Effect of Age and Diet on Insulin Secretion and Insulin Action in the Rat

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SUMMARY

The effects of aging on various aspects of insulin secretion and action were studied in male Sprague-Dawley rats, maintained from 1½ to 12 mo of age on conventional rat chow, sucrose-rich, or calorie-restricted diets. In chow-fed rats, islet volume increased as the animals grew from 1½ to 12 mo of age, but glucose-stimulated insulin secretion (per volume islet) declined over the same interval. In addition, in vivo insulin-stimulated glucose utilization fell in these rats. However, the plasma insulin response to an oral glucose challenge was sufficient to prevent frank decompensation of glucose tolerance (presumably due to an increase in total pancreatic endocrine cell mass). All these changes, with the exception of the decline in glucose-stimulated insulin secretion per volume islet, were accentuated by feeding sucrose. Thus, 12-mo-old sucrose-fed rats had larger islets and higher plasma insulin levels in response to an oral glucose challenge, and the rats were more insulin-resistant than chow-fed rats. However, glucose-stimulated insulin release per volume islet was similar in 12-mo-old chow-fed and sucrose-fed rats. In contrast, calorie restriction led to an amelioration in all but one of the age-related changes, i.e., islets from calorie-restricted rats were comparable in size to those of 2-mo-old rats, the animals had lower plasma insulin levels in response to an oral glucose load, and they were less insulin resistant than the other two groups of 12-mo-old rats. On the other hand, glucose-stimulated insulin secretion per volume islet was similar to that of the other 12-mo-old rats. These results suggest that aging leads to marked changes in both insulin secretion and insulin action. The decline in glucose-stimulated insulin secretion per unit endocrine pancreas appears to be an inevitable consequence of the aging process. In contrast, the age-related changes in islet size, insulin response to a glucose load, and in vivo insulin-stimulated glucose uptake are extremely responsive to variations in amount and kind of calories. DIABETES *32*:175–180, February 1983.

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We have previously indicated that profound changes occur in both the structure and function of the islets of Langerhans when normal Sprague-Dawley rats age under laboratory conditions.[1,2] Specifically, we documented a progressive decrease in glucose-stimulated insulin secretion by collagenase-isolated islets, associated with an increase in the number of beta-cells (and insulin content) per islet. We interpreted these findings to mean that the insulin secretory capacity of the individual beta-cell declined with age, and that the increase in number of beta-cells represented a compensatory effort necessary to maintain glucose homeostasis. If this formulation is correct, it might be anticipated that the increase in beta-cell mass that occurs with age would vary as a function of the insulin secretory needs of the organism. As a consequence, changes in insulin sensitivity (which determine insulin requirements) should profoundly modify the magnitude of beta-cell hyperplasia. In contrast, since it is postulated that a reduction in beta-cell function is a fundamental age-related defect, there is no reason to think that changes in insulin sensitivity would have any effect on the ability of the individual beta-cell to secrete insulin. Although we have presented indirect evidence in support of this general hypothesis,[2] there is no direct experimental support that confirms the proposed disparate effects of age-related changes in in vivo insulin sensitivity on islet structure and function. Consequently, we initiated the current experiments, in which we quantified the effect of dietary manipulation on in vivo insulin sensitivity of aging rats and correlated the observed changes with measurements of islet structure and

function. The results provide direct experimental confirmation for the pivotal role played by variations in insulin sensitivity in modulating the effect of age on the endocrine pancreas.

METHODS AND MATERIALS

Animals. Male Sprague-Dawley rats (cesarian derived-barried sustained) were obtained from the Charles River Breeding Laboratories (Wilmington, Massachusetts) at 40 days of age. The rats were randomly divided into three groups of 13, and were maintained in our facility until they were 12–13 mo of age. One group was fed conventional rat chow (Ralston Purina Co., St. Louis, Missouri) containing 4.2 kcal/g. A second group consumed a pelleted calorie-restricted diet, consisting of 1 part chow and 2 parts cellulose (Alphacel), containing 1.4 kcal/g. The third group ate a pelleted diet that contained 3.6 kcal/g, and consisted of (as percent calories) 60% sucrose, 29% protein, and 11% fat (Teklad, Madison, Wisconsin). In addition, a fourth group of seven rats was maintained on conventional rat chow and studied at 2 mo of age.

The animals were maintained throughout the study on a 12-h light (1800–0600 h)/12-h dark cycle (0600–1800 h), and kept in laminar flow hoods to minimize infection. Food was withdrawn at 0800 on the day of experimentation to assure a 4-h fast before the initiation of study. At 12 mo of age, an oral glucose tolerance test was performed on all rats in each category. Subsequently (3–6 wk later), six chow and sucrose-fed rats and six calorie-restricted rats were used for measurement of in vivo insulin-stimulated glucose uptake. The remaining seven rats per group were used to estimate glucose-stimulated insulin secretion by isolated islets.

EXPERIMENTAL MEASUREMENTS

Oral glucose tolerance tests. Since plasma glucose and insulin responses to an oral glucose challenge vary considerably as a function of the size of the administered glucose load,[3] it is difficult to devise an oral glucose challenge for rats that differ a great deal in weight. Therefore, we have not attempted to compare 2- and 12-mo-old rats whose body weights differ approximately threefold. However, since the greatest difference in mean weights between the groups of 12-mo-old rats was less than 200 g, we thought it possible to assess differences in the plasma glucose and insulin responses of chow-fed, sucrose-fed, and calorie-restricted rats. Several considerations were taken into account in an effort to devise the magnitude of the glucose challenge. It has been established that the percentage of body weight, as fat, increases as rats age.[4–6] Indeed, from studies of both weight gain and weight loss, it appears that approximately two-thirds of the increase in weight that occurs as mature rats grow older is due to an increase in fat mass.[5,6] Furthermore, since glucose disposal by adipose tissue is negligible,[7] we felt it was inappropriate to administer the glucose challenge on a per weight basis. An alternative course was to administer the glucose on an approximation of lean body mass. Since precise figures for lean body mass were not available for the animals in this study, we administered the glucose load as if the entire difference in weight between animals was due to fat. Specifically, we gave each rat 0.9 g of glucose, based on a standard load of 190 mg/kg and a mean weight of 470 g for 12-mo-old calorie-restricted rats. We chose this approach in an effort to err on the side of giving the larger rats less glucose per estimated fat-free mass, and, in this manner, avoiding the production of artifactual hyperinsulinemia in these animals. Consequently, higher plasma insulin levels in 12-mo-old chow-fed and sucrose-fed rats could not be attributed to an inappropriately large glucose challenge.

To perform this test, unanesthetized rats were loosely wrapped in towels, and blood obtained from the tail vein before and 30, 60, 120, and 180 min after administering the glucose load into the stomach for determination of plasma glucose[8] and insulin[9] concentrations.

In vivo insulin-stimulated glucose uptake. Rats were anesthesized with an intraperitoneal injection of sodium thiamylal (6 mg/100 g body wt), and the right internal jugular vein exposed and cannulated with polyethylene tubing for administration of the infusate (to be described subsequently). Anesthesia was maintained during the procedure by a continuous, subcutaneous infusion of sodium thiamylal, administered at approximately 40% of the original dose per hour. This was begun 30–45 min after the initial intraperitoneal dose, and discontinued or restarted, as judged by the prevailing respiratory rate, response to painful stimuli, and the color of appendages. Normal body temperature was maintained throughout the procedure.

Rats received a continuous infusion (Harvard infusion pump) of epinephrine (0.08 μg/kg/min), propranolol (1.7 μg/kg/min), glucose (8 mg/kg/min), and 5.0 mU/kg/min of insulin for young rats and 2.5 mU/kg/min for old rats. The use of this technique to measure in vivo insulin action is based on a method previously developed in man[10] and dog,[11] and adapted for use in the rat.[12,13] The amounts of epinephrine and propranolol used have been shown to suppress endogenous insulin release,[13] permitting assessment of insulin-stimulated glucose utilization under conditions in which circulating exogenous insulin levels are comparable in all subjects. Preliminary studies from our laboratory with this method had indicated that insulin sensitivity, but not maximal insulin-stimulated glucose uptake, was reduced in 12-mo-old rats.[14] The greatest difference between insulin sensitivity of young and old rats was seen at steady-state insulin concentrations of approximately 100 μU/ml. In order to approach these levels, we knew from previous experience[13] that it would be necessary to infuse young rats at a rate of 5.0 mU/kg/min and old rats at a rate of 2.5 mU/kg/min.

Infusions were carried out for 3 h, and tail vein blood was obtained at 10-min intervals during the last hour of the study for determination of plasma glucose[8] and insulin[9] concentrations. Steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations for each study were determined by averaging the values of the seven measurements taken during the last 60 min of the infusion. Experiments in which the coefficient of variation for either SSPG or SSPI exceeded 10% were excluded.

In the past,[10–12] we have used the height of the SSPG concentration as a direct assessment of the ability of insulin to stimulate disposal of the infused glucose load. However, this approach neglects the fact that the height of the SSPG concentration itself promotes glucose disposal.[15–17] In an effort

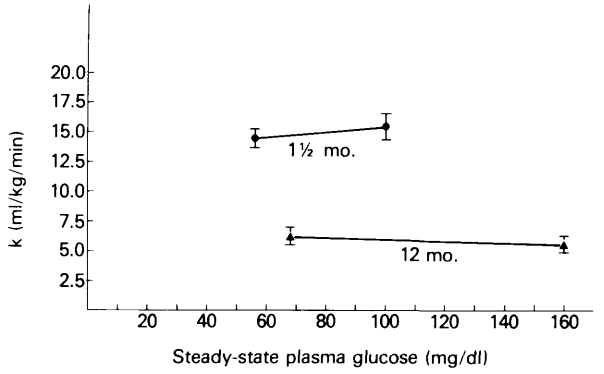

FIGURE 1. The relationship between glucose clearance (k) and steady-state plasma glucose concentration. Steady-state plasma insulin was ~100 mU/ml and was achieved by infusing young (1½ mo old) rats with 5 mU insulin/min and older (12 mo old) rats with 2.5 mU insulin/min. Although levels for k differed between young and older rats, within each category, k remained steady over a wide range of plasma glucose levels.

to control for this variable, we have also attempted to compare insulin-stimulated glucose utilization of groups of animals on the basis of the efficiency of glucose uptake, i.e., the glucose clearance (k). This approach is also independent of the amount of glucose infused.

Glucose clearance (k) was calculated from the following formula: k = glucose uptake/SSPG. Since these experiments were carried out under steady-state conditions, we assumed that glucose uptake was equal to the rate of glucose infusion. However, this is only true if values for urinary glucose loss and hepatic glucose output are negligible. We directly determined the lack of significant glycosuria during these infusions, but we did not measure hepatic glucose output. However, there is considerable evidence in man that comparable steady-state levels of glucose and insulin totally suppress hepatic glucose output.[10,15–19] It seems reasonable to assume that glucose uptake rate approximates glucose infusion rate.

To compare individuals on the basis of glucose clearance, it was essential that clearance be independent of the SSPG concentration. Although there is evidence in both normal[15,16] and diabetic[17] man that this is true at the steady-state plasma levels of glucose and insulin used in these studies, we thought it important to also validate this point in the rat. To do this, studies were carried out at different glucose infusion rates in both young (1½ mo old) and aging (12 mo old) rats. These data appear in Figure 1, and indicate that glucose clearance is independent of SSPG concentration when the steady-state plasma insulin concentration is approximately 100 μU/ml, e.g., the level employed in these studies.

Glucose-stimulated insulin secretion by isolated islets. Islets from aging animals of each of the experimental categories, as well as from young, 2-mo-old rats (obtained directly from Charles River Co.) were isolated by a spinner flask modification of the method of Lacy and Kostianovsky,[20] currently in use in our laboratory.[21] Since we know that islets from any one geographic region of the pancreas of aging 12-mo-old rats are similar to islets obtained from other regions of the pancreas (in both insulin secretory capacity and morphology),[21] the entire pancreas of each rat was used as starting material. Islets from the more obese 12-mo-old chow- and sucrose-fed rats, which appeared to disintegrate after collagenase treatment,[2] were avoided, and only compact islets with smooth contours were used. Insulin release over a 60-min period at 2.8 and 25.0 mM glucose was measured as previously outlined,[1,2] except that Hepes buffer (10 mM, pH 7) was included in both preincubating and incubating solutions. Twenty randomly selected, intact, nonincubated islets of each preparation were measured for size as previously described,[1,2] and corrected for variations in connective tissue content determined from paraffin sections of pancreases from similarly treated rats.[22] Thus, the averaged volumes of each preparation of islets from calorie-restricted, chow-fed, and sucrose-fed rats were reduced by 13%, 25%, or 42% to account for the contribution of connective tissue. The connective tissue content of 2-mo-old rats[22] was found to be less than 5%. Insulin release was expressed per corrected islet volume for each experiment performed.

Statistical analysis. Student's two-tailed *t* test was used to define the statistical significance of differences that existed between the experimental groups.

RESULTS

The aging rats of this study tolerated the dietary manipulations well and gained weight in a predictable fashion:[2] i.e., rats that had been consuming the chow-fed, sucrose-fed, or cellulose-enriched diet ad libitum for 10½ mo showed a mean (± SEM) weight of 656 ± 7, 663 ± 12, and 470 ± 12 g, respectively, at 12 mo of age.

The plasma glucose and insulin responses to oral glucose of the three groups of 12-mo-old rats are seen in Figure 2. Basal plasma glucose levels of the three groups were quite comparable, as was the pattern of the response to the glucose challenge. However, some differences were noted: e.g., mean plasma glucose levels of the 12-mo-old calorie-restricted rats were significantly ($P < 0.01$) lower than that of the other two groups at the 60-min time point. Furthermore, the total plasma glucose response (area under the curve) of the calorie-restricted rats was significantly lower ($P < 0.05$) than that of chow-fed or sucrose-fed rats. Although plasma glucose levels were somewhat higher in sucrose-fed as compared with chow-fed rats, neither the individual time points nor the total responses of these two groups of animals was significantly different.

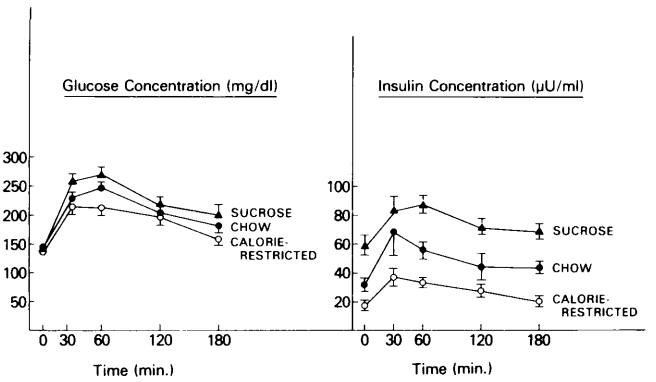

FIGURE 2. Plasma glucose and insulin levels in 12-mo-old chow-, sucrose-, and calorie-restricted rats given an oral glucose challenge of 0.9 g glucose/rat. The data points represent mean (±SEM) values from 13 rats in each experimental category.

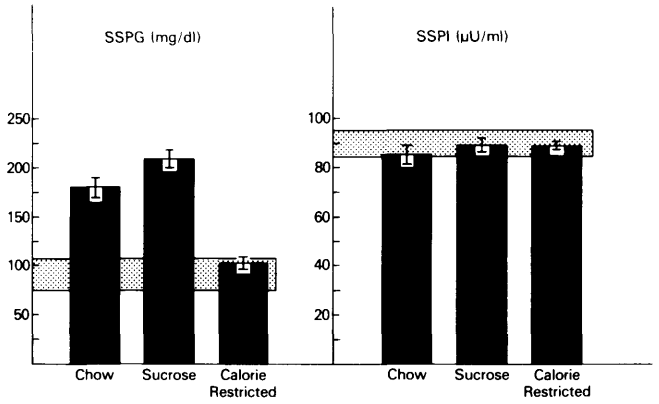

FIGURE 3. Steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations achieved in 12-mo-old chow-fed, sucrose-fed, and calorie-restricted rats during the infusion of epinephrine, propranolol, glucose, and insulin (see METHODS). Each black bar represents the mean (±SEM) data from six 12-mo-old rats maintained on the different experimental regimens of the previous 10½ mo. The stippled horizontal bar represents SSPG and SSPI values (mean ± 2 SEM) obtained from 1½–2-mo-old rats measured by the same techniques.

Plasma insulin concentrations following the oral glucose challenge are seen in the right panel of Figure 2, and it is clear that the differences between the plasma insulin responses of the three groups were more dramatic. First, basal (postabsorptive) plasma insulin concentrations were significantly different among the groups, and were lowest in the calorie-restricted animals (~20 μU/ml) and highest in the sucrose-fed rats (~60 μU/ml). Although the pattern of the insulin response to a glucose load was similar in all categories of rats studied, insulin levels in sucrose-fed rats were higher at every point tested that those of chow-fed animals. Chow-fed rats, in turn, had higher insulin values at every point than did the calorie-restricted animals. Total (mean ± SEM) insulin response (area under the curve) was 525 ± 61, 831 ± 68, and 306 ± 25 μU/ml • h for chow-fed, sucrose-fed, and calorie-restricted rats, respectively, and the results from each group of rats were significantly ($P < 0.01$) different from the others. Finally, it should be remembered that the same oral glucose load was given to all rats, and the greater insulin responses in the larger rats occurred in spite of the fact that these relatively obese rats received proportionally less glucose than did the calorie-restricted animals.

Insulin-induced glucose uptake. SSPG and SSPI concentrations during the infusion studies are seen in Figure 3. It is evident from the data in the right panel that similar steady-state concentrations of exogenous insulin were achieved during the infusions in all three groups of 12-mo-old rats, and that the values were also comparable to the SSPI levels of young rats. Despite this similarity in SSPI concentrations, it is evident from the data in the left panel that considerable variation did exist in the SSPG concentrations of the experimental groups. Thus, SSPG concentrations of 12-mo-old chow-fed rats were almost twice as high as values for young rats ($P < 0.001$). This age-related increase in SSPG level was accentuated in sucrose-fed rats, whose SSPG concentrations were significantly greater ($P < 0.01$) than those of chow-fed rats of similar age. In marked contrast, SSPG levels of calorie-restricted rats were markedly lower ($P < 0.001$) than in the other two groups of 12-mo-old rats, and were not significantly different from values of young rats.

Higher SSPG concentrations in 12-mo-old chow-fed and sucrose-fed rats could be due to a decrease in insulin-stimulated peripheral glucose utilization, and/or a decrease in the ability of the liver to reduce glucose efflux or increase glucose uptake in response to insulin. It is obvious that these results do not provide insight as to which mechanisms are operative, but only indicate that the net ability of insulin to stimulate disposal of a defined load of exogenous glucose is diminished in 12-mo-old chow-fed rats, and that this defect is accentuated in 12-mo-old sucrose-fed rats and alleviated in 12-mo-old calorie-restricted rats.

Furthermore, the data in Figure 3 do not take into consideration the effect that variations in the height of SSPG concentration may have on glucose disposal. In order to address this issue, we also calculated values for glucose clearance (k) of the various experimental groups. These data appear in Figure 4, and it can be seen that changes in either kind or amount of calorie intake profoundly altered insulin-stimulated glucose clearance of 12-mo-old rats. Thus, glucose clearance was significantly lower ($P < 0.05$) in 12-mo-old rats fed a diet high in sucrose as compared with conventional chow. A more dramatic dietary effect was seen in the calorie-restricted group, and insulin-stimulated glucose clearance was almost double that ($P < 0.01$) of 12-mo-old chow-fed rats. Indeed, Figure 4 indicates that the glucose clearance of 12-mo-old calorie-restricted rats approximated that of 1½–2-mo-old rats studied by the same technique.

Insulin secretion by isolated islets. The results in Figure 5 show that the size (corrected volume) of islets from young (2-mo-old) and aging (12-mo-old) calorie-restricted rats are comparable, whereas the size of islets from either chow- or

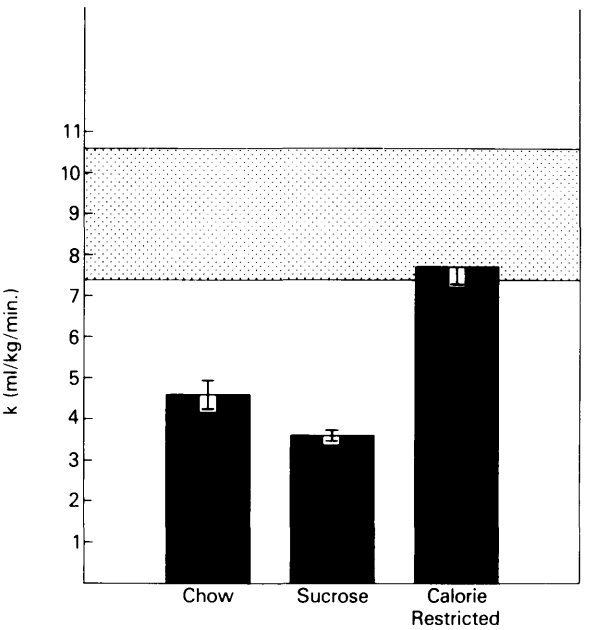

FIGURE 4. A comparison of glucose clearance (k) values calculated from estimates of glucose disposal rates and the SSPG results shown in Figure 3. As in Figure 3, each black bar represents the mean (±SEM) data from six 12-mo-old rats maintained on the different experimental regimens, and the stippled horizontal bar represents values (mean ± 2 SD) from 1½–2-mo-old rats.

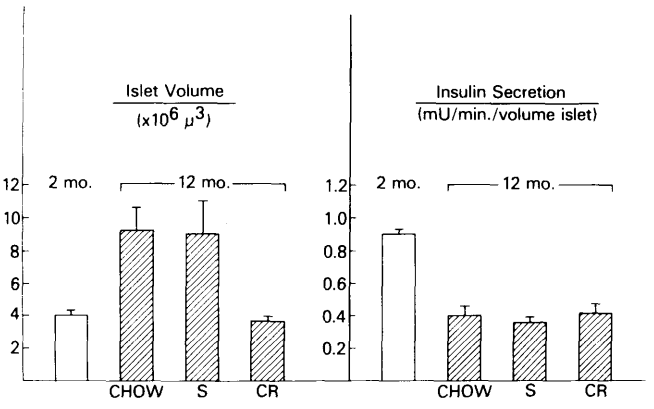

FIGURE 5. Size (volume) of collagenase-isolated islets and glucose-stimulated insulin release (per volume islets) from 2-mo-old rats fed chow, or 12-mo-old rats who were calorie-restricted (CR) or maintained on chow or sucrose-enriched diets (S) for the previous 10½ mo. Islet volume was determined from diameter measurements, and was further corrected for connective tissue content utilizing average values obtained from tissue sections.[22] Insulin release was measured after stimulation with 25 mM glucose for 60 min. Each bar represents measurements from seven experiments.

sucrose-fed rats are more than double this value. When insulin secretion from the same preparations of islets is expressed per islet volume, secretion rates from islets of all 12-mo-old rats are identical (regardless of differences in body weight or in the kind of calories that had been consumed by the rats). However, insulin secretion from all the older groups of rats is less than 50% of that of young rats.

DISCUSSION

Although the results presented indicate that both insulin secretion and insulin action are profoundly modified by age, the implication of these events is quite different. When islet volume is considered, it seems that islet secretory capacity is reduced in older rats, and this age-related decline cannot be altered by environmental manipulation; that is, the insulin secretory defect appears to be an inevitable consequence of the aging process. In contrast, age-related changes in in vivo insulin-stimulated glucose uptake are extremely sensitive to environmental manipulations. For example, the decrease in the ability of insulin to promote glucose utilization seen in 12-mo-old chow-fed rats is significantly attenuated in calorie-restricted rats of the same age, but is exaggerated in 12-mo-old rats fed a high sucrose diet. These direct estimates of in vivo insulin action are consistent with the results of the oral glucose tolerance tests seen in Figure 2. Thus, plasma insulin levels are lowest in calorie-restricted rats, intermediate in chow-fed rats, and highest in rats fed a diet rich in sucrose. Since the plasma glucose responses of the three groups were comparable, the height of the plasma insulin levels can be interpreted to be a reflection of the degree of insulin sensitivity. Thus, by both direct and indirect estimates, calorie-restricted rats are more insulin sensitive than equally old rats fed conventional chow, and, in turn, sucrose-fed rats are more insulin-resistant. These combined data emphasize the degree to which insulin-stimulated glucose uptake can be regulated by environmental changes.

Given these data, we can propose an integrated hypothesis concerning the effects of aging on carbohydrate metabolism. Thus, chow-fed rats, allowed to become obese as they age, demonstrate a reduction in both insulin secretion per volume of endocrine pancreas (beta-cell) as well as in total body insulin-stimulated glucose uptake, as measured by either SSPG concentration or glucose clearance. These rats do not become hyperglycemic because they secrete enough insulin to maintain reasonably normal plasma glucose levels (Figure 2). Since insulin secretion per beta-cell is reduced dramatically, this must mean that 12-mo-old rats have an increase in number of beta-cells and/or a defect in insulin catabolic rate. The data in Figure 5 (as well as ref. 2) provide support for the first alternative, and illustrate the degree of islet hypertrophy that develops with aging in these rats. There is also evidence that aging may be associated with a decrease in rate of insulin degradation,[13] and this phenomenon may also contribute to the maintenance of plasma insulin concentrations in 12-mo-old chow-fed rats.

On the other hand, calorie restriction prevents the deterioration in in vivo insulin action that occurs with age in chow-fed rats. As a result, glucose tolerance is easier to maintain and less insulin secretion is required to fulfill this function. Consequently, the degree of pancreatic beta-cell hyperplasia need not be as great, nor should plasma insulin levels be as high as in chow-fed rats of the same age. It is obvious from the experimental results that these predictions are realized. In contrast are the results from sucrose-fed rats. These rats are more insulin-resistant than 12-mo-old chow-fed rats, they have the highest plasma insulin response during the glucose tolerance test, and showed a substantial degree of islet hypertrophy (Figure 5).

In conclusion, these results indicate that rats have two means of compensating for the loss of insulin secretion per beta-cell, which is apparently an inevitable consequence of aging. On the one hand, normal glucose tolerance can be maintained by minimizing the loss of in vivo insulin action that is seen in sedentary and obese 12-mo-old rats. In this manner, an intrinsically defective endocrine pancreas can still manage to supply the amount of insulin needed to maintain glucose tolerance. Alternatively, hyperplasia of the endocrine pancreas can help compensate for the loss of insulin secretory function per beta-cell, and enable the organism to secrete enough insulin to keep up with its needs. However, it must be emphasized that our studies have not extended beyond 12 mo and 1-yr-old rats have only reached "middle-age." It is possible that these compensatory mechanisms may not be sufficient as rats age further, and that the combination of diminished beta-cell function and increased insulin resistance will eventually lead to frank decompensation of glucose tolerance. This possibility is currently under study in our laboratory.

ACKNOWLEDGMENTS

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