Fat, Carbohydrate, and Calories in the Development of Diabetes and Obesity in the C57BL/6J Mouse

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We have previously shown that the C57BL/6J (B6) mouse will develop obesity and diabetes if raised on a high-fat diet. Because high fat feeding is associated with hyperphagia, the present study was designed to separate the effects of fat from those of excess caloric consumption in this animal model. B6 mice were fed a low-fat diet (LF group) diet, high-fat diet (HF group) diet, or high-fat-restricted diet (HFR group), in which intake animals were pair-fed a high-fat diet to caloric level consumed by LF for 11 weeks. Within 3 weeks, HFR were significantly heavier than LF and, after 11 weeks, weight and glucose levels, but not insulin, were significantly increased in HFR when compared to LF. Body composition analysis showed the weight increase in HFR arose from an increase in percent fat conumed. We conclude that reducing the number of kilocalories consumed from a high-fat diet attenuates but does not prevent the development of type 2 diabetes and obesity in the B6 mouse.

RECENT EPIDEMIOLOGIC studies report the increasing prevalence of obesity and diabetes throughout the world.[1-4] Data summarized by Bray and Popkin[5] from several ecologic studies show a significant positive relationship between the percent energy from fat and the percent of the population that is obese (body mass index [BMI] $>$ 25). However, the development of obesity is not necessarily the result of increased caloric intake associated with high-fat diets. Oscai et al[6] found no difference in total caloric intake over 60 weeks between rats were fed a high-fat diet (42% to 60% kcal fat) versus Purina Rat Chow (Purina Mills, St Louis, MO). Yet the body weight of the animals fed the high-fat diet was 128% that of the low-fat group and carcass analysis showed that most of this weight difference was due to excess body fat in the high-fat–fed group. Boozer et al[7] found a positive relationship between percent kilocalories from fat in the diet and percent body fat when rats were fed isocaloric diets. Furthermore, this increase in body fat was disproportional to the increased caloric intake in animals prone to develop obesity.[8]

It is also known that high-fat diets disregulate glucose metabolism. Level of dietary fat is associated with the conversion from impaired glucose tolerance to type 2 diabetes in humans.[9,10] Early work by Ip et al[11] and Olefsky and Saekow[12] showed the number of adipocyte insulin receptors was decreased in rats eating a high-fat diet (67% lard) when compared to those fed a diet high in simple carbohydrates. In addition, insulin resistance is increased[13] and insulin sensitivity decreased.[14,15]

We have established a novel diet-induced mouse model of diabetes and obesity in the B6 strain. The B6 mouse becomes hyperglycemic, hyperinsulinemic, obese,[16,17] and hypertensive[18] when fed a high-fat diet. Pancreatic islet function is compromised, ie, glucose-stimulated insulin release is blunted.[19,20] The weight gained is grossly disproportionate to the additional calories provided by the high-fat diet.[17,21] The obesity results from both adipocyte hypertrophy and hyperplasia.[17,21] In addition, UCP2 expression is decreased[22] and β_3-adrenergic receptor expression and function are downregulated.[23] Interestingly, this syndrome is prevented when B6 mice are fed a low-fat diet[16,17] and, even after it is fully established, the disease can be reversed by treatment with a low-fat diet.[21] However, although we have shown that feed efficiency is increased in B6 animals fed a high-fat diet, hyperphagia is also present and when obese animals are switched to a low-fat diet, they consume fewer calories.[17,21] The present study used a pair-feeding design to assess separately the effects of calories and fat in the development of obesity and diabetes in the B6 mouse.

MATERIALS AND METHODS

Mice and Diet

The 4-week old male B6 mice purchased from Jackson Labs (Bar Harbor, ME) were housed individually in a temperature-controlled facility (71 to 73°F). The room was equipped with a 12-hour light/dark cycle (lights on at 7 AM). The mice, 10 per group, were assigned to 1 of 3 dietary treatments for 11 weeks: low-fat diet fed ad libitum (LF group); high-fat diet fed ad libitum (HF group); or a high-fat-restricted diet (HFR group). Research Diets (New Brunswick, NJ) manufactured the diets. In the HFR group, the mice were pair-fed. These mice were fed the high-fat diet but restricted to the number of calories consumed by animals fed low fat during the previous 24-hour period. The percentages kilocalories from fat were 11% and 58% in the LF and HF, respectively. The protein content was 16% in both diets and the balance carbohydrate. The animals were weighed weekly and food intake was measured daily.

Plasma Samples

Plasma samples were collected at 4, 8, and 10 weeks during the experiment. Samples were collected via retroorbital sinus puncture in nonanesthesized animals. Food was removed 6 to 8 hours before the samples were collected.

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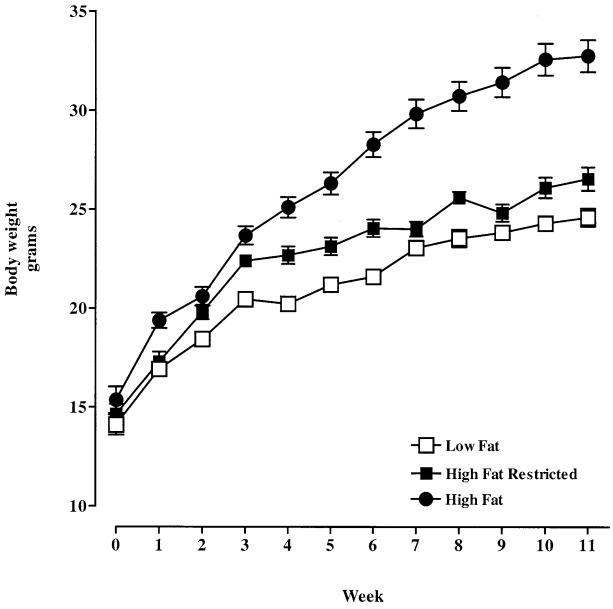

Fig 1. Body weight of mice fed low-at, high-fat calorically restricted, or high-fat ad libitum diets. HFR mice were significantly heavier within 3 weeks of treatment when compared to LF. Data points represent the mean ± SEM of 10 mice per group.

Biochemical Analysis

Glucose. Glucose concentration (milligrams per deciliter) was determined using the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA).

Insulin. Insulin levels (microunits per milliliter) were measured by double-antibody radioimmunoassay (Linco Research, St Louis, MO). The assay was based on a rat standard.

Body composition. The percent fat was determined at the end of the experiment by dual-energy x-ray absorptiometry (DEXA) using the PIXImus densitometer (GE Medical Systems, Waukesha, WI). The animals were anesthetized with a cocktail of ketamine/medetomidine (60 mg/kg + 1.0 mg/kg intraperitoneally) for this procedure. Atipamezole (1 mg/kg intraperitoneally) was used to reverse the anesthesia.

Statistical Analysis

A repeated-measures analysis of variance (ANOVA) was used to analyze data. Following a significant overall result, comparisons between treatments were made using the Newman-Keuls test. *P* values greater than .05 were considered not significant.

Table 1. Food Intake, Feed Efficiency, and Percent Body Fat of Mice on Three Diet Treatments

Diet Treatment	Total Food Intake (kcal)	Feed Efficiency (%)	Body Fat (%)
Low fat ad libitum	719 ± 4[a]	1.05 ± .06[a]	18.5 ± 1.3[a]
High fat restricted	726 ± 5[a]	1.18 ± .07[a]	24.1 ± 1.0[b]
High fat ad libitum	879 ± 15[b]	1.66 ± .06[b]	34.8 ± 1.8[c]

NOTE. Values are the mean ± SEM for 10 animals per groups for food intake and feed efficiency measurements and 6 animals per group for body fat. Means with different superscripts are significantly different ($P < .001$).

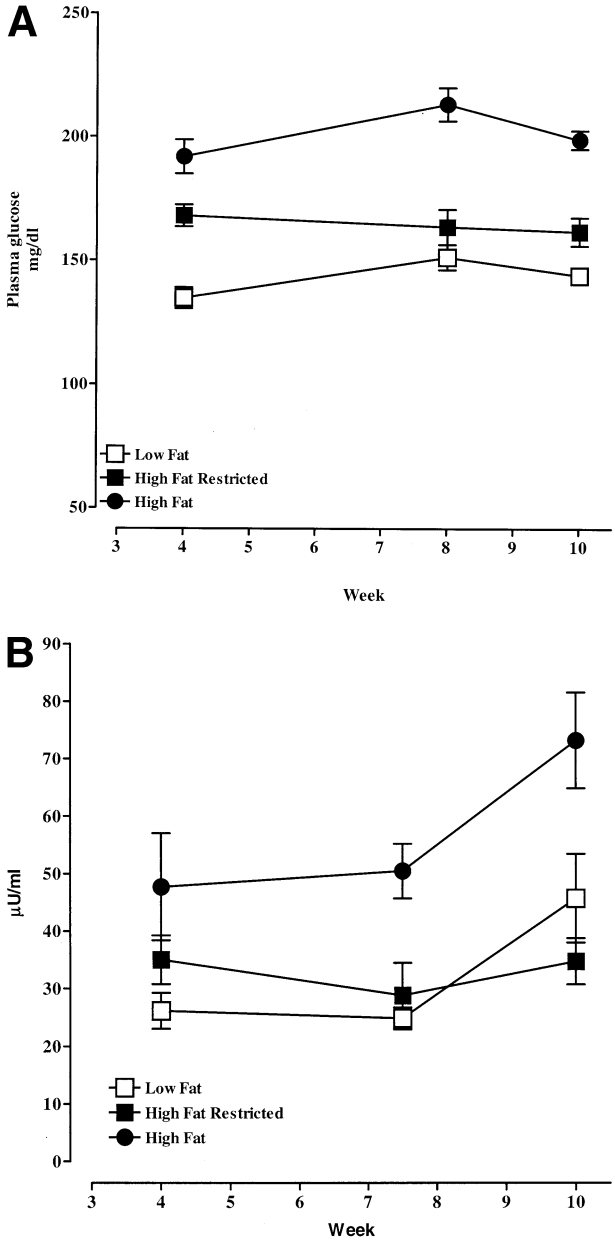

Fig 2. (A) Plasma glucose was affected by diet. Glucose concentrations were increased with the addition of dietary fat but caloric restriction attenuated the increase. Data points represent the mean ± SEM of 10 mice per group. Significance levels are described in the text. (B) There were no significant differences in insulin levels between mice fed low-fat ad libitum and mice fed calorically restricted high-fat diets. Significance levels are described in the text. Data points represent the mean ± SEM of 7 to 8 mice per group.

RESULTS

Body Weight

Repeated-measures ANOVA showed a significant effect of treatment on body weight over time. Although the caloric intake of the HFR group was restricted to the level consumed by LF, the mice in the HFR group were significantly heavier

when compared to the LF group ($P < .01$). The weight of the HF group was increased when compared to LF ($P < .001$) and HFR ($P < .001$). By the end of the experiment the weight of the HF group was dramatically increased (Fig 1).

Food Intake and Feed Efficiency

The caloric intake of the animals is shown in Table 1. There was a slight increase in feed efficiency (body weight gain in grams per kilocalories consumed) in the HFR group when compared to the LF group (Table 1). Although this increase was not statistically significant ($P = .19$), the HFR group gained more weight than the LF group while eating the same number of calories.

Plasma Glucose

The plasma glucose concentration was significantly increased by dietary fat. Repeated-measures ANOVA revealed a significant effect of treatment ($P < .001$). All groups differed from each other (LF *v* HFR: $P < .001$; HFR *v* HF: $P < .001$; LF *v* HF: $P < .001$). The treatment $\times$ time interaction was not significant, indicating that the effects of diet did not change over time. This is consistent with previous reports.[16,17] As seen with body weight, caloric restriction of fat attenuated but did not prevent the rise in plasma glucose attributable to the high-fat diet. There were no significant changes in plasma glucose within groups during the experiment, showing that the effects of diet were stable (Fig 2A).

Plasma Insulin

Plasma insulin concentrations are shown in Fig 2B. Repeated-measures ANOVA showed an effect of treatment ($P < .001$). The treatment $\times$ time interaction was not significant. Post-hoc analysis showed insulin levels in the HF group were significantly higher than in the LF ($P < .001$) and HFR ($P < .001$) groups.

Body Composition

The increased body weight observed in the HF and HFR groups was due to an increase in fat tissue. The percent fat as determined by DEXA analysis was significantly increased in the HFR group when compared to the LF group (HFR 24.1 $\pm$ 1.0% *v* LF 18.5 $\pm$ 1.3%; $P < .001$). As in total body weight, the percent fat was increased in the HF group (Table 1).

DISCUSSION

The present study clearly shows that fat has an effect on the development of obesity and diabetes in the B6 mouse that is independent of increased caloric intake. Our results support previous studies that have shown that other animals gain more weight when pair-fed a high-fat diet. In addition, we show that high fat feeding has an effect on plasma glucose that is also independent of caloric intake in this animal model.

The differences in body weight and body composition found in these 2 studies are predicted by the research of Flatt[24] and Horton et al[25] who found differences in energy storage and nutrient oxidation when subjects were overfed fat or carbohydrate in isocaloric amounts relative to the individual's baseline intake. When overfed fat, carbohydrate oxidation was decreased and there was no change in fat oxidation. Yet when the same subjects were overfed carbohydrate, carbohydrate oxidation was increased and fat oxidation was decreased. In this report the high-fat calorically restricted animals weighed more and accumulated significantly more body fat than mice consuming equal energy of the low-fat diet.

To the extent that we can generalize from mice to humans, the data in these studies provide important considerations for weight management. Energy restriction without restriction of percent kilocalories from fat is likely to be unsuccessful. Animals fed a high-fat diet but calorically restricted to the consumption of animals fed low fat were heavier within 2 weeks and by the end of the study had significantly increased percent body fat. A study by Toubro and Astrup[26] supports these data. They found that subjects who had participated in a weight reduction program were better able to maintain the weight loss for at least 1 year when the maintenance diet was low fat/high carbohydrate ad libitum compared to a more conventional diet that was calorically restricted. Although complete details of the maintenance diets were not given, food diaries indicated there was no difference in energy content between the 2 diets but the calorie-controlled group consumed more fat. Increased fat mass contributed a larger portion of the regained weight in the energy-restricted group compared to the ad libitum low-fat/high-carbohydrate group (75% *v* 67%).

In summary, data from this study show that fat, and not caloric intake, is the crucial stimulus for obesity and diabetes in the B6 mouse. While one should be careful extrapolating these findings to humans, there are numerous human studies that support the notion that fat, and not carbohydrate, is the critical factor in the development and maintenance of obesity and type 2 diabetes.

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