Diabetogenic Response to Streptozotocin Varies among Obese Yellow and Among Lean Agouti (BALB/c × VY)F_1 Hybrid Mice

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Abstract. **To test the hypothesis that the elevated insulin levels in obese neoplasia-susceptible yellow $A^{vy}/-$ mice might be a major factor stimulating tumor formation, it is necessary to use normoinsulinemic yellow mice. Although our attempt to obtain normoinsulinemic, euglycemic mice by streptozotocin treatment was unsuccessful, we did observe significant differences in the responsiveness to this treatment among mice of identical genotype. These differences were observed among female yellow A^{vy}/A and agouti A/a (BALB/c × VY)F_1 hybrid mice in the responses of body weight gain, plasma glucose, and plasma insulin levels to a single intraperitoneal injection of either 150 or 200 mg/kg streptozotocin (STZ) at 4 weeks of age followed by a 22-week observation period. Among animals treated with the high streptozotocin dose, 80% of the yellow mice gained almost no weight and became grossly hyperglycemic and hypoinsulinemic; however, only 55% of the agouti mice exhibited such a strong response. In the low dose group, 25% of the yellow mice responded with reduced body weight gain, decreased insulin, and elevated glucose levels whereas none of the agouti mice exhibited such responses. More pancreatic islet tissue mass was present in the untreated yellow control mice than among the comparable agouti mice by the end of the study. In both streptozotocin dose groups and in both genotypes, islet tissue mass was reduced to a much greater extent in the more responsive mice than in the less responsive mice. There appeared to be no correlation between islet tissue mass and insulin level. The phenotypic variation in responsiveness to an exogenous agent among test animals of a single inbred or F_1 hybrid genotype reported here is not unique to this F_1 hybrid since it is seen in most chronic bioassays when relatively low levels of agent are used.**

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Obesity of adult yellow A^{vy}/A mice is accompanied by and may be preceded by hyperplasia of the pancreatic islets of Langerhans and elevated circulating insulin levels (1, 2). In contrast to hyperinsulinemia in other genetically obese mice, such as *ob/ob* (obese) and *db/db* (diabetes), the relatively mild hyperinsulinemia in obese yellow mice maintains blood glucose concentration within the normal range. The A^{vy} mutation increases somatic growth (3) as well as tumorigenic response to endogenous and exogenous carcinogens, resulting in earlier and more rapid formation of neoplasms and increased lesion prevalence (4, 5).

When allogeneic tumor cells were implanted in yellow A^{y}/a or A^{vy}/a and black a/a mice, the resulting solid tumors tended to be larger in the yellow mice than in the black siblings (6). This could be interpreted either as indicating a relative deficiency in cell-mediated immunity and an associated decrease in the capacity to

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reject antigenic transplantable tumor cells or as an inherent genotypic or phenotypic difference in host capacity to support cell proliferation. In an earlier study (7), the capacity to make an IgG anamnestic response to the T cell-dependent antigen tetanus toxoid was found to be decreased in obese yellow A^{vy}/a (YS × VY)F_1 hybrid female mice as compared with their lean pseudoagouti A^{vy}/a and lean black a/a siblings. These findings indicate that although a diminished T cell-dependent response appears to be associated with the obese phenotype, it is not a direct consequence of the presence of the A^{vy} gene. Additionally, Stutman (8) found that the presence of the A^{vy} gene was associated with increased tumor growth and weight gain, whether the mice were T cell-deficient (nu/nu) or immunologically intact ($nu/+$ or $+/+$). Collectively, these findings suggest that increased tumor growth in yellow mice is a consequence of an increased capacity to support cell proliferation rather than of decreased immune surveillance.

Since insulin is required for somatic growth and is known to support cell proliferation *in vitro*, we postulated that the higher circulating insulin level in the yellow mice might be a major factor in the tumor-promoting characteristic of this phenotype (2). To test this hypothesis, it was first necessary to ascertain whether it would be possible to prevent development of the hyperinsulinemia in young pre-obese yellow mice while maintaining normal growth and body weight. If such a normoinsulinemic state could be achieved in the yellow mice, insights regarding the relation of insulin to tumor growth *in vivo*, as well as the relation of insulin level to the initiation of obesity in these mice, might be obtained.

One means of testing this hypothesis was to destroy a small but sufficient proportion of the β cell population in the islets of Langerhans of yellow mice in an attempt to maintain similar insulin concentrations and rates of body weight gain in both yellow and agouti mice without inducing general toxicity. For this purpose streptozotocin (2-deoxy-2{[(methyl-nitrosoamino) carbonyl] amino}-D-glucopyranose) (STZ), which is specifically cytotoxic to β cells (9), was administered.

Materials and Methods

Mottled yellow A^{vy}/A and agouti A/a (BALB/cStCrlfC3Hf/Nctr × VY/WffC3Hf/Nctr-A^{vy})F_1 hybrid mice were produced as described previously (10). They were maintained in a clean, conventional animal room. Four female mice of the same genotype were housed in each polycarbonate cage (6.5 × 21.6 × 12.7 cm) covered with a molded filter bonnet of spun-bonded polyester. Sterilized hardwood chips (Sani-Chips; P. J. Murphy Forest Products Corp., Rochelle Park, NJ) served as bedding. The mice were fed sterilized NIH-31 meal (Teklad, Inc., Madison, WI) *ad libitum*. Filtered water, heated to 93.3°C and then cooled to ambient temperature, was available *ad libitum*. Cages, feed, and water bottles were changed weekly. Room temperature was maintained at 22 ± 2°C and relative humidity at 50 ± 5%, with 15–17 changes of air per hour. Twelve-hour continuous fluorescent light cycles were automatically regulated.

Streptozotocin. Streptozotocin (STZ), originally isolated from *Streptomyces achromogenes* fermentation broth (11), was purchased from Sigma Chemical Co., St. Louis (lot 123F-0301, minimum purity of 90+%). Solutions of 10 mg/ml in citrate-buffered saline (pH 4.5) were prepared within 1 hr of use and stored in amber bottles.

Treatment. At 25–32 days of age 40 mottled yellow A^{vy}/A and 40 agouti A/a (BALB/c × VY)F_1 hybrid female mice were each injected intraperitoneally with a single dose of 0.2–0.3 ml of STZ solution to give administered STZ doses of 146.6 ± 4.4 mg/kg body wt or 196.0 ± 3.3 mg/kg to the yellow mice and 142.6 ± 2.5 mg/kg or 205.9 ± 2.5 mg/kg to the agouti mice. These are referred to as the low (150 mg/kg) and high (200 mg/kg) STZ doses. They were chosen on the basis of a preliminary dose-finding study in which groups of four yellow and four agouti mice were injected with single doses of 50, 100, 150, 200, or 250 mg/kg STZ and were assayed weekly for body weight and plasma glucose and plasma insulin concentrations for 3 weeks.

In the present study, control groups of 20 yellow and 20 agouti mice were injected with vehicle (citrate-buffered saline, pH 4.5), the exact volume for each animal being matched to its body weight as with the treated animals. The animals were allocated to the study in three equal weekly replicates. For evaluating the effect of STZ treatment on pancreatic islet tissue, an additional group of 12 yellow and 12 agouti females was sacrificed before treatment at Week 0 and a similar group of mice at Week 3 of the study.

Because of their greater weight, yellow mice in the high dose group received about 8% more total STZ, on average, than the comparable agouti mice. The yellow mice in the low dose group received about 14% more total STZ, on average, than their agouti siblings. However, when expressed as dosage (mg/kg body weight), yellow mice received only 3% more STZ than the agouti mice at the low dose and 5% less at the high dose. Thus, it appears unlikely that differences in total dose or dosage account for differences in responsiveness between the two genotypes.

Glucose, Insulin, and Body Weight. Nonfasting plasma glucose and plasma insulin concentrations in each mouse were measured on blood obtained from the retro-orbital sinus (under mild CO_2 anesthesia) between 9 and 11 AM before dosing at the start of the experiment (Week 0) and at 2, 6, 10, 14, 18, and 22 weeks after dosing. Glucose was measured by the kinetic hexokinase method (12) at 340 nm on a centrifugal analyzer (GEMSAEC; Electro-Nucleonics, Fairfield, NJ). The plasma glucose concentration was proportional to the reduction of nicotinamide adenine dinucleotide phos-

phate in the assay. Plasma insulin was determined by a double antibody technique (13). Body weights were recorded weekly. All mice were sacrificed by CO_2 euthanasia 22 weeks after treatment with STZ.

Islet Tissue. Islet tissues from 12 mice in each treatment and control group sacrificed at Weeks 0 and 3 and from 20 mice from each group at Week 22 were examined histologically. Each pancreas was removed and formed into a 3- to 4-mm-thick circular mass on filter paper prior to fixation in 10% neutral buffered formalin. After fixation, specimens were dehydrated, embedded in paraffin, sectioned at 5 μm thickness, stained with hematoxylin and eosin, and examined microscopically.

A subjective semiquantitative approach was devised to estimate the amount of islet tissue present. All collections of pancreatic tissue were made by the same person. All measurements on one 5-μm section from each pancreas were performed by the same pathologist. Bias was minimized by evaluating pancreata in replicates consisting of one animal of the same age from each treatment group without knowledge of the treatment group. Islets of less than five cells were arbitrarily disregarded. All others were subjectively evaluated for size and placed in categories of "small," "normal," or "large." The relative amount of tissue on the slide was also subjectively evaluated with a correction factor (Q = 0 to 1.0 with 1.0 being the largest specimen) assigned to normalize the data. Data collected for each animal consisted of (i) number of small, normal, and large islets and (ii) the relative quantity of pancreatic tissue on the slide. The weighted total number of islets counted in one 5-μm section of pancreas per animal was derived as follows: ([number of small islets × 1] + [number of normal islets × 2] + [number of large islets 9× 3]) × 1/Q and is designated as "islet units" in Table I.

Statistical Analysis. Initially, body weights, glucose and insulin levels, and pancreatic islet units were analyzed for dose effects within each genotype using all animals regardless of degree of response. For each parameter and genotype, a univariate, unbalanced, mixed effects analysis of variance was used. Only data for animals sacrificed 22 weeks after dosing were included. Bonferroni t tests were performed for all dose combinations where the analysis of variance indicated a significant difference among dose level means. The rates of change of body weight and of glucose and insulin levels were analyzed for dose effects using regression analysis. A two-sided t test was performed on the difference in slopes for each pairwise comparison of dosed animals (low and high) with controls. Pearson correlation coefficients were calculated for each combination of these four dependent variables.

Subsequently, dosed animals sacrificed at 22 weeks were grouped into two categories, low responders (LR) and high responders (HR), based on whether or not they had developed gross hyperglycemia (HR = >290 mg/dl plasma glucose). The insulin levels and islet units for groups sacrificed at 0 and 3 weeks and for the LR and HR mice sacrificed at 22 weeks were each analyzed separately for genotype differences by univariate, unbalanced, mixed effects analyses of variance. A Mantel-Haenszel test was used to test for an overall difference in the proportion of animals developing gross hyperglycemia (HR compared with LR) in the two genotypes.

Results

Dose-Finding Study. In the preliminary dose-finding study, 50 mg/kg STZ had no observable effect on body weights, plasma glucose, or insulin concentrations of the yellow mice. Single doses of 100 and 150 mg/kg STZ decreased the rate of body weight gain to a similar degree for 2 weeks after treatment; between the second and third week, however, the rate of weight gain was similar to that of mice treated with 50 mg/kg STZ.

Table I. Islet Tissue in 5-μm Sections Compared with Insulin Levels

Week	Yellow		Agouti	
	Islet units	Insulin (microunits/ml)	Islet units	Insulin (microunits/ml)
		0 mg/kg streptozotocin		
0	20.0 ± 2.7 (12)[a]	28.8 ± 9.5 (7)[a]	24.2 ± 2.4 (12)	6.8 ± 1.7 (12)
3	34.0 ± 4.7 (12)	24.8 ± 5.7 (11)	33.6 ± 2.8 (11)	6.6 ± 1.3 (12)
22	103.8 ± 8.7 (20)	52.3 ± 12.5 (17)	63.2 ± 8.0 (20)	6.4 ± 1.9 (16)
		150 mg/kg streptozotocin		
3	37.8 ± 6.7 (12)	29.0 ± 6.3 (12)	32.6 ± 4.4 (12)	7.0 ± 0.6 (11)
22, LR[b]	60.4 ± 5.9 (15)	28.5 ± 10.2 (14)	44.5 ± 4.9 (20)	9.5 ± 3.8 (19)
22, HR[b]	35.0 ± 4.3 (5)	23.2 ± 17.4 (4)	—	—
		200 mg/kg streptozotocin		
3	34.1 ± 5.9 (12)	19.6 ± 4.1 (18)	29.0 ± 4.2 (12)	10.2 ± 2.0 (7)
22, LR	82.0 ± 16.1 (4)	12.2 ± 6.5 (4)	48.3 ± 4.0 (9)	6.6 ± 2.0 (9)
22, HR	33.0 ± 2.6 (14)	3.6 ± 1.5 (14)	32.4 ± 2.9 (11)	0.4 ± 0.2 (7)

[a] Mean ± SE. Values in parentheses, no. of animals.
[b] Low responders, <290 mg/dl plasma glucose; high responders, >290 mg/dl plasma glucose.

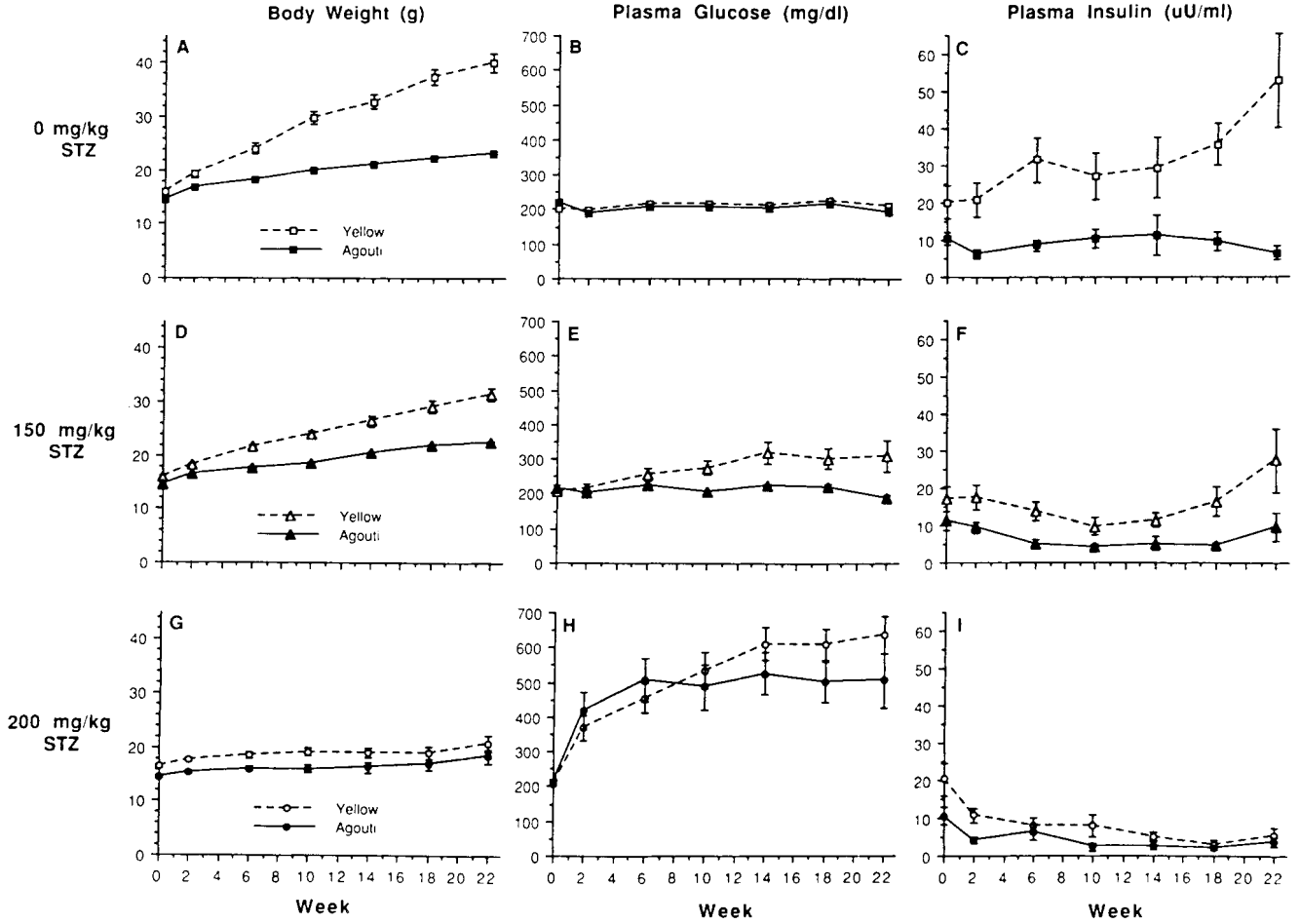

Figure 1. Body weight, plasma glucose, and insulin concentration curves in female yellow and agouti (BALB/c × VY)F_1 hybrid mice following single intraperitoneal injections of vehicle, 150 or 200 mg/kg body wt streptozotocin, at 4 weeks of age. Data points = mean ± SE. N_{yellow}: 0 mg/kg = 19, 150 mg/kg = 20, 200 mg/kg = 20; N_{agouti}: 0 mg/kg = 20, 150 mg/kg = 20, 200 mg/kg = 20.

Statistical significance of differences between slopes of the regression lines:

	Body weight	Plasma glucose	Plasma insulin
Yellow mice			
0 cf. 150	($P < 0.0001$)	($P < 0.001$)	($P = 0.02$)
0 cf. 200	($P < 0.0001$)	($P < 0.0001$)	($P < 0.0001$)
150 cf. 200	($P < 0.0001$)	($P < 0.0001$)	($P > 0.001$)
Agouti mice			
0 cf. 150	($P > 0.05$)	($P > 0.05$)	($P > 0.05$)
0 cf. 200	($P < 0.0001$)	($P = 0.001$)	($P > 0.05$)
150 cf. 200	($P < 0.0001$)	($P > 0.001$)	($P > 0.05$)

Plasma insulin concentration in the 50 mg/kg group increased from 21.2 ± 2.1 microunits/ml to 114.0 ± 3.5 microunits/ml by the third week after treatment but remained relatively constant at 25–33 microunits/ml in the 100 and 150 mg/kg groups during this time. Both body weight gains and insulin concentrations were decreased in the 200 mg/kg group. Among the mice in the 250 mg/kg STZ group only one of four yellow mice survived to Week 2 and only one of four agouti mice survived to Week 3.

Vehicle-Treated Controls. During the subsequent 22-week study, mean body weight of the vehicle-treated yellow mice increased from 16.0 ± 0.2 g to 39.8 ± 1.6 g, while that of the agouti mice increased from 14.5 ± 0.2 g to 23.1 ± 0.3 g (Fig. 1A). Plasma glucose concentrations remained in the same range for both yellow and agouti mice (Fig. 1B). Insulin levels remained essentially constant in the control agouti mice, but gradually increased in the yellow controls from 20.0 ± 4.4 microunits/ml to 52.3 ± 12.5 microunits/ml over the course of the study (Fig. 1C). Thus, normal plasma glucose levels were associated with higher insulin levels in the yellow than in the agouti mice throughout the study. In yellow mice, but not in agouti mice, the insulin level increased with age, suggesting that yellow mice were relatively more resistant to insulin action than their agouti siblings at the start of the study and that this resistance increased with age.

Low Dose Groups. The 150-mg/kg STZ dose decreased the rate of body weight gain considerably ($P < 0.0001$) in the yellow mice but had no effect in the agouti mice when compared with the respective vehicle-

treated controls (Fig. 1, A and D). The glucose level was elevated ($P < 0.001$) in the yellow mice but was not changed in the agouti animals (Fig. 1, B and E). In this dose group, insulin levels were decreased in the yellow mice ($P = 0.02$) compared with control levels; however, no differences in insulin levels were observed between the treated and control agouti mice (Fig. 1, C and F).

High Dose Groups. The 200-mg/kg STZ dose reduced the rates of weight gain in both genotypes below those of their respective controls ($P < 0.0001$) and to approximately the same level (Fig. 1, A and G). Glucose concentrations in both genotypes increased rapidly to grossly hyperglycemic levels ($P < 0.0001$) (Fig. 1H) and toward the end of the study diuresis was a frequent observation. In yellow mice, insulin levels were decreased significantly ($P < 0.001$) below the comparable levels in the low dose STZ group (Fig. 1I). In contrast, among the agouti mice no statistically significant differences in insulin levels were observed between the controls and the two dose groups (Fig. 1, F and I).

Islet Mass. The number of islet units per animal was similar in yellow and agouti control mice during the first 3 weeks of the study (Table I). However, between Weeks 3 and 22, the number of islet units increased over 4-fold in yellow controls and only about 2-fold in agouti controls.

STZ treatment had no effect on the increase in number of islet units in either yellow or agouti mice during the first 3 weeks. After this period, STZ effects on the number of islet units depended on the degree of responsiveness of the individual mice to the treatment; these are reported below. In no case did STZ reduce the number of islet units below that seen in controls at Week 3. These data indicate that STZ did not cause destruction of preexisting islets.

Islet Mass and Insulin Level. Plasma insulin levels were not proportionally correlated with the number of islet units per mouse (Table I), suggesting that the rates of insulin synthesis, release, and/or degradation were more important than the amount of islet tissue in the regulation of circulating insulin levels. This was especially apparent in contrasting yellow and agouti mice during Weeks 0 and 3 when the numbers of islet units were very similar but insulin levels in the two genotypes differed.

Furthermore, among the control agouti mice, the number of islet units per animal increased significantly during the study whereas, in contrast to the yellow mice, their insulin level remained constant. This suggests that either the total β cell fraction of the islets remained constant and that the release of insulin was the primary regulator of plasma insulin or that the rate of insulin degradation was higher in the agouti mice.

Among the vehicle-treated yellow mice, the number of islet units per animal increased considerably more between the 3rd and 22nd weeks than in the vehicle-treated agouti mice. The insulin levels did not change up to Week 3 among the control yellow mice but appeared to increase between Weeks 3 and 22. Here, insulin concentration more or less paralleled the increase in number of islet units per mouse. This difference between the agouti and yellow mice was probably a compensatory response related to a decreased number of insulin receptors associated with the overt obesity (14) of the yellow mice which had developed by Week 22 (Fig. 1A).

Differential Responsiveness. Examination of data from individual mice revealed differential responsiveness to STZ treatment of pancreatic islet units, plasma glucose level, and body weight gain within each genotype. Analogous observations of differential responsiveness to exogenous agents by members of genetically homogeneous inbred and F_1 hybrid populations have been reviewed recently (15).

Between Weeks 3 and 22, there was no apparent increase in the number of islet units in the HR mice (Table I). The apparent degree of inhibition of proliferation was more pronounced in yellow than in agouti mice. The low STZ dose reduced the increase in islet units in HR yellow mice to 23% of that seen in the yellow controls; in HR agoutis, on the other hand, it was reduced to 41% of that seen in agouti controls. The high STZ dose reduced the increase in islet units among HR yellow mice to 14% of the control value and in HR agouti mice to 34% of controls. Thus, by the end of the study, HR yellow and agouti mice treated with 200 mg/kg STZ had very nearly equal numbers of islet units.

Individual mice of both genotypes responded with decreased insulin concentrations and hyperglycemia (HR). Other mice of the same genotype, however, appeared to be relatively resistant (LR) to these effects of STZ. It was not possible to subjectively identify prospective HR and LR mice by any simple criterion among the vehicle-treated control mice of either genotype.

Weight Gain. Inspection of Figures 2B and 3B indicates that in the LR yellow and agouti mice treated with 150 mg/kg STZ the rates of body weight gain were decreased beginning about 2 weeks after treatment and lasting until about 10 weeks after treatment. This may reflect some treatment-related toxicity. From 10 weeks to the end of the study, the rates of weight gain appeared to be similar to those of the control mice of the respective genotypes, suggesting recovery from the suspected toxicity.

Yellow LR mice treated with 200 mg/kg STZ exhibited a decreased rate of weight gain for 14 weeks but then resumed a more rapid rate of gain (Fig. 2B). The rate of weight gain of high dose agouti LR mice did not appear to differ from those treated with 150 mg/kg STZ (Fig. 3B).

Glucose Levels. The greater responsiveness of the yellow mice to toxicants (1, 5, 16) was confirmed in this study. In contrast with the agouti mice, 25% of the yellow mice treated with the low STZ dose developed

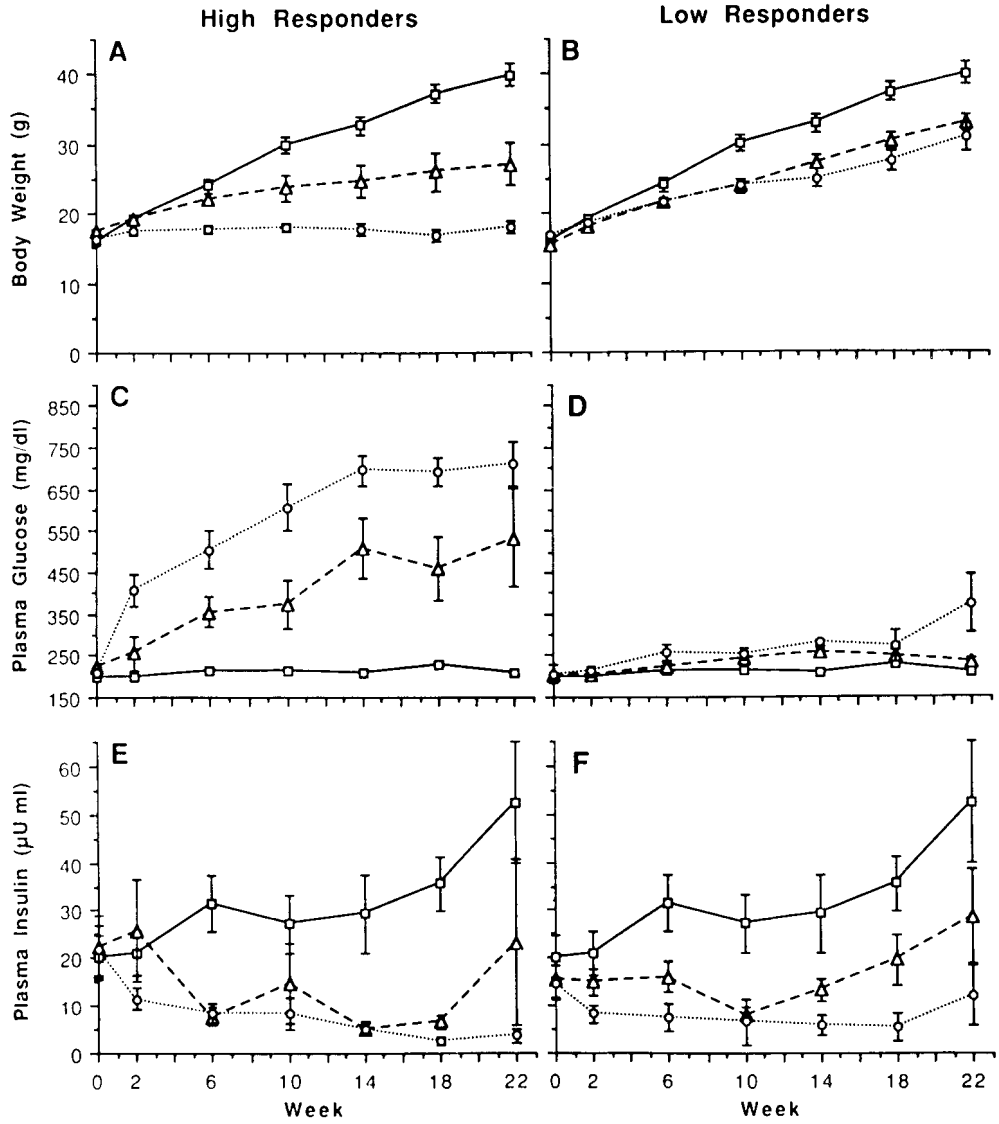

Figure 2. Body weight, plasma glucose, and insulin concentration curves in HR and LR female yellow A^{vy}/A (BALB/c × VY)F_1 hybrid mice following single intraperitoneal injections of 150 or 200 mg/kg streptozotocin at 4 weeks of age. The relevant 0 mg/kg curves from Figure 1 are repeated for convenience of comparisons. Data points = mean ± SE. N_{HR}: 150 mg/kg = 5, 200 mg/kg = 16; N_{LR}: 150 mg/kg = 15, 200 mg/kg = 4. —□—, 0 mg/kg STZ; – –△– –, 150 mg/kg STZ; · · ·○· · ·, 200 mg/kg STZ.

Table II. Proportions of Mice Exhibiting Low and High Responses to Streptozotocin Treatment

Dose (mg/kg)	Low response[a]		High response[a]	
	Yellow A^{vy}/A	Agouti A/a	Yellow A^{vy}/A	Agouti A/a
150	15 (75%)	20 (100%)	5 (25%)	0
200	4 (20%)	9 (45%)	16 (80%)	11 (55%)

[a] Low response, <290 mg/dl glucose; high response, >290 mg/dl glucose.

gross hyperglycemia (Table II), indicating decreased insulin levels (17), presumably due to toxic injury of β cells. At this STZ dose, the glucose levels in the agouti mice remained at control levels (Fig. 1E). In the 200 mg/kg STZ group, hyperglycemia developed in 80% of the yellow mice but in only 55% of the agouti mice (Table II). Apparently, agouti mice were relatively more resistant to the cytotoxic/diabetogenic effects of STZ than yellow mice ($P = 0.01$).

Insulin Levels. In the yellow mice, streptozotocin induced a much less dramatic difference in insulin levels than in glucose levels in the LR group (Fig. 2, D and F) as compared with the HR group (Fig. 2, C and E).

Toxicity. The data suggest that, even at the low dose, STZ treatment exerted toxic effects in the LR mice, both yellow and agouti, since the body weight gain curves were lower and the glucose curves were higher than those of the control mice (Figs. 2B and D and 3B and D). The elevation of the glucose curve correlates well with the decrease in plasma insulin levels (Fig. 2F).

Discussion

A report by Mahler (17) that adult obese *ob/ob* mice could be treated with alloxan to decrease their

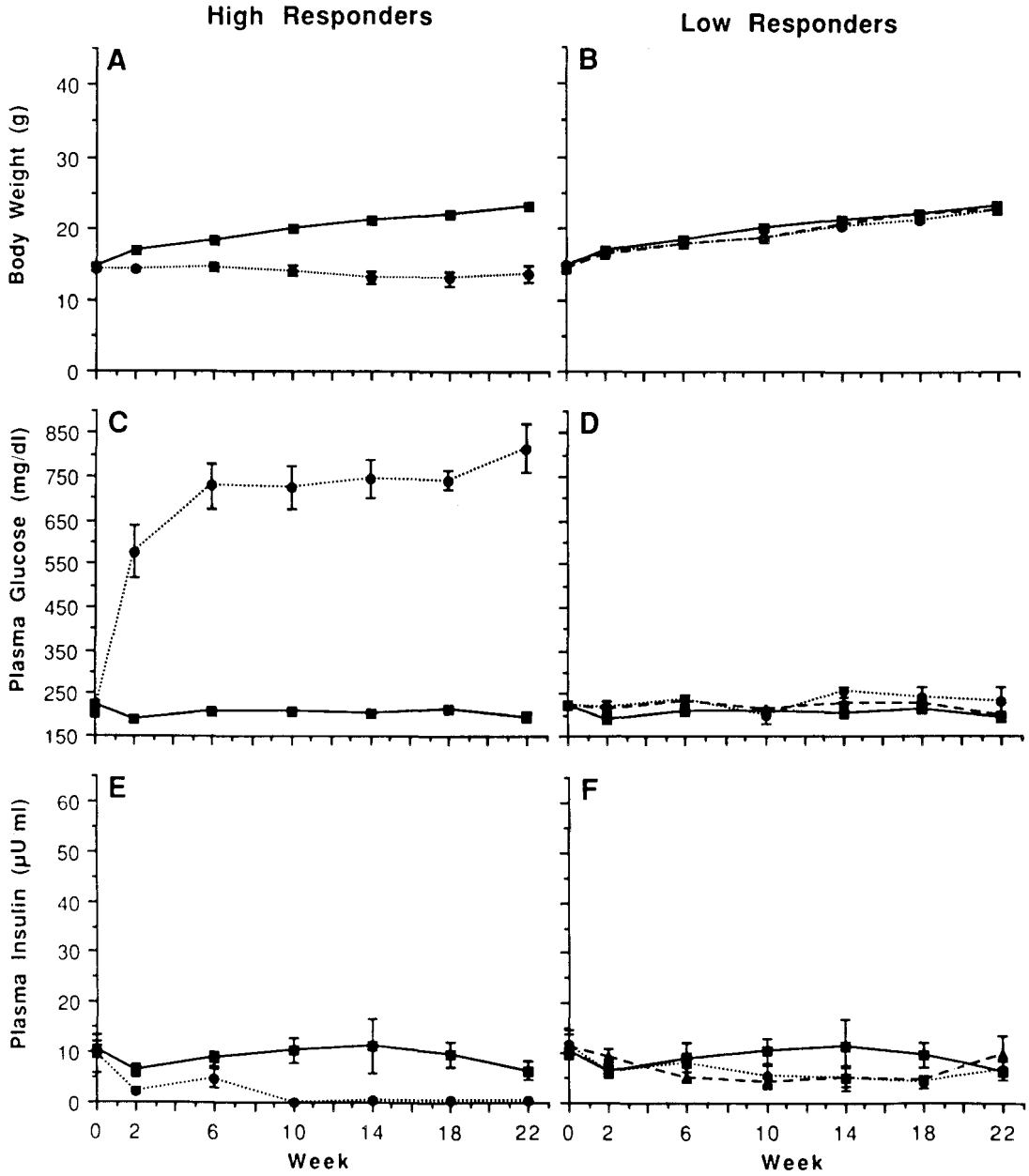

Figure 3. Body weight, plasma glucose, and insulin concentration curves in HR and LR female agouti *A/a* (BALB/c × VY)F_1 hybrid mice following single intraperitoneal injections of 150 or 200 mg/kg streptozotocin at 4 weeks of age. The relevant 0 mg/kg curves from Figure 1 are repeated for convenience of comparisons. Data points = mean ± SE. N_{HR}: 150 mg/kg = 0, 200 mg/kg = 11; N_{LR}: 150 mg/kg = 20, 200 mg/kg = 9. —■—, 0 mg/kg STZ; – –▲– –, 150 mg/kg STZ; · · ·●· · ·, 200 mg/kg STZ.

insulin levels to normal without affecting their body weight prompted the present study. No reports were found in the literature on the effects of streptozotocin or alloxan treatment on obese yellow $A^y/-$ or $A^{vy}/-$ mice nor on weanling *ob/ob* or pre-obese yellow mice. To attain our objective of producing normoinsulinemic, euglycemic, lean yellow mice, it was essential that the plasma insulin and glucose levels of the treated yellow mice remain constant and that their body weight increase only at the rate of that of the agouti littermates. In the present study, these criteria were approximated only at the high STZ dose in the LR mice (Fig. 2, B and F). However, toward the end of the study evidence of hyperglycemia appeared (Fig. 2D). Their growth rate (Fig. 2B) also appeared to accelerate somewhat in comparison to that of the untreated agouti mice (Fig. 1A). Thus, a longer period of evaluation would be required to ascertain whether these mice would become frankly diabetic and thus be inappropriate for a chronic carcinogenesis study. The relatively small proportion of yellow LR mice in the test population makes this approach of questionable practical utility. Thus, the primary objective of this study was not achieved. Instead, differences in responsiveness to STZ between the yellow A^{vy}/A and agouti A/a mice, as well as differences in the degree of responsiveness between subgroups within each genotype, were observed.

Yellow mice are more responsive to STZ than their

agouti littermates as demonstrated in Table II. Whereas none of the agouti mice in the low dose group had a hyperglycemic response to STZ, 25% of the yellow mice in the low dose group exhibited such a response. Likewise, in the high dose group, 80% of the yellow mice but only 55% of the agouti mice became grossly hyperglycemic. The rate of body weight gain in both dose groups of the yellow LR mice was lower than in the vehicle-treated mice until Week 14 (Fig. 2B). This suggests that, even though there appeared to be no hyperglycemic response, there may have been an acute but low-level toxic response to STZ administration from which these mice did not immediately recover. In contrast, the rate of body weight gain of the agouti LR mice did not appear to be affected to the same extent.

Differences in relative insulin resistance between the HR and LR yellow mice were suggested by the dramatic differences in plasma glucose levels (Fig. 2, C and D) which were associated with relatively minor differences in insulin levels (Fig. 2, E and F). The same is true of the HR and LR agouti mice (Fig. 3, C to F). Such differences in insulin resistance could be attributable to differences in the number of insulin receptors (11). The differences in the rates of body weight gain of HR and LR mice (Figs. 2A and B and 3A and B) may have resulted from this postulated difference in cellular responsiveness to insulin.

An analogous situation has been described recently in A^{vy}/A (C3H × VY)F_1 hybrid male mice. Individual mice responded to identical long-term treatment with sodium phenobarbital with different levels of hepatic 7-pentoxyresorufin-*O*-dealkylase activity (18). Induction of this isozyme activity is dependent on the induction of transcription of the P-450IIB gene by sodium phenobarbital. Thus, there appear to be differences either in the regulatory programs for transcription of specific genes or in the posttranscriptional/posttranslational regulation of gene-specific polypeptide activity among individuals of the same genotype.

Two questions arise: (i) Why is there a difference in the proportions of LR and HR mice between two genotypes which differ by only one gene at the agouti locus? and (ii) Why do not all mice of the identical genotype respond similarly?

The best characterized phenotypic effects of the A^{vy} mutation at the tissue and organismic levels are dysregulated timing of pigment formation by hair bulb melanocytes, increased somatic growth, more rapid formation and growth of neoplasms, and increased efficiency of nutrient utilization (19) accompanied by obesity. In contrast with *ob/ob* and *db/db* mice, the obesity is not an obligatory component of the expressed mutant phenotype since it is absent in the lean pseudoagouti A^{vy}/a mice. However, increased somatic growth is still expressed in these lean A^{vy}/a mice as indicated by their slightly greater carcass weight when compared with black a/a siblings (3).

Increased growth of tumor cells, increased fat cell mass, and dysregulation of the hair pigment pattern are all mediated by unknown effects of the A^{vy} gene on microenvironmental conditions in the host tissues of the affected cells (2). Thus, the gene apparently alters the host tissue, affects the response of the cells to those conditions, or possibly both, depending on the particular tissue and cell type.

The specific effects of agouti locus mutations have been most clearly defined at the level of pigment synthesis. Normally, eumelanin granules are produced by melanocytes in the hair bulb and fed into the forming hair shaft by the melanocytes. At a specific time point in this process, the same melanocytes cease eumelanin synthesis and begin phaeomelanin synthesis for a short period before resuming eumelanin synthesis. Thus, a subterminal yellow band is formed which is characteristic of the species-type hair pattern "agouti." The A^{vy} mutation disrupts this regulated pattern of pigment synthesis (20). Phaeomelanin is produced most of the time with irregular periods of eumelanin synthesis resulting in the mottled yellow appearance of the animal. Apparently, the agouti locus controls a timing signal, mediated by the hair bulb, which coordinates the production of pigment with the onset of rapid growth of the hair (21).

If, by analogy, the agouti locus is assumed to have a similar "timing" function in other tissues, its possible role in the regulation of other cellular processes becomes clearer. The species-type agouti allele, *A*, may serve as a "traffic policeman" in directing metabolic substrates into alternative processing pathways which either retard or enhance cellular metabolism and/or proliferation. Under this scenario, the agouti locus would play an important homeostatic role in maintaining "normal" physiologic and metabolic conditions in the organism. The A^{vy} mutation could decrease homeostatic buffering by directing the substrates into more anabolic pathways, resulting in increased metabolic efficiency (obesity) and cell proliferation (increased normal growth, hyperplasia, neoplasia). Decreased homeostatic capacity would result in greater physiologic and metabolic variability and could thus account for the increased susceptibility of the yellow mice to the effects of streptozotocin as well as other exogenous agents.

Consideration of the second question requires a reexamination of the common supposition that, because each inbred or F_1 hybrid population consists of individuals with the same genetic constitution, all of these individuals might be expected to respond similarly to a given dose level of a toxicant for a given length of time. That this is not the case is demonstrated by the results of almost every subchronic and chronic bioassay. When inbred or F_1 hybrid animals are exposed chronically to low doses of a toxicant, only a certain proportion react to this treatment with a characteristic toxic response such as formation of tissue-specific neoplasms (3).

Genetic identity stipulates only that the DNA se-

quences of the genes of the organisms are identical. It does *not* imply that the temporal or quantitative expression of all genes is necessarily completely identical in all individuals of a genetically homogeneous inbred or F_1 hybrid population or even in monozygous twins. As mentioned previously, the inducibility of transcription of specific genes by exogenous agents and/or the post-transcriptional regulation of specific mRNA levels may differ considerably among mice of the same genotype. Additionally, the specific activities of gene-specified proteins are determined by their conformations which, in turn, are dependent on each protein's amino acid sequence and the particular characteristics of the intra- or extracellular milieu in which the proteins act. Thus, complete identity of the metabolic, physiologic, and morphologic characteristics of the organisms is not a necessary consequence of genetic identity (15). As an example, the regulatory function of the agouti locus alleles is modulated by local tissue-specific conditions so that, for example, totally black hairs occur in specific localized areas in yellow mice (22).

In the present case, the data indicate that there are definite differences in the degree of response to streptozotocin, in the resistance of the β cells to the pharmacologic effects of STZ, or in the ability of the surviving β cells to compensate for the effects of cytotoxicity by increased compensatory proliferation. There may also be differences in insulin resistance among the mice.

Conceptually, all of these differences could originate during prenatal development when modifications of the normal regulatory programs for gene transcription are imprinted in individual embryos as a result of local microenvironmental conditions in the dam's reproductive tract. Thus, if there were local differences in the immediate maternal microenvironment of specific embryos during phenocritical periods of differentiation, individual embryos with the same genotype might develop somewhat different characteristics; such phenotypic variants would differ physiologically and metabolically from each other throughout their lifetimes (15).

While human populations are genetically heterogeneous, the accumulating evidence in inbred and F_1 hybrid laboratory animal populations suggests that certain aspects of disease syndromes may vary in different individuals in response to the same level of an environmental agent or condition, not only because of their different genetic constitutions, but also because of microenvironmental modulation of transcriptional, post-transcriptional, or posttranslational regulatory effects on the same genes or gene products.

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