at baseline, at 4 months, and at 8 months for determining the weight of four fat pads: inguinal (ING), retroperitoneal (RP), epididymal (EPI), and mesenteric (MES). The fat pads were dissected from each animal according to defined anatomic landmarks. All subcutaneous fat between the lower part of the rib cage and mid-thigh was considered ING fat, whereas all fat found along the mesentery starting at the lesser curvature of the stomach and ending at the sigmoid colon was considered MES fat. The weight of fat pads was expressed in relative terms (ING%, RP%, EPI%, and MES%) by dividing the individual fat pad weight (in grams) by eviscerated body weight (in grams). An adiposity index was calculated as follows: ([ING + RP + EPI + MES fat pad weights in grams]/eviscerated body weight in grams) × 100.

Fasting Plasma Glucose and Insulin

Blood was obtained from the 4-week-old baseline mice and from all of the other animals at 4 months and 8 months for glucose and insulin measurements. Blood was drawn after an 8-hour fast via retroorbital sinus puncture in nonanesthetized mice. Plasma glucose concentration was determined using a Beckman Glucose Analyzer 2 (Palo Alto, CA). Plasma insulin was measured with a double-antibody radioimmunoassay kit based on a rat standard (Linco Research, St Louis, MO).

Data Analysis

For comparisons of the baseline measures of body weight, adiposity, glucose, and insulin in the 4-week-old mice, unpaired two-tailed t tests were used. For individual fat pads, adiposity index, glucose, and insulin measurements, a 2 × 2 × 2 ANOVA (strain × diet × time on diet) was used to assess differences between the low- and high-fat diets in both strains at both time points (4 and 8 months) and a 2 × 4 ANOVA with strain and diet (L8, H8, L4H4, H4L4) as the factors was used to assess differences at 8 months. Post-hoc Scheffé's tests were used for means comparisons. Body weight was assessed at 4 and 8 months with two-way ANOVAs (strain × diet). The relationship of adiposity index with glucose and insulin at 8 months was assessed with Pearson's correlation coefficients. Cumulative food intake was determined for the first 4 months of the study and FE was calculated for this period. Both food intake and FE were assessed with a two-way ANOVA (strain × diet). Since FE and food intake were measured per cage, statistics could only be performed on the data from the first 4 months, since after that point, only two cages per group remained.

RESULTS

Baseline Measures

Baseline measurements are listed in Table 2. A/J mice had significantly higher fasting plasma glucose ($t = 2.836$, $P = .011$) and adiposity ($t = 6.472$, $P = .000004$) measures than B6 mice at the age of 4 weeks.

Body Weight

At 4 Months

Table 2 lists body weights at 4 months. There were significant main effects for strain ($F_{1,116} = 112.96$, $P < .000001$) and diet ($F_{1,116} = 543.93$, $P < .000001$), and a significant interaction between strain and diet ($F_{1,116} = 166.84$, $P < .000001$). Post-hoc tests showed that both strains were heavier on the high-fat diet versus the low-fat diet ($P < .000001$ for both strains). Also, B6 mice on the high-fat diet were heavier than the A/J mice on the same diet ($P < .000001$).

At 8 Months

Body weights after 8 months are shown in Fig 1. There were significant main effects for strain ($F_{1,71} = 56.75$, $P < .000001$)

Table 2. Plasma Glucose and Insulin, Adiposity, and Body Weight of A/J and B6 Mice at Baseline and After 4 Months on the Diets

Strain	Plasma Glucose (mg/dL)	Plasma Insulin (μU/mL)	Adiposity Index	Body Weight (g)
A/J				
Baseline	177 ± 4*	30.2 ± 5.6*	6.9 ± 0.4*	18.0 ± 0.6*
L4	145 ± 3 (29)	25.0 ± 1.8 (29)	9.9 ± 0.4*	27.9 ± 0.4†
H4	148 ± 2†	34.8 ± 3.0†	18.6 ± 1.1*	33.5 ± 0.6†
B6				
Baseline	156 ± 6*	31.1 ± 5.7*	4.1 ± 0.2*	16.6 ± 0.4*
L4	122 ± 3†	20.1 ± 4.2 (28)	7.4 ± 0.2*	26.7 ± 0.3†
H4	221 ± 4†	106.1 ± 8.0†	21.2 ± 0.7*	46.1 ± 0.7†

NOTE. Values are means ± SEM. Significance levels are shown in text.

*n = 10, †n = 30; otherwise n is shown in parentheses.

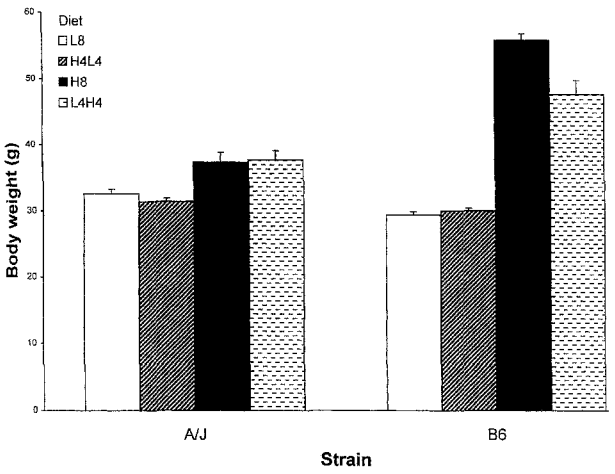

Fig 1. Body weight in A/J and B6 mice after 8 months on 4 diets. Values are means ± SEM (n = 9 for B6 mice on L4H4 diet; n = 10 for all other groups). Significance levels are given in the text.

and for diet ($F_{3,71} = 106.17$, $P < .000001$), and a significant interaction between strain and diet ($F_{3,71} = 42.09$, $P < .000001$). Post-hoc tests showed that in A/J mice, the L4H4 group was heavier than the H4L4 group ($P < .04$). Within B6 mice, the two groups ending with the high-fat diet were heavier than the two groups ending with the low-fat diet (H8 and L4H4 *v* L8 and H4L4, $P < .000001$ for all comparisons). Also in B6 mice, the group which was on the high-fat diet for 8 months (L8) weighed more than the group of mice switched from the low-fat diet to the high-fat diet (L4H4) ($P < .002$). Between strains, B6 mice were heavier than A/J mice in both the groups ending with the high-fat diet (H8, $P < .000001$; L4H4, $P < .00006$).

Food Intake

Food intake is shown in Fig 2. There were main effects for strain ($F_{1,20} = 21.67$, $P < .001$) and diet ($F_{1,20} = 168.08$, $P < .000001$), as well as a significant interaction of strain and diet ($F_{1,20} = 5.64$, $P < .03$) for cumulative food intake during the first 4 months of the study. Post-hoc tests showed that both A/J and B6 strains consumed more kilocalories on the high-fat diet ($P < .00001$ and $P < .000001$, respectively) than on the low-fat diet. Also, B6 mice consumed more kilocalories on the high-fat diet than A/J mice ($P < .001$).

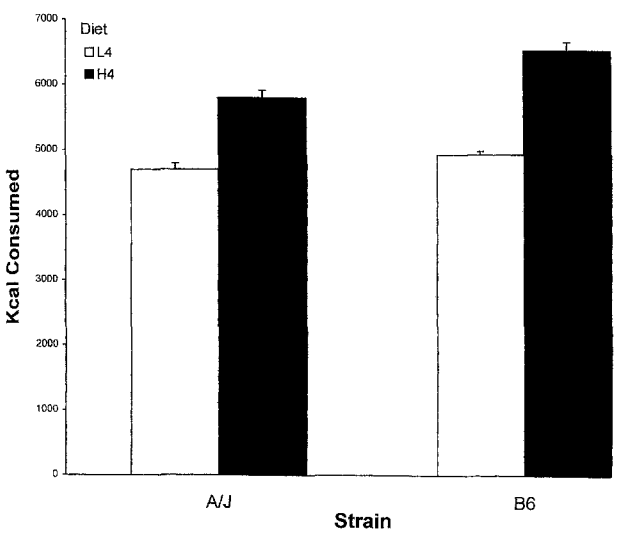

Fig 2. Cumulative food intake in A/J and B6 mice after 4 months on the low- and high-fat diets. Values are means ± SEM (n = 6 cages, 5 animals per cage, for all 4 groups). Significance levels are given in the text.

FE

FE is shown in Fig 3. There were main effects for strain ($F_{1,20} = 23.67$, $P < .0001$) and diet ($F_{1,20} = 309.85$, $P < .000001$), and a significant interaction between strain and diet ($F_{1,20} = 122.20$, $P < .000001$) for FE. Post-hoc comparisons showed that both strains had a higher FE on the high-fat diet than on the low-fat diet ($P < .01$ for A/J and $P < .000001$ for B6). B6 mice had a significantly higher FE on the high-fat diet than A/J mice ($P < .000001$) and A/J mice had a higher FE on the low-fat diet as compared with B6 mice ($P < .01$).

Regional Fat Distribution

ING Fat Pad

Low- versus high-fat diets. See Table 3. There were main effects for diet ($F_{1,72} = 457.47$, $P < .000001$) and time on diet ($F_{1,72} = 22.36$, $P < .0001$), as well as significant interactions between strain and diet ($F_{1,72} = 49.25$, $P < .000001$) and strain by diet by time on diet ($F_{1,72} = 8.67$, $P < .005$). Within both A/J and B6 mice, ING% was greater on the high-fat diet versus the low-fat diet at 4 months (both strains, $P < .000001$) and 8 months (A/J, $P = .0001$; B6, $P < .000001$). In B6 mice, ING% was greater after 8 months on the high-fat diet as compared with 4 months on the high-fat diet ($P = .007$). Between strains, AJ mice had a greater ING% than B6 mice after 8 months on the low-fat diet ($P = .002$), while B6 mice had a greater ING% than A/J mice after 8 months on the high-fat diet ($P = .003$).

All four diet groups at 8-month end point. See Table 3. There was a main effect for diet ($F_{3,70} = 151.13$, $P < .000001$) and an interaction between strain and diet ($F_{3,70} = 18.82$,

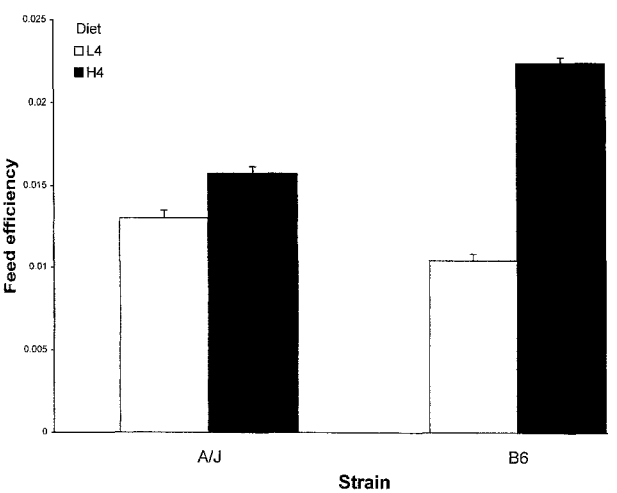

Fig 3. FE in A/J and B6 mice after 4 months on the low- and high-fat diets. Values are means ± SEM (n = 6 cages, 5 animals per cage, for all 4 groups). Significance levels are given in the text.

Table 3. Relative Weight of Four Fat Pads in A/J and B6 Mice After 4 Months and After 8 Months on the Diets

Strain	ING (g)	RP (g)	EPI (g)	MES (g)
A/J				
4 months				
L4	3.5 ± 0.2	0.9 ± 0.1	3.1 ± 0.2	2.3 ± 0.1
H4	6.9 ± 0.5	1.7 ± 0.1	7.0 ± 0.3	3.0 ± 0.2
8 months				
L8	4.8 ± 0.2	1.4 ± 0.1	4.5 ± 0.1	2.8 ± 0.1
H4L4	3.4 ± 0.2	1.0 ± 0.1	3.4 ± 0.2	2.3 ± 0.1
H8	7.2 ± 0.5	1.6 ± 0.1	5.4 ± 0.3	3.4 ± 0.3
L4H4	6.9 ± 0.4	1.8 ± 0.1	6.3 ± 0.2	3.5 ± 0.2
B6				
4 months				
L4	2.2 ± 0.1	0.6 ± 0.0	2.9 ± 0.1	1.7 ± 0.1
H4	7.3 ± 0.3	1.8 ± 0.1	8.3 ± 0.4	3.9 ± 0.2
8 months				
L8	2.7 ± 0.1	0.8 ± 0.1	3.7 ± 0.2	1.9 ± 0.1
H4L4	2.5 ± 0.2 (9)	0.7 ± 0.1 (9)	3.6 ± 0.3 (9)	1.3 ± 0.1 (9)
H8	9.2 ± 0.2	2.0 ± 0.1	6.8 ± 0.3	3.9 ± 0.1
L4H4	7.9 ± 0.3 (9)	2.0 ± 0.1 (9)	8.6 ± 0.3 (9)	4.1 ± 0.3 (9)

NOTE. Relative weight calculated as (fat pad weight in grams/ eviscerated body weight in grams). Values are means ± SEM. Significance levels are shown in text. n = 10 unless indicated otherwise in parentheses.

$P < .000001$). In both A/J and B6 mice, both diet groups that ended with the high-fat diet showed a greater ING% than both groups that ended with the low-fat diet (A/J: H8 *v* L8 $P = .0002$, H8 *v* H4L4 $P < .000001$, L4H4 *v* L8 $P = .002$, L4H4 *v* H4L4 $P < .000001$; B6: $P < .000001$ for all comparisons). Between strains, A/J mice showed a higher ING% than B6 mice after 8 months on the low-fat diet ($P = .003$), while B6 mice had a greater ING% than A/J mice after 8 months on the high-fat diet ($P = .005$).

RP Fat Pad

Low- versus high-fat diets. See Table 3. There were main effects for strain ($F_{1,72} = 4.33$, $P < .05$), diet ($F_{1,72} = 311.67$, $P < .000001$), and time on diet ($F_{1,72} = 18.34$, $P < .00006$), as well as significant interactions between strain and diet ($F_{1,72} = 43.38$, $P < .000001$), diet and time on diet ($F_{1,72} = 12.03$, $P < .001$), and strain by diet by time on diet ($F_{1,72} = 7.49$, $P < .008$). Post-hoc tests show that in A/J and B6 mice, RP% is greater on the high-fat diet versus the low-fat diet at 4 months ($P < .000001$ for both strains) and at 8 months in B6 mice only ($P < .000001$). In A/J mice, RP% is higher after 8 months versus 4 months on the low-fat diet ($P < .002$). Between strains, A/J mice have a greater RP% than B6 mice after 8 months on the low-fat diet ($P < .0004$).

All four diet groups at 8-month end point. See Table 3. There was a main effect for diet ($F_{3,70} = 97.35$, $P < .000001$) and an interaction between strain and diet ($F_{3,70} = 16.5$, $P < .000001$). In A/J mice, the group that switched from the high to the low-fat diet (H4L4) had a smaller RP% than all three of the other diet groups (H4L4 *v* L8, $P < .04$; H4L4 *v* H8, $P < .00004$; H4L4 *v* L4H4, $P < .000001$). Also in A/J mice, the L4H4 group had a larger RP% than the L8 group ($P < .05$). In B6 mice, both diet groups ending with the high-fat diet showed a greater RP% compared with both diet groups ending with the low-fat diet ($P < .000001$ for all comparisons). Between strains, A/J mice had a greater RP% than B6 mice after 8 months on the low-fat diet ($P < .001$).

EPI Fat Pad

Low- versus high-fat diets. See Table 3. There were main effects for strain ($F_{1,72} = 4.20$, $P < .05$) and diet ($F_{1,72} = 318.72$, $P < .000001$), as well as significant interactions between strain and diet ($F_{1,72} = 25.03$, $P < .00001$) and between diet and time on diet ($F_{1,72} = 49.22$, $P < .000001$). In both A/J and B6 mice, EPI% was greater on the high-fat diet versus the low-fat diet at 4 months ($P < .000001$ for both strains) and at 8 months in B6 mice only ($P < .000001$). Also, both A/J and B6 mice showed a smaller EPI% after 8 months on the high-fat diet versus after 4 months on the high-fat diet (A/J, $P < .03$; B6, $P < .05$).

All four diet groups at 8-month end point. See Table 3. There were main effects for strain ($F_{1,70} = 17.73$, $P < .00008$) and diet ($F_{3,70} = 108.74$, $P < .000001$), and a significant interaction between strain and diet ($F_{3,70} = 16.06$, $P < .000001$). In A/J mice, the group on the low-fat diet for 8 months had a higher EPI% compared with the H4L4 group ($P < .0002$), while the L4H4 group had a higher EPI% versus both the L8 group ($P < .002$) and the H4L4 group ($P < .000001$). In B6 mice, both groups ending with the high-fat diet showed a higher EPI% versus both groups ending with the low-fat diet ($P < .000001$ for all comparisons). Between strains, B6 mice showed a greater EPI% than A/J mice in both the groups ending with the high-fat diet (H8, $P < .04$; L4H4, $P < .00002$).

MES Fat Pad

Low- versus high-fat diets. See Table 3. There were main effects for diet ($F_{1,72} = 116.26$, $P < .000001$) and time on diet ($F_{1,72} = 5.45$, $P < .03$), as well as a significant interaction between strain and diet ($F_{1,72} = 37.60$, $P < .000001$). Within B6 mice, MES% was greater on the high-fat diet versus the low-fat diet at 4 and 8 months (both comparisons, $P < .000001$). Across strains, A/J mice had a larger MES% than B6 mice after 8 months on the low-fat diet ($P < .05$).

All four diet groups at 8-month end point. See Table 3. There was a main effect for diet ($F_{3,70} = 46.96$, $P < .000001$) and an interaction between strain and diet ($F_{3,70} = 9.52$, $P < .00003$). Within A/J mice, the L4H4 group had a higher MES% versus the H4L4 group ($P < .02$). In B6 mice, both groups ending with the high-fat diet showed a greater MES% than both groups ending with the low-fat diet ($P < .000001$ for all comparisons).

Adiposity Index

Low- Versus High-Fat Diets

There were main effects for diet ($F_{1,72} = 478.39$, $P < .000001$) and time on diet ($F_{1,72} = 7.82$, $P < .007$), as well as significant interactions between strain and diet ($F_{1,72} = 58.08$, $P < .000001$), diet and time on diet ($F_{1,72} = 9.79$, $P < .003$), and strain by diet by time on diet ($F_{1,72} = 4.13$, $P < .05$). In both strains, after 4 months, adiposity was greater on the high-fat diet versus the low-fat diet (A/J, $P < .000001$; B6, $P < .000001$; Table 2), demonstrating the development of obesity. Adiposity in both strains was also higher on the high-fat

diet versus the low-fat diet at 8 months (A/J, $P < .009$; B6, $P < .000001$), although it was not significantly different from adiposity after 4 months on the high-fat diet for either strain. Between strains, B6 mice had greater adiposity on the high-fat diet after 8 months compared with A/J mice ($P < .006$), while A/J mice had greater adiposity on the low-fat diet compared with B6 mice at 8 months ($P < .004$).

All Four Diet Groups at 8-Month End Point

There was a significant main effect of diet ($F_{3,70} = 161.38$, $P < .000001$) and an interaction between strain and diet ($F_{3,70} = 23.20$, $P < .000001$) (Fig 4). Post-hoc tests showed that in the A/J mice and the B6 mice, both groups that ended with the high-fat diet had higher adiposity than both groups that ended with the low-fat diet (A/J: H8 *v* L8 $P < .009$, H8 *v* H4L4 $P < .000001$, L4H4 *v* L8 $P < .0005$, L4H4 *v* H4L4 $P < .000001$; B6: $P < .000001$ for all comparisons). Note that in both A/J and B6 mice, the adiposity that developed after 4 months on the high-fat diet was reversed in animals that were then switched to the low-fat diet for 4 months (H4L4 and L8 groups are not statistically different for either strain). Also, in both strains, adiposity occurred to the same extent regardless of when the diet was started (L4H4 and H8 groups are the same within both strains). Across strains, B6 mice had greater adiposity than A/J mice in both the diet groups that ended with high-fat (H8, $P < .006$; L4H4, $P < .02$), while A/J mice had greater adiposity than B6 mice in the L8 group ($P < .004$).

Fasting Plasma Glucose

Low- Versus High-Fat Diets

There were significant main effects for strain ($F_{1,151} = 37.92$, $P < .000001$), diet ($F_{1,151} = 195.40$, $P < .000001$), and time on diet ($F_{1,151} = 14.49$, $P < .0003$), as well as significant interactions between strain and diet ($F_{1,151} = 220.96$, $P < .000001$), strain and time on diet ($F_{1,151} = 5.09$, $P < .03$), and diet and time on diet ($F_{1,151} = 10.58$, $P < .002$). A/J mice showed no differences in glucose levels regardless of diet or time on the diet. However, in B6 mice, glucose was elevated on the high-fat diet as compared with the low-fat diet at 4 months, demonstrating the development of hyperglycemia ($P < .000001$; Table 2.)

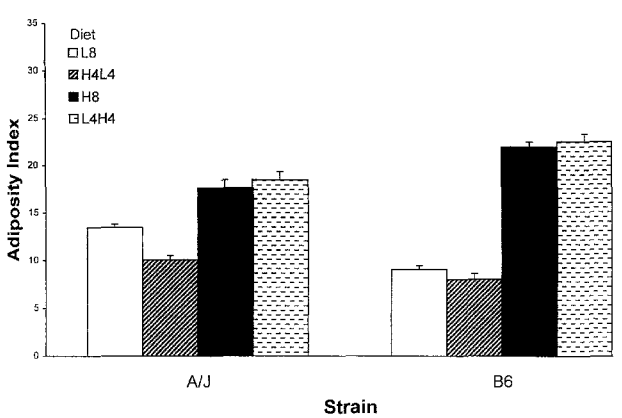

Fig 4. Adiposity index in A/J and B6 mice after 8 months on 4 diets. Values are means ± SEM (n = 9 for B6 mice on L4H4 diet; n = 10 for all other groups). Significance levels are given in the text.

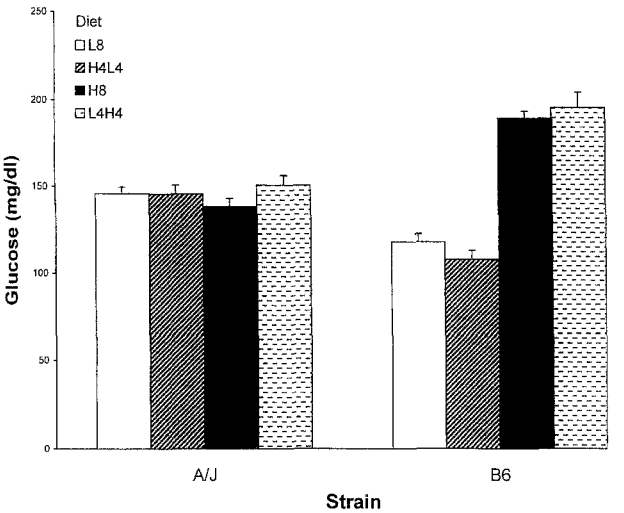

Fig 5. Fasting plasma glucose in A/J and B6 mice after 8 months on 4 diets. Values are means ± SEM (n = 9 for B6 mice on H4L4 and L4H4 diets, n = 10 for all other groups). Significance levels are given in the text.

While glucose remained elevated in B6 mice after 8 months on the high-fat diet as compared with the low-fat diet ($P < .000001$), it was lower than the glucose level after 4 months on the high-fat diet ($P < .0003$). Comparing strains, at both 4 and 8 months on the high-fat diet, B6 mice had significantly higher glucose levels than A/J mice ($P < .000001$ for both time points), while on the low-fat diet A/J mice had higher glucose levels than B6 mice at both 4 months ($P < .0002$) and 8 months ($P < .05$).

All Four Diet Groups at 8-Month End Point

Fasting plasma glucose values after 8 months are shown in Fig 5. There were significant main effects for diet ($F_{3,70} = 36.16$, $P < .000001$) and strain ($F_{1,70} = 3.98$, $P < .05$), but, more importantly, there was a highly significant interaction between diet and strain ($F_{3,70} = 37.81$, $P < .000001$). While glucose levels in A/J mice were not significantly different from each other regardless of diet, B6 mice demonstrated increased glucose levels in both groups that ended with the high-fat diet, H8 and L4H4, as compared with both groups that ended with the low-fat diet, L8 and H4L4 ($P < .000001$ for all comparisons). Note that in B6 mice, the hyperglycemia that developed after 4 months on the high-fat diet was completely reversed when the animals were subsequently switched to the low-fat diet (H4L4 and L8 groups are not statistically different). Also, hyperglycemia occurred to an equal extent in the B6 mouse, regardless of whether the diet was introduced at weaning or 4 months later (H8 and L4H4 groups are not different). Between strains, B6 mice had a higher glucose level than A/J mice in both groups that ended with the high-fat diet (H8, $P < .000006$; L4H4, $P < .0002$) while A/J mice had a higher glucose level than B6 mice on the H4L4 diet ($P < .0002$).

Fasting Plasma Insulin

Low- Versus High-Fat Diets

There were significant main effects for strain ($F_{1,148} = 36.72$, $P < .000001$), diet ($F_{1,148} = 79.41$, $P < .000001$), and time on

diet ($F_{1,148} = 6.72$, $P < .02$), as well as significant interaction between strain and diet ($F_{1,148} = 52.49$, $P < .000001$). In A/J mice, there were no significant differences in insulin across diets. In B6 mice, insulin was higher after 4 months on the high-fat diet as compared with the low-fat diet, demonstrating the development of hyperinsulinemia ($P < .000001$; Table 2). Insulin remained elevated in B6 mice after 8 months on the high-fat diet versus the low-fat diet ($P < .00001$), but was not significantly different from insulin after 4 months on the high-fat diet. Between strains, B6 mice had higher insulin levels than A/J mice on the high-fat diet at both 4 months ($P < .000001$) and 8 months ($P < .0002$).

All Four Diet Groups at 8-Month End Point

There were significant main effects of diet ($F_{3,69} = 11.58$, $P < .000004$) and strain ($F_{1,69} = 21.36$, $P < .00002$), as well as a significant interaction between diet and strain ($F_{3,69} = 6.00$, $P < .002$) (Fig 6). A/J mice showed no differences in insulin among the four diet groups. Among B6 mice, insulin was higher in both groups that ended with the high-fat diet as compared with both groups that ended with the low-fat diet (H8 *v* L8, $P < .002$; H8 *v* H4L4, $P < .005$; L4H4 *v* L8, $P < .003$; L4H4 *v* H4L4, $P < .007$). Note that in B6 mice, the hyperinsulinemia present after 4 months on the high-fat diet was completely reversed after mice subsequently spent 4 months on the low-fat diet (H4L4 and L8 groups are not different). Also, hyperinsulinemia occurred to the same extent in B6 mice regardless of whether the high-fat diet was started at weaning or 4 months later (H8 and L4H4 groups are not different). Between strains, B6 mice had higher insulin than A/J mice in the two groups that ended on the high-fat diet (H8, $P < .02$; L4H4, $P < .02$).

Relationship of Adiposity With Glucose and Insulin at 8 Months

In A/J mice, there was no significant correlation between glucose and adiposity and a significant positive correlation between insulin and adiposity ($r = .45$, $P < .05$, n = 40). In B6 mice, there was a significant positive correlation between glucose and adiposity ($r = .92$, $P < .05$, n = 38) and also a significant positive correlation between insulin and adiposity ($r = .72$, $P < .05$, n = 37).

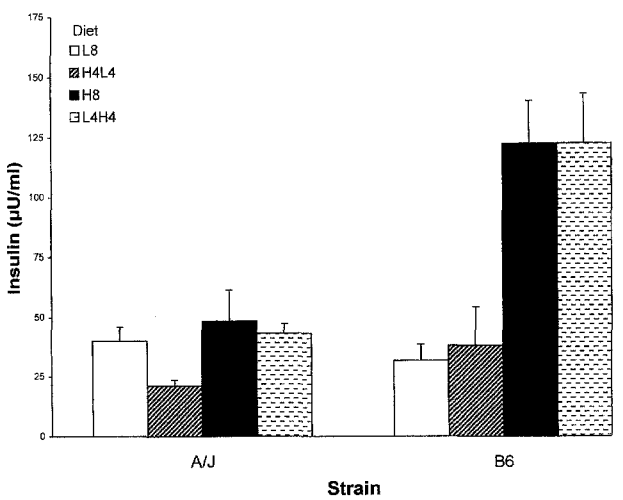

Fig 6. Fasting plasma insulin in A/J and B6 mice after 8 months on 4 diets. Values are means ± SEM (n = 9 for B6 mice on L8, H4L4, and L4H4 diets; n = 10 for all other groups). Significance levels are given in the text.

DISCUSSION

Our results demonstrate the complete reversal of diet-induced obesity and diabetes in B6 mice when obese animals were switched to a low-fat diet. This is notable given that B6 mice show adipocyte hyperplasia on high-fat diets,[16,17] and other reports have suggested that hyperplastic obesity in rodents cannot be reversed.[23,24] However, our study is in part consistent with a report by Hill et al,[23] who found that in rats a 17-week period of high-fat feeding induced hyperplastic obesity that could be reversed with ad libitum feeding of a low-fat diet, but that when high-fat feeding was extended to 30 weeks, obesity was not reversible. This suggests that the longer duration of the obesity or of the high-fat feeding resulted in a more permanent alteration in body composition. In the present study, we found that with a longer period of fat feeding (4 months *v* 8 months), neither diabetes nor obesity (as measured by adiposity index) became more severe. It is also notable that adiposity and diabetes occurred regardless of whether B6 mice were weaned onto the high-fat diet or began the high-fat diet 4 months later. This suggests that diabetes in B6 mice is tightly linked to dietary variables, a conclusion supported by the strong positive correlations found between adiposity and both fasting plasma glucose and insulin levels in B6 mice. In contrast, A/J mice, like normal humans,[33,34] only showed a moderate correlation between adiposity and insulin and no relationship between obesity and glucose. This is consistent with a previous study in which we demonstrated that insulin resistance and hyperglycemia are controlled by different genetic factors, both of which are needed in order for diabetes to appear.[15] However, one limitation of our study is that we failed to include body growth parameters other than body weight. Since our experimental diets were introduced at weaning, the possibility that body growth may have been differentially influenced by the diets cannot be ruled out.

Several differences in the metabolic response to fat feeding between B6 and A/J mice may explain why fat causes diabetes and severe obesity in the B6 strain. First of all, B6 mice demonstrate depressed adenylyl cyclase activity in both white and brown adipocytes in response to beta-adrenergic stimulation when on a high-fat diet.[35] The consequences of this type of defect would likely include reduced lipolysis in white fat, as well as reduced thermogenesis in brown fat, resulting in the preservation of adipose tissue in B6 mice consuming a high-fat diet. Also, we have shown that B6 mice have a significantly smaller leptin response to the initiation of fat-feeding as compared with A/J mice.[36] Since leptin stimulates sympathetic outflow to brown fat,[37] B6 mice may be less able to dissipate calories as heat via thermogenesis when a high-fat diet is begun. Finally, we have recently identified a novel uncoupling protein[38] (UCP2) that is genetically linked to diabetes and obesity in B6 mice.[39] UCP2 is upregulated in white adipocytes in A/J, but not B6, mice in response to fat-feeding.[38] Thus, the differential

expression of this protein may partially explain the observed interaction of dietary fat and the B6 genetic background.

In this study, we examined the effect of the diets on four individual fat pads, expressed relative to the eviscerated carcass weight. After 8 months on the high-fat diet, the ING and EPI fat pads were relatively greater in B6 mice than in A/J mice, while there was no significant difference in either the RP or MES fat pad between strains. Interestingly, in B6 mice, all of the fat pads increased on the high-fat diet versus the low-fat diet at 4 months, but in A/J mice, all fat pads except the MES fat pad increased. This finding is consistent with our previous work showing that, relative to A/J mice, B6 mice have a tendency to deposit fat in the mesentery on high-fat diets.[16,17]

Contrary to our prior reports,[16,40] we found that B6 mice consumed more calories than A/J mice on the high-fat diet. In the present study, food intake was measured on a daily basis, which was a more accurate assessment than measuring food intake for only one 24-hour period per week, as was done previously.[16,40] However, our method for assessing food intake in this study was still subject to error, since pellets that spilled out of the cage's food dispenser were not measured. It is important to note that the difference in cumulative caloric intake between the strains was relatively small (6,532 ± 131 *v* 5,803 ± 118 kcal), and since B6 mice were significantly heavier than A/J mice after 4 months on the high-fat diet (46.1 ± .07 g *v* 33.5 ± 0.6 g), B6 mice actually consumed fewer calories per gram of body weight than A/J mice. This point is emphasized by the finding of a much higher FE in B6 mice as compared with A/J mice on the high-fat diet. Therefore, hyperphagia alone does not account for the greater weight gain of B6 mice on the high-fat diet. We have previously shown that the greater obesity of B6 mice on the high-fat diet cannot be attributed to reduced spontaneous motor activity either, as B6 mice were actually more active than A/J mice on both low- and high-fat diets.[40] While the high-fat diet used in this study contained sucrose and the low-fat diet did not, we have previously shown that sucrose added to a low-fat diet did not produce diabetes or obesity in B6 mice.[16] Therefore it seems clear that in the present study the critical difference between the two diets was fat content and not sucrose content. In other studies we have shown that a low-sucrose diet containing 35.8% of calories from fat in the form of lard also induces diabetes and obesity in B6 mice.[14,17]

While the development of diabetes in B6 mice is the outcome of the interaction between both genetic and environmental variables, we have shown that the manipulation of environment alone can actually eliminate the manifestation of the disease in this animal model. Our results demonstrate that the syndrome of obesity and diabetes that occurs on a high-fat diet in B6 mice is reversible simply by reducing the amount of fat in the diet. This finding may have implications for the treatment of type 2 DM in humans as well. Several studies have demonstrated that an isocaloric low-fat diet taken acutely improves glycemic control in persons with diabetes,[29-31] but there is less literature available on the effects of chronic treatment with a low-fat diet. While one study has demonstrated the utility of long-term treatment with a low-fat diet in patients with type 2 DM,[47] there is also reason to believe that once glucose toxicity develops in type 2 DM, glucose metabolism is irreversibly compromised. In that we did not observe pancreatic failure in B6 mice, our model may not be comparable to some forms of human type 2 DM. Nevertheless, our results suggest that dramatically restricting dietary fat may prove to be an effective treatment for some patients, particularly in the early stages of type 2 DM.

REFERENCES

1. West DB, Boozer CN, Moody DL, et al: Dietary obesity in nine inbred mouse strains. Am J Physiol 262:R1025-R1032, 1992
2. Schemmel R, Michelsen O, Gill JL: Dietary obesity in rats: Body weight and body fat accretion in seven strains of rats. J Nutr 100:1041-1048, 1970
3. Dreon DM, Frey-Hewitt B, Ellsworth N et al: Dietary fat: carbohydrate ratio and obesity in middle-aged men. Am J Clin Nutr 47:995-1000, 1988
4. Miller WC, Lindeman AK, Wallace J, et al: Diet composition, energy intake, and exercise in relation to body fat in men and women. Am J Clin Nutr 52:426-430, 1990
5. Tucker LA, Kano MJ: Dietary fat and body fat: A multivariate study of 205 adult females. Am J Clin Nutr 56:616-622, 1992
6. Klesges RC, Klesges LM, Haddock CK, et al: A longitudinal analysis of the impact of dietary intake and physical activity on weight change in adults. Am J Clin Nutr 55:818-822, 1992
7. Marshall JA, Hamman RF, Baxter J: High-fat, low-carbohydrate diet and the etiology of non-insulin-dependent diabetes mellitus: The San Luis Valley diabetes study. Am J Epidemiol 134:590-603, 1991
8. Marshall JA, Hoag S, Shetterly S, et al: Dietary fat predicts conversion from impaired glucose tolerance to NIDDM: The San Luis Valley diabetes study. Diabetes Care 17:50-56, 1994
9. Feskens EJM, Virtanen SV, Räsänen L, et al: Dietary factors determining diabetes and impaired glucose tolerance: A 20-year follow-up of the Finnish and Dutch cohorts of the seven countries study. Diabetes Care 18:1104-1112, 1995
10. Morris RD, Rimm DL, Hartz AJ, et al: Obesity and heredity in the etiology of non-insulin-dependent diabetes mellitus in 32,662 adult white women. Am J Epidemiol 130:112-121, 1989
11. Cassano PA, Rosner B, Vokonas PS, et al: Obesity and body fat distribution in relation to the incidence of non-insulin-dependent diabetes mellitus: A prospective cohort study of men in the Normative Aging study. Am J Epidemiol 136:1474-1486, 1992
12. Chan JM, Stampfer MJ, Rimm EB, et al: Obesity, fat distribution and weight gain as risk factors for clinical diabetes in men. Diabetes Care 17:961-969, 1994
13. Carey VJ, Walters EE, Colditz GA, et al: Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women: The nurses' health study. Am J Epidemiol 145:614-619, 1997
14. Surwit RS, Kuhn CM, Cochrane C, et al: Diet-induced type II diabetes in C57BL/6J mice. Diabetes 37:1163-1167, 1988
15. Surwit RS, Seldin MF, Kuhn CM, et al: Control of expression of insulin resistance and hyperglycemia by different genetic factors in diabetic C57BL/6J mice. Diabetes 40:82-87, 1991
16. Surwit RS, Feinglos MN, Rodin J, et al: Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism 44:645-651, 1995
17. Rebuffé-Scrive M, Surwit R, Feinglos M, et al: Regional fat distribution and metabolism in a new mouse model (C57BL/6J) of non–insulin-dependent diabetes mellitus. Metabolism 42:1405-1409, 1993
18. Peckham SC, Entenman C, Carroll HW: The influence of a

hypercaloric diet on gross body and adipose tissue composition in the rat. J Nutr 77:187-197, 1962

19. Salmon DMW, Flatt JP: Effect of dietary fat content on the incidence of obesity among ad libitum fed mice. Int J Obes 9:443-449, 1985

20. Bartness TJ, Polk DR, McGriff WR, et al: Reversal of high-fat diet-induced obesity in female rats. Am J Physiol 263:R790-R797, 1992

21. Bell RR, Spencer MJ, Sherriff JL: Diet-induced obesity in mice can be treated without energy restriction using exercise and/or a low fat diet. J Nutr 125:2356-2363, 1995

22. Harrington ME, Coscina DV: Early weight gain and behavioral responsivity as predictors of dietary obesity in rats. Physiol Behav 30:763-770, 1983

23. Hill JO, Dorton J, Sykes MN, et al: Reversal of dietary obesity is influenced by its duration and severity. Int J Obes 13:711-722, 1989

24. Lladó I, Proenza AM, Serra F, et al: Dietary-induced permanent changes in brown and white adipose tissue composition in rats. Int J Obes 15:415-419, 1991

25. Schaefer EJ, Lichtenstein AH, Lamon-Fava S, et al: Body weight and low-density lipoprotein cholesterol changes after consumption of a low-fat ad libitum diet. JAMA 274:1450-1455, 1995

26. Siggaard R, Raben A, Astrup A: Weight loss during 12 weeks' ad libitum carbohydrate-rich diet in overweight and normal weight subjects at a Danish work site. Obes Res 4:347-356, 1996

27. Fukuda M, Tahara Y, Yamamoto Y, et al: Effects of very-low-calorie diet weight reduction on glucose tolerance, insulin secretion, and insulin resistance in obese non-insulin-dependent diabetics. Diabetes Res Clin Pract 7:61-67, 1989

28. Gumbiner B, Polonsky KS, Beltz WF: Effects of weight loss and reduced hyperglycemia on the kinetics of insulin secretion in obese non-insulin dependent diabetes mellitus. J Clin Endocrinol Metab 70:1594-1602, 1990

29. Anderson JW: High carbohydrate, high fiber diets for patients with diabetes. Adv Exp Med Biol 119:263-273, 1979

30. Simpson RW, Mann JI, Eaton J et al: Improved glucose control in maturity-onset diabetes treated with high-carbohydrate-modified fat diet. BMJ 1:1753-1756, 1979

31. Hjøllund E, Pedersen O, Richelsen B, et al: Increased insulin binding to adipocytes and monocytes and increased insulin sensitivity of glucose transport and metabolism in adipocytes from non–insulin-dependent diabetics after a low-fat/high-starch/high-fiber diet. Metabolism 32:1067-1075, 1983

32. Genuth SM: Insulin secretion in obesity and diabetes: An illustrative case. Ann Intern Med 87:714-716, 1977

33. Storlien LH, James DE, Burleigh KM, et al: Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. Am J Physiol 251:E576-E583, 1986

34. Lovejoy J, DiGirolamo M: Habitual dietary intake and insulin sensitivity in lean and obese adults. Am J Clin Nutr 55:1174-1179, 1992

35. Collins S, Daniel KW, Petro AE, et al: Strain-specific response to β_3-adrenergic receptor agonist treatment of diet-induced obesity in mice. Endocrinology 138:405-413, 1997

36. Surwit RS, Petro AE, Parekh P, et al: Low plasma leptin in response to dietary fat in diabetes- and obesity-prone mice. Diabetes 46:1516-1520, 1997

37. Collins S, Kuhn CM, Petro AE, et al: Role of leptin in fat regulation. Nature 380:677, 1996

38. Fleury C, Neverova M, Collins S, et al: Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia. Nat Genet 15:269-272, 1997

39. Seldin MF, Mott D, Bhat D, et al: Glycogen synthase: a putative locus for diet-induced hyperglycemia. J Clin Invest 94:269-276, 1994

40. Brownlow BS, Petro A, Feinglos MN, et al: The role of motor activity in diet-induced obesity in C57BL/6J mice. Physiol Behav 60:37-41, 1996

41. Barnard RJ, Massey MR, Cherny S, et al: Long-term use of a high-complex-carbohydrate, high-fiber, low-fat diet and exercise in the treatment of NIDDM patients. Diabetes Care 6:268-273, 1983