Structure and function changes in the endocrine pancreas of aging rats with reference to the modulating effects of exercise and caloric restriction.

E P Reaven, G M Reaven

J Clin Invest. 1981;68(1):75-84. https://doi.org/10.1172/JCI110256.

Research Article

The current study was conducted to determine if physical activity and/or weight control could influence the age-related decrease in beta cell insulin response noted in earlier studies. As such, virgin, male Sprague-Dawley rats were maintained in our facility for 1 yr on three differential experimental programs; in the first group, control rats lived under standard laboratory conditions; the second group of rats ran several miles a day in exercise wheels, and the third group was given a calorie-restricted diet designed to keep the rats weight-matched with the exercising rats. The results showed that the 12-mo-old sedentary control rats weighed an average of 800g. From the time these rats were 4 mo old, they were significantly hyperinsulinemic, with mean (+/- SEM) serum insulin levels of 55 +/- 6 microU/ml. Morphological studies on the pancreas of these rats at the end of the year revealed enlarged, multilobulated, fibrotic islets. After collagenase digestion, the most normal-appearing islets from the 12-mo-old controls were used for insulin secretion studies, these islets showed significantly reduced glucose-induced insulin release (0.83 microU insulin/min per volume islet) compared with islets from young rats (1.80 microU insulin/min per volume islet). In contrast, 12-mo-old exercised or calorie-restricted rats weighed approximately 500 g and did not show the changes in serum insulin levels or pancreas pathology exhibited by the sedentary control animals. […]

Structure and Function Changes in the Endocrine Pancreas of Aging Rats with Reference to the Modulating Effects of Exercise and Caloric Restriction

EVE P. REAVEN and GERALD M. REAVEN, *Department of Medicine, Stanford University School of Medicine, and Geriatric Research, Education and Clinical Center, Veterans Administration Medical Center, Palo Alto, California 94304*

ABSTRACT The current study was conducted to determine if physical activity and/or weight control could influence the age-related decrease in beta cell insulin response noted in earlier studies. As such, virgin, male Sprague-Dawley rats were maintained in our facility for 1 yr on three differential experimental programs: in the first group, control rats lived under standard laboratory conditions; the second group of rats ran several miles a day in exercise wheels; and the third group was given a calorie-restricted diet designed to keep the rats weight-matched with the exercising rats. The results showed that the 12-mo-old sedentary control rats weighed an average of 800 g. From the time these rats were 4 mo old, they were significantly hyperinsulinemic, with mean (±SEM) serum insulin levels of 55±6 μU/ml compared with their base-line levels at 1½ mo of 20±3 μU/ml. Morphological studies on the pancreas of these rats at the end of the year revealed enlarged, multilobulated, fibrotic islets. After collagenase digestion, the most normal-appearing islets from the 12-mo-old controls were used for insulin secretion studies, these islets showed significantly reduced glucose-induced insulin release (0.83 μU insulin/min per volume islet) compared with islets from young rats (1.80 μU insulin/min per volume islet). In contrast, 12-mo-old exercised or calorie-restricted rats weighed ~500 g and did not show the changes in serum insulin levels or pancreas pathology exhibited by the sedentary control animals. However, islets from the calorie-restricted group functioned in vitro no better than islets from the sedentary control group. Islets from the exercised rats were somewhat improved in this regard. In summary, we believe exercise and weight control diminishes the animals' need for insulin—resulting in youthful-appearing islets after a year's time. However, these regimes do not appear able to correct the beta cell decline in function previously described.

Received for publication 8 January 1981 and in revised form 16 March 1981.

INTRODUCTION

In previous studies from this laboratory we have shown that as rats age from 2 to 18 mo, their islets of Langerhans change morphologically and functionally (1, 2). That is, islet size (volume) increases with age as a result of an increased number of endocrine cells. Also, the average beta cell contains more beta granules with progressing age, and as a consequence, the average islet from an aging rat stores more insulin than would be estimated from its size alone. Despite these increased storehouses of insulin, isolated islets from aging rats secrete insulin less efficiently in response to stimulation by glucose or leucine than do islets from young animals. This age-related decrease in insulin secretory rates noted for islets is even more dramatic when expressed per beta cell, and it is clear that the impairment in insulin secretion per beta cell is progressive, worsening with each 6-mo interval studied.

Although these results indicate that alterations in islet structure and function occur as rats grow from 2 to 18 mo, it is not clear whether the islet changes are due to aging itself, or the result of some additional process which is merely associated with increasing age. For example, laboratory rats are committed to life-long inactivity, and, under these conditions, Sprague-Dawley rats tend toward obesity. The impact of inactivity and obesity on islet size and insulin secretion in our previous study is not known. In addition, it is possible that sexual activity affects islet morphology and function, as reported by Wexler in a number of studies (3, 4). Since the aging rats in our original study had been acquired as retired breeders, the possible

influence of breeding on the changes in islet volume and insulin secretion could not be evaluated.

Therefore, we conducted the current study, in which we used virgin rats, and attempted to control for obesity and/or decreased physical activity in the following manner. Sprague-Dawley rats were obtained at a very early age, and divided into three experimental groups. In the first, rats were maintained under standard laboratory conditions as before; the second group of rats ran several miles a day in exercise wheels and were permitted to eat standard lab chow ad lib.; the third group was maintained on a calorie-restricted diet designed to keep the rats weight-matched with the lower weight of the exercising rats. With this approach we hoped to differentiate between the effects of age per se, as distinguished from age-related environmental variables on islet structure and function.

METHODS

Experimental protocol

Male Sprague-Dawley rats (caesarean-originated, barrier-sustained) were obtained from the Charles River Breeding Laboratories Co. (Wilmington, Mass.) at 40 d of age. Within a few days of arrival, the rats were individually tagged with ear clips, weighed, and bled for determination of base-line serum glucose and insulin concentrations. They were then divided into three categories of 24 rats each.

Control rats were housed three to a cage (18″ × 8″) and permitted to eat standard laboratory rat chow (pelleted Purina chow, Ralston Purina Co., St. Louis, Mo.; 350 kcal/100 g) ad lib. Exercised rats were housed in individual exercise wheels (Wahmann Co., Timonium, Md.) on which revolutions were automatically recorded (5). The rats were screened and only those animals which maintained a level of spontaneous running of >4 mi/d for a period of 2 wk were kept in the experiment. Discarded runners were replaced with new rats until 24 good runners could be identified. These rats were allowed free access to the same standard chow given to the control animals. Calorie-restricted rats were given a pellet diet (114 kcal/100 g) containing ⅓ Purina chow and ⅔ cellulose (Alphacell; Tek Labs, Madison, Wis.). Preliminary experiments had indicated that if this diet was given ad lib., rats would maintain weights similar to those of exercising group. Accordingly, the animals of this group were permitted to eat as much of this diet as they wished. They were housed in the same manner as the control group.

It was the intent of these studies to maintain the rats on their exercise or feeding programs until they were 12 mo of age. However, the exercising rats tended to run less as they aged. It was decided, therefore, that if an individual rat ran <2 mi/d over a period of 2–3 wk, the rat was removed from the study, as was a rat chosen at random from each of the other groups. As a consequence, the number of observations decreased as the study progressed. In all cases, the rats of this study were maintained on an automatically controlled light/dark cycle (6:00 a.m./6:00 p.m.) and were kept in laminar flow hoods (Lab Products, Inc., Rochelle Park, N. J.) to protect against infection.

Body weight was recorded monthly. Tail bleeding for serum glucose and insulin values were obtained between the 3rd and 4th mo, and then at 2–3-mo intervals; all blood samples were taken between 2:00 and 4:00 p.m. Food consumption over a 24-h period was recorded every 3–4 mo. For convenience, these various values have been grouped into four periods: 1½ mo (base-line), 3–4 mo, 6–8 mo, and 10–12 mo.

At the termination of the study, the remaining 12-mo-old rats of this series were used for insulin secretion studies as described below. To control for possible technical discrepancies, a group of young (2-mo-old) rats were killed at the same time and, on any given day, rats from different groups were selected at random for study.

In addition to these rats used for functional studies, a small number of extra rats were permitted to grow to 12 mo of age while being maintained on the two different diets. At the termination of the study, these animals, as well as a group of 2-mo-old rats, were bled for blood chemistries and their pancreata were excised for mophological examination. Because of limited space, exercised rats were not included in this protocol.

Serum values

Tail-blood was taken from unanesthesized rats before the experiment at 45–50 d, and also at 3–4 mo, 6–8 mo, and 10–12 mo. At the end of the 12-mo period, the animals were anesthesized with methoxyfluorane (Metafane; Pitman-Moore, Inc., Washington Crossing, N. J.), and aortic blood was obtained before the pancreas was removed. Aliquots of serum from these various samples were stored frozen for subsequent determination of glucose and insulin. At the termination of the study, all samples were assayed together. Serum glucose values were determined with a glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.), and serum insulin was determined by a modification of the method of Desbuquois et al. (6). When more than one sample of blood was obtained from an animal within the arbitrary time periods chosen for Table I, the values were averaged and only the mean value is reported.

Insulin secretion

In general, islets were isolated by collagenase digestion and utilized for insulin secretion studies as previously described (1). On any given day, two secretion studies were conducted, and for each experiment, either two 2-mo-old or one 12-mo-old rat was used. Collagenase (CLS IV, Lot 40A282, Worthington Biochemical Corp., Freehold, N. J.) was used throughout, and in general, it was found that tissues from 12-mo-old rats (exercised or calorie-restricted) required only slightly more incubation time with collagenase (6½ min) than did young, 2-mo-old rats (6 min). However, initial experiments with the 12-mo-old control rats revealed that a substantial proportion of the isolated islets were abnormal in appearance. When viewed with the dissecting microscope, many islets from these rats appeared to be loose conglomerates of exceedingly small bits of islet tissue; this was in sharp contrast to the 0.2–0.4-mm compact globular structures that one customarily identifies as individual islets. Attempts to vary the conditions of the collagenase isolation procedure (in particular, to decrease incubation time with collagenase) did not alter the appearance of the islets from these animals. It was subsequently decided to avoid the abnormal appearing islets when islets were selected for secretion studies. As a result, islets used for secretion studies from the aging control rats were not truly representative of the pancreatic population of islets of these animals. In the case of the other experimental groups, however, 20 islets were randomly chosen from the isolated group of islets for each of 10 incubation vials (see

TABLE I

Caloric Consumption and Body Weight of Rats Growing to 12 mo of Age on Different Diet and Exercise Programs

Category	Age		24-h calorie consumption	Body weight*
	mo		*cal*	*g*
Controls	1½	(20)‡	—	205±3
	3–4	(18)	87±1.8	553±10
	6–8	(16)	91±2.0	683±23
	10–12	(12)	109±12.0	810±23
Exercised	1½	(20)‡	—	199±3
	3–4	(18)	112±2.8§	429±11§
	6–8	(16)	126±5.0§	523±15§
	10–12	(12)	126±7.0§	548±22§
Calorie-restricted	1½	(20)‡	—	200±3
	3–4	(18)	57±0.3§	453±11§
	6–8	(16)	71±6.8§	542±14§
	10–12	(12)	78±6.8§	544±13§

Values are mean±SE; parentheses contain number of animals studied.

* Body weight values represent a mean from several measurements taken during the specified time interval.

‡ Values at 1½ mo are base line (before rats were placed on different diet and exercise programs).

§ $P < 0.01$ compared with control rats of comparable age.

reference 1 for details); duplicate vials were used for incubation with increasing concentrations of glucose in the media. Prior to incubation with glucose, islets from one vial were fixed with glutaraldehyde (1) and preserved for electron microscopy. Following incubation, two vials of islets from each experiment were fixed in buffered formalin and used for measurements of islet diameter and estimation of islet volume; these estimates were subsequently used to correct the secretory data for the differences due to islet size.

Morphological methods

Electron microscopy on isolated islets. Glutaraldehyde-fixed islets were processed as previously described (1), and viewed for evidence of pathology. Various morphometric measurements were made on these islets using volume density techniques (1, 7).

Light microscopy on the intact pancreas. At the termination of the study, intact pancreata were obtained from the small extra group of 2- and 12-mo-old rats on different diets. The pancreas of each animal was injected through the bile duct with Bouin's fixative (6 ml for the pancreas 2-mo-old rats and 10 ml for the pancreas of 12-mo-old rats on different diets). Subsequently, the pancreata were removed intact, fixed further for 24 h in Bouin's fixative, processed, and embedded in paraffin. 5-μm sections, taken at random through the embedded blocks without regard to region, were stained with hematoxylin and eosin or with a standard trichrome method.

Using an eyepiece grid, volume density measurements were made on the composition of the islets as well as on the islet and connective tissue content of the sections (1). To avoid bias, the individual making of these morphometric measurements did not know the identity of the animal at the time the measurements were being made.

RESULTS

General description

The virgin control rats were sedentary, chronically consumed a large number of calories, and became obese with advancing age. As seen in Table I, mean body weight of these rats at 1 yr was 800 g, ~300 g more than that of exercising or calorie-restricted rats. It should be noted that the mean weight of rats from the extra group of six controls (see Methods) was also high (826±20 g). Exercised rats, on the average, consumed more calories than the aging control rats at any given age period, but still weighed less at every time interval. Calorie-restricted rats ate a large quantity of low calorie pellets; however, total calorie consumption averaged between 65 and 70% of that of aging control rats, and this group also weighed less throughout the experimental period.

Approximately one-third of the exercising rats were gradually eliminated from the study when their spontaneous running dropped below 2 mi/d. Fig. 1 indicates the spontaneous running achieved by those rats that remained. The large standard error bars are a result of substantial variations over time in the running behavior of individual rats, as well as variations between rats.

Serum glucose and insulin levels

As seen in Table II, base-line glucose concentrations of 1½-mo-old rats ranged between 92 and 99 mg/dl.

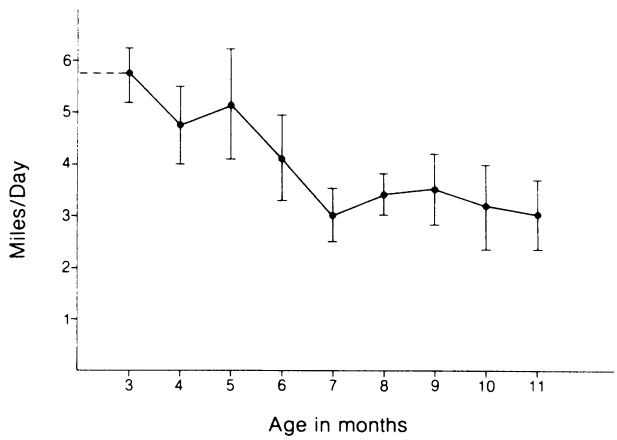


FIGURE. 1 Mean±SE running activity of the 12 rats that remained in the exercise group until the termination of the study at 12 mo. Although the large SE bars represent the substantial variation in running between the rats, many of the individual rats also changed their running behavior during the study; some rats ran more at the beginning than later, others reversed this process to some extent.

TABLE II

Serum Glucose and Insulin Concentration of Rats Growing to 12 mo of Age on Different Diet and Exercise Programs

Category	Age		Serum glucose	Serum insulin
	mo		*mg/dl*	*μU/ml*
Controls	1½	(20)*	99±2	22±3
	3–4	(18)	100±3	48±6
	6–8	(16)	105±3	55±6
	10–12	(12)	102±4	61±6
Exercised	1½	(20)*	95±2	22±4
	3–4	(18)	95±3	16±2‡
	6–8	(16)	103±2	25±5‡
	10–12	(12)	101±3	21±4‡
Calorie-restricted	1½	(20)*	92±2	25±4
	3–4	(18)	89±2	17±2‡
	6–8	(16)	96±3	21±3‡
	10–12	(12)	87±5‡	20±3‡

Values are means±SE; parentheses contain number of animals studied.

* Values at 1½ mo are basal values before rats were placed on the different diet and exercise programs.

‡ $P < 0.01$ as compared with control rats of same age.

Slight variations in these values occurred during the 12-mo experimental period, but in general, plasma glucose levels remained stable.

In contrast, serum insulin concentrations showed dramatic changes between experimental groups, as seen in Table II. Base-line insulin levels ranged between 22 and 25 μU/ml. In the control rats eating standard lab chow, insulin values doubled by 3–4 mo and tripled by 10–12 mo. In contrast, no increase in plasma insulin levels occurred in exercised and calorie-restricted rats during the experimental period.

In vitro insulin secretion

Table III indicates that the mean volume of islets obtained from 12-mo-old rats was substantially greater than the volume of islets of young control rats. This occurred, despite the fact that the largest and most bizarre islets from 12-mo-old rats were not selected for these studies (see Methods). On the other hand, islets from 12-mo-old rats that had either exercised or been subjected to caloric restriction were only slightly larger than islets obtained from 2-mo-old rats.[1]

Insulin secretion, per volume islets, is also seen in Table III, and it is clear that the larger islets of 12-mo-old rats secrete significantly less insulin than do islets from 2-mo-old rats at stimulating concentrations of glucose ($P < 0.01$). The situation in 12-mo-old rats that have been exercised and calorie-restricted is more complex. Glucose-stimulated insulin secretion by islets from 12-mo-old exercised rats most closely resembles that of islets from 2-mo-old rats. Indeed, the differences between the two groups are only of marginal significance. ($0.10 < P > 0.05$), and islets from 12-mo-old exercised rats secrete significantly more insulin than do islets from 12-mo-old control rats ($P < 0.01$).

[1] It should be noted that the mean volume of islets from the young rats of this study is less than that reported previously from our laboratory (1). Although we feel this discrepancy is due to some technical change in our procedure of isolating or processing of the islets, we cannot pinpoint the particular cause at this time. We find that the batch of collagenase used, and the precise method of isolation, can contribute to this kind of variation; e.g., islets from young rats of our current experiments are again somewhat larger than those reported here. In view of this kind of problem, we use a given batch of collagenase and scrupulously maintain the same method of isolation and treatment of islets for any given set of experiments. As a result, we feel the absolute values for islet volumes may vary among studies, but comparisons within studies are valid.

TABLE III

Size and Insulin Secretory Response of Isolated Islets from 2- and 12-mo-old Rats on Different Diet and Exercise Programs

Category*	Islet volume	Insulin secretion in response to different glucose concentrations			
		2.8 mM	8.3 mM	16.7 mM	25 mM
	$\times 10^6 \mu^3$	*μU insulin/min/volume islet*			
2-mo controls (7)	2.3±0.27	0.14±0.03	0.79±0.09	1.50±0.14	1.79±0.16
12-mo controls (12)	3.7±0.20‡	0.10±0.03	0.48±0.05‡	0.90±0.06‡	0.83±0.10‡
12-mo exercised (12)	2.6±0.27	112±0.01	0.48±0.07‡	1.28±0.12	1.50±0.16
12-mo calorie-restricted (12)	2.7±0.27	0.08±0.02	0.37±0.05‡	1.02±0.14‡	1.12±0.15‡

Values are mean±SE.

* Parentheses contain number of different experiments conducted in each animal group.

‡ $P < 0.01$ as compared with islets from 2-mo controls at same glucose concentrations.

The beneficial effects of calorie restriction on insulin secretion do not appear to be as great. Thus, insulin secretion by islets from 12-mo-old carlorie-restricted rats is only slightly better than that of islets from 12-mo-old control rats ($0.01 < P > 0.05$) and significantly less than the response of islets from 2-mo-old rats ($P < 0.01$). These results can be summarized by saying that exercising and caloric restriction are equally effective in preventing the age-related increase in islet volumes, whereas exercising seems to be more effective in preventing the age-related decrease in insulin secretion.

Morphological observations

General observations on sections of intact pancreas. Light microscopic examination of random sections through the pancreata of the aging control rats revealed many pleomorphic and enlarged islets. Figs. 2A and B depict the alterations typical of these islets, and show a substantial increase in connective tissue elements within the islets. The result is a multinodular islet, in which clusters of endocrine cells are widely separated from each other by traversing bands of connective tissue. Elongated cells, presumed to be fibroblasts, are present in these connective tissue areas, and an occasional inflammatory cell can be found. Hemosiderin inclusions are present in numerous cells (macrophages?) found on the outskirts of the islets. In addition, islet cell size and cytoplasmic stainability of the islet cells varied widely in these islets. Occasionally, one also saw "budding" of islet cells from small intralobular ducts.

In contrast, sections of the largest islets from calorically-restricted 12-mo-old rats seem quite normal in appearance (Figs. 3A and B). These islets appear to have basically rounded contours, and are composed of uninterrupted planes of endocrine cells.

Morphometric analyses on sections of intact pancreas. It is important to note that not all pancreatic islets of the 12-mo-old controls appeared enlarged and abnormal; variations in islet structure and size were present in pancreata from different rats and also within any given pancreatic slice. To more fully understand the extent of changes relating to the different groups of animals, we obtained quantitative measurements on islet size, percentage of connective tissue present in islets, and percentage of total pancreas tissue occupied by islet tissue, using volume density techniques on trichrome-stained sections of pancreas.

Table IV indicates that the average volume of islets from 12-mo-old controls is more than three times ($P < 0.025$) that of islets from 2-mo-old control rats. Part of this change is due to the dramatic increase in the amount of connective tissue within the islets. On the other hand, a simple calculation reveals that islets from 12-mo-old control rats also contain more endocrine tissue. Thus, in islets from 12-mo-old rats, ~85% of the islet is not connective tissue (100% − 15.2%); as a result, the total volume of the average islet that is composed of endocrine tissue would be equal to 0.85 times the islet volume ($10.9 \times 10^6 \mu^3$), or $9.3 \times 10^6 \mu^3$ A similar calculation shows the endocrine tissue mass of islets from 2-mo-old control rats to be approximately one-third less, or $2.8 \times 10^6 \mu^3$. Therefore, the enlarged islets of the 12-mo-old control rats contribute a substantial increase in endocrine tissue mass to the pancreas, and this may account for the differences seen in the final column of Table IV.

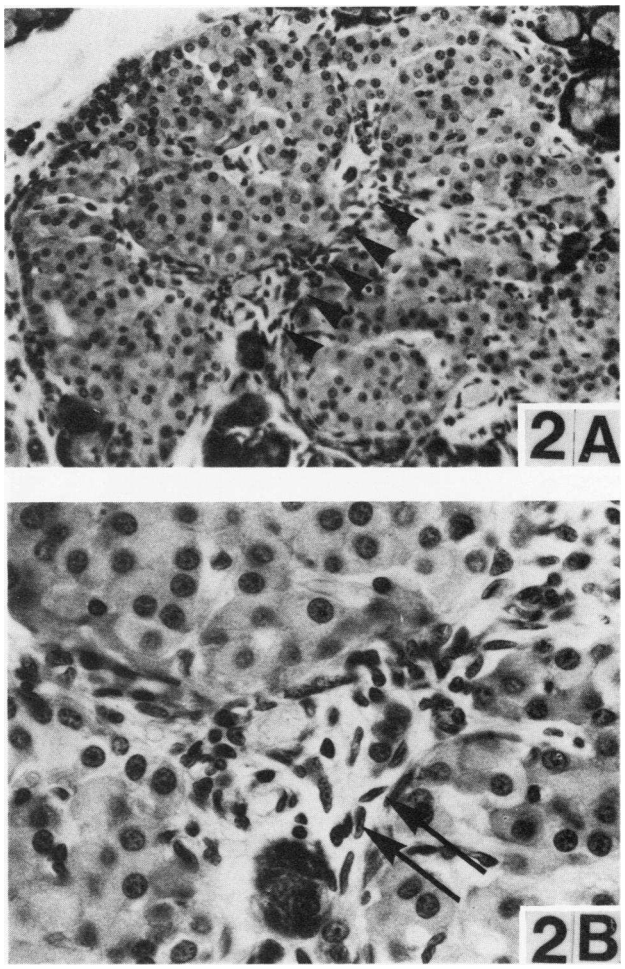


FIGURE 2 Typical light microscopic photographs of pleomorphic islets from the 12-mo-old control groups of rats. 2A illustrates the size and multinodular appearance of the islets with areas of connective tissue infiltration marked by arrowheads (×150). 2B shows a higher power view of the spindle-shaped nuclei of fibroblasts within the connective tissue (arrows). Hematoxylin and eosin (×400).

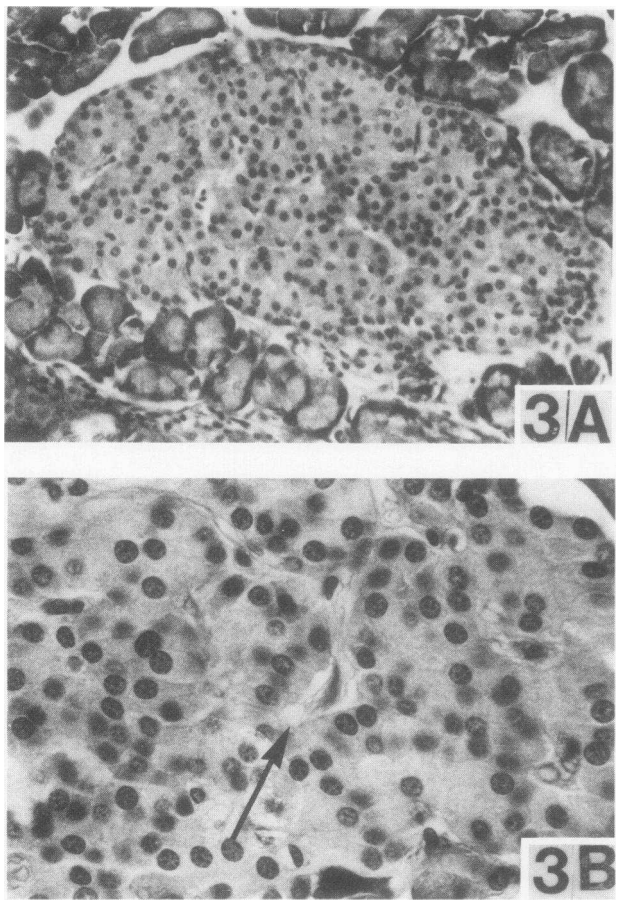


FIGURE 3 Light microscope photographs of one of the largest islets encountered in a section of pancreas from a calorically restricted rat. 3A shows the smaller size and rounded appearance of the islet as compared with the multinodular islets in 2A (×150). 3B indicates that blood channels separate clusters of endocrine cells (arrow), but that spindle-shaped connective tissue cells seen in Fig. 2B are not present here. Hematoxylin and eosin (×400).

When the same quantitative procedures are applied to slices of pancreas from 12-mo-old calorie-restricted animals, the data in Table IV indicate that there is no significant change in islet size or islet content per pancreas, and only a moderate change in connective tissue content per islet[2] as compared with measurements in young animals.

Ultrastructural changes in isolated islets. Collagenase-isolated islets obtained from the insulin secretory studies were routinely examined by electron microscopy in an effort to relate functional and structural changes. As might be anticipated from the description of the light microscopic studies, islets from

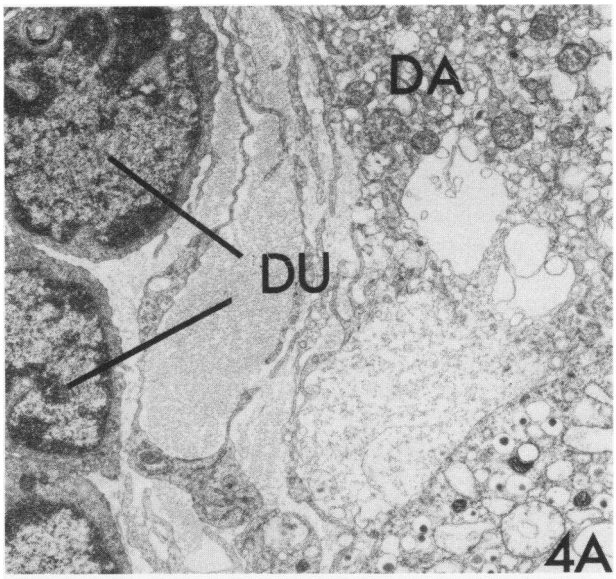
[2] In this respect, islets of the 12-mo-old, calorie-restricted animals of this study are similar to islets of the 12-mo-old retired breeders used previously (1).

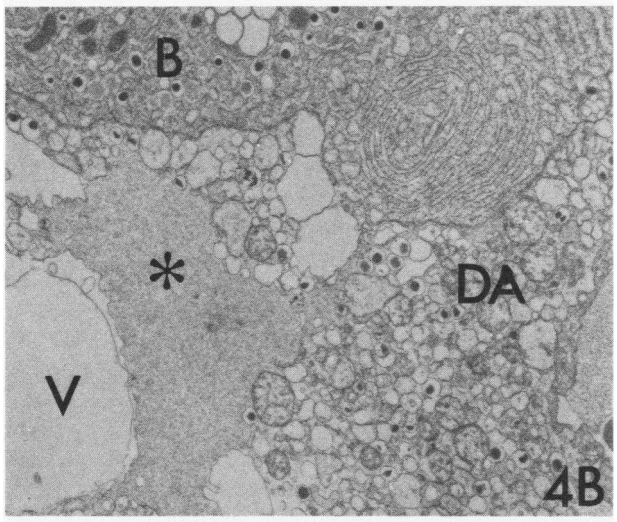
TABLE IV
Composition of the Pancreas of 2- and 12-mo-old Rats on Different Diet Programs

Category*	Islet volume‡	Connective tissue content of islets	Proportion pancreas as islet tissue
	$\times 10^6 \mu^3$	%	%
2-mo controls (5)	2.9±0.3	4.6±0.9	0.95±0.16
12-mo controls (5)	10.9±3.1§	15.2±2.3§	1.70±0.40
12-mo calorie-restricted (5)	3.7±0.7	6.9±1.4	0.93±0.17

Values are mean±SE.
* Values in parentheses represent the number of animals on which measurements were made.
‡ Islet volume was calculated from volume density measurements on sections of pancreas: these figures were corrected by the Giger-Riedweyl equation (7).
§ $P < 0.025$ as compared with 2-mo controls.

the 12-mo-old control group of rats were strikingly different from islets of the other rats. In addition to the obvious changes in connective tissue elements, casual observation showed these islets to have occasional infiltration of pancreatic duct cells (Fig. 4A) and a large number of abnormal-appearing beta cells (compare Figs. 4A and B with Fig. 5). Some of the beta cells were extremely vacuolated with swollen mitochondria; other cells had progressed beyond this stage, had lost part or all of their plasma membrane (Figs. 4A and B), and a portion of their cytoplasm was filled with amorphous or filamentous material (Fig. 4C). Because of the connective tissue infiltration within these islets and the probable effect of collagenase on this intra-islet connective tissue, it was not possible to obtain reliable stereological comparisons of the content of different cellular elements of the islets. We feel, however, that not all the observed structural changes could be attributed to the in vitro effect of collagenase: the location of amorphous and filamentous elements within beta cells suggested that beta cell alteration and damage had occurred also in vivo, prior to the use of collagenase isolation procedures. In an effort to quantitate the extent of this alteration in beta cells, we estimated the ratio of "damaged" to "nondamaged" beta cells in islets of the four different categories of rats. For this purpose, we considered the altered beta cells without plasma membranes, and with abnormal or fibrous elements within the cytoplasm, to be "damaged," and all other beta cells, regardless of vacuolation and mitochondrial changes, to be"nondamaged." Table V indicates that despite this arbitrary decision, which would tend to minimize changes in islets of 12-mo-old control rats, the islets from these animals contained far more damaged beta cell cytoplasm than did the islets from rats of any of the other categories.





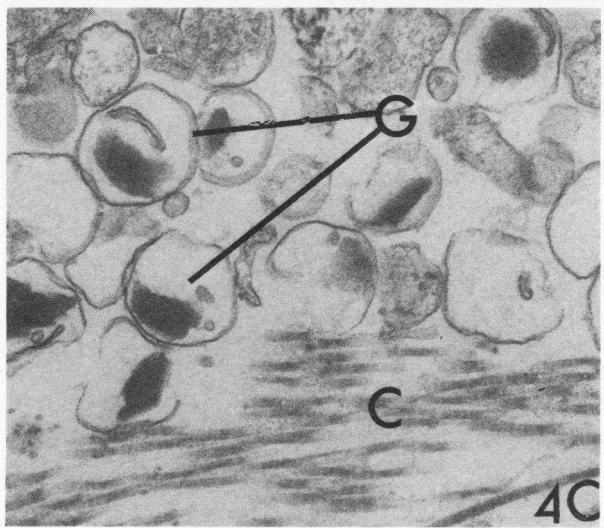


FIGURE 4 Electron micrographs of portions of collagenase-isolated islets from 12-mo-old control rats. In 4A, ductal cells (DU) are found within an islet which includes the cytoplasm of damaged beta cells (DA) (×7,800). In 4B, a normal-appearing beta cell (B) is found adjacent to a damaged beta cell (DA) in which a large vacuole (V) and amorphous material (*) fill space formerly occupied by beta granules (×7,800). 4C is a higher magnification (×48,000) view of a damaged beta cell showing cytoplasm in which collagen (C) mingles with remnants of beta granules (G).

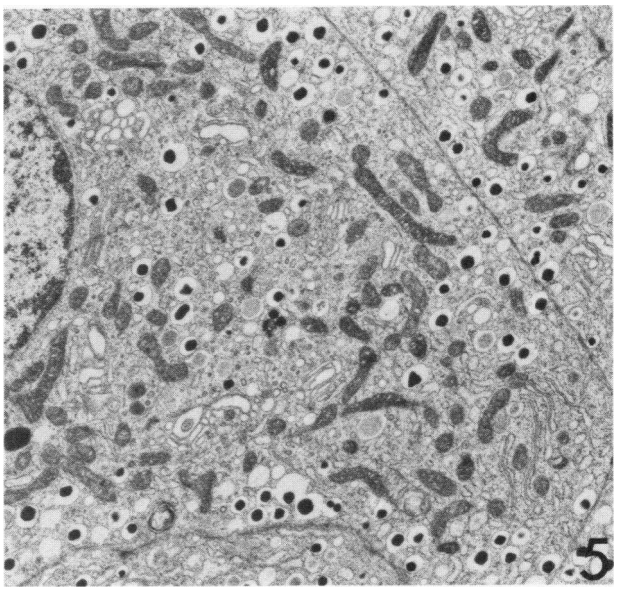


FIGURE 5 Typical low magnification electron micrograph of a portion of a collagenase-isolated islet from a calorically restricted 12-mo-old rat. These beta cells resemble cells from similarly prepared islets of 2-mo-old animals (×7,800).

DISCUSSION

The current study was conducted to determine if physical activity and/or weight control could influence the age-related decrease in beta cell insulin secretory capacity noted in earlier studies (1). As such, virgin male, Sprague-Dawley rats were maintained in our facility for 1 yr on three different diet and exercise programs. The findings of the study are reasonably straightforward, but due to unexpected changes in the control group of animals, unequivocal statements as to the benefits of physical activity and weight control on beta cell insulin secretion are not possible. On the other hand, considerable new information and important insights relevant to the endocrine pancreas of the aging rat have been gained.

The most surprising event to us was the degree to which obesity developed in the 12-mo-old control rats. The average weight of sedentary rats eating standard rat chow in our facility was 800 g at 1 yr. This is in

TABLE V
Beta Cell Damage in Isolated Islets

Category*	Damaged beta cells‡/ Nondamaged beta cells
	%
2-mo controls (5)	0.3
12-mo controls (5)	15.3
12-mo exercised (5)	0.6
12-mo calorie-restricted (5)	1.2

* Numbers in parentheses represent the number of different islet preparations from each category on which measurements were made.

‡ "Damaged beta cells" are cells in which part or all of the plasma membrane is missing and the cytoplasm of the cell contains abnormal amorphous or fibrous elements. "Nondamaged beta cells" are all other beta cells.

excess of 250 g of the retired breeders of the same age, strain, and stock previously obtained from the Charles River Breeding Laboratories (1). We can explain this weight difference only on conditions surrounding breeding, since rats at Charles River eat the same diet and are housed in even closer quarters than are the rats in our own facility.

Whatever the reason for this difference, the development of marked obesity in 12-mo-old sedentary rats eating ad lib. was associated with more dramatic changes in islet structure than we had noted during our previous study (1). Thus, a large number of the islets in these rats did not simply increase in volume, but also underwent major pathological changes ending in beta cell damage and fibrosis. The morphological findings of this study are similar to those described by Hadju and Rona (8, 9) in a large survey of nonbreeder aging male rats, by Wexler and Fischer (3, 4) in repeatedly bred male rats, and by Shino et al. (10) in fatty Zucker rats. We suspect the common denominator here is not genetic makeup, breeding, or lack of same, but of obesity.

Associated with these dramatic changes in islet structure were functional changes comparable to those we described earlier with age (1). Thus, the data in Table III indicate again that isolated islets from 12-mo-old rats secrete less insulin per volume islet than do islets isolated from 2-mo-old rats. On the other hand, this does not mean that less total insulin is secreted from the pancreas of 12-mo-old rats in vivo. Indeed, the data in Table II suggest the converse. As control rats age, they develop hyperinsulinemia; there is a striking increase in serum insulin levels as early as 4 mo of age, and by 12 mo, serum insulin concentration is approximately three times that of the young rat. Serum glucose concentrations remain relatively constant throughout this period, and it seems reasonable to suggest that the increase in serum insulin concentration represents a compensatory effort on the part of the pancreas to overcome the loss of normal insulin sensitivity associated with obesity (11). Furthermore, it seems likely that it is the development of insulin resistance in the sedentary rats allowed to eat ad lib. that is primarily responsible for the bizarre changes observed in islet structure. Thus, as these rats age (and become obese), there is an increasing demand placed upon the beta cell to secrete more insulin. At the same time, as we noted previously (1), there is a progressive decline in beta cell capacity. The combination of these events (beta cell decline plus increased total demand) might be expected to put a great deal of stress on the beta cell, and this may account for the changes that we noted. Thus, it is possible that beta cell "exhaustion" occurs as control rats age, leading to beta cell damage and death, and connective tissue infiltration at 1 yr of age. New beta cell differentiation could occur in an effort to maintain glucose homeostasis, and the repetition of this sequence of events could lead to the production of the enlarged, fibrotic islets that we observed in the pancreata of 12-mo-old control rats. This formulation is based upon the notion that the chronic need to increase insulin secretory capacity can lead to "exhaustion" of the beta cell. Although we cannot prove that this event takes place as rats age, there is at least some precedent for this notion from recent studies in man. Obesity is a potent cause of insulin resistance, and normal glucose tolerance is often maintained at the expense of greatly increased insulin secretion (11). However, obese subjects with frank decompensation of glucose tolerance (fasting plasma glucose > 200 mg/dl) are absolutely insulin deficient (12–14). On the other hand, the insulin response of these patients returns toward normal when glucose tolerance is improved following weight loss (12–14). These data are certainly consistent with the notion of functional "exhaustion" of the beta cell. Whether or not a similar change takes place as rats age is not known. No doubt there are other explanations for our findings, and we are currently carrying out sequential structural and function studies on aging rats in an effort to deal with various alternatives.

Given the above formulation, the findings in the exercised and calorie-restricted rats are not surprising. Although these rats were from the same litters, raised in the same facility, and precisely the same age when studied, all of the changes described for the control animals are absent in the exercising and dieting group: the weights of these animals averaged some 300 g less than that of the control animals at 1 yr; serum insulin remained at base-line levels throughout the year for both groups of animals; and islet morphology at 1 yr, viewed after collagenase treatment or in the intact pancreas, was essentially unchanged from that of 2-mo-old control rats. The most likely explanation for these differences is that exercised and calorie-restricted rats

retain near-normal insulin sensitivity throughout the duration of the experiment, and there are several reasons to account for this. In the first place, obesity can cause insulin resistance (11), and the reduced rate of weight gain in both of these groups of rats as compared to the control rats would undoubtedly help to maintain insulin sensitivity. There are additional factors in the exercised and calorie-restricted rats that would alleviate the demand on the beta cell. Exercise training by itself leads to enhanced insulin sensitivity (5, 15), and this phenomenon would aid in the maintenance of normal glucose tolerance despite the increased caloric intake of the exercised animals. The calorie-restricted rats, on the other hand, eat only two-thirds as much food as the control rats, and as such, would require less insulin to dispose of this food intake. In addition, the high fiber content of the diet might tend to delay the absorption of nutrients (16), and further minimize the demand on the beta cells. The combination of all of these events provides a reasonable explanation for the ability of the exercised and calorie-restricted rats to maintain serum insulin levels equal to those of young rats, and for the "youthful appearance of their islets of Langerhans at the end of 1 yr of either regime.

In our earlier reports (1), we were able to relate insulin secretion of isolated islets to the number of beta cells present. Unfortunately, the extreme changes in morphology of many of the islets in the 12-mo-old control rats do not permit this method of data analysis. The increased connective tissue content of these islets was undoubtedly responsible for the bizarre islets obtained after collagenase digestion. Although the most dramatically altered islets were not included in our functional studies, other islets which we did use could also have been altered; as a result, we view the data from these secretory studies on the 12-mo-old rats with some caution. Given these reservations, it is clear that isolated islets from 12-mo-old control rats secreted significantly less insulin. However, we do not know if this was due to a decrease in beta cell capacity with age, or simply a function of the reduced content of functional beta cells in the islets due to cell damage and increased connective tissue infiltration. Since we have previously demonstrated that age leads to a decrease in insulin secretion per beta cell, we would suggest that the observed reduction in insulin secretion was due to a combination of both factors. Despite this lack of an adequate aging control, it was interesting nevertheless to compare the functioning of islets from the 12-mo-old exercised and calorically restricted rats with each other, and with islets from young rats. Despite their normal appearance, neither group of islets secreted insulin as efficiently as did islets from young rats. Insulin secretion by islets from the calorically restricted rats were significantly decreased as compared with young controls at every glucose concentration used. Indeed, these islets functioned no better than islets from the 12-mo-old control group of rats. Although somewhat improved, islets from the exercised group still performed less efficiently at every glucose concentration than islets from the young controls. Although this difference was not statistically significant at maximal glucose stimulation, we remain somewhat skeptical about the ability of exercise to completely reverse the age-related defect previously noted, and feel further studies are necessary before the extent of this effect can be properly evaluated.

With these reservations in mind, we submit the following analysis of the data. The ability of the beta cell to secrete insulin declines with age. If the animals gain excessive weight, and/or become significantly insulin resistant with age, more and more insulin secretion is demanded of the pancreas. This demand, in combination with the age-related decline in beta cell capacity, eventually leads to injury and death of some cells. In order to compensate, new cells are formed, resulting in enlarged, multilobulated islets. Both exercise and diet reduce the animals' requirement for insulin, and thus permit the endocrine pancreas to cope, despite the putative decline in the efficiency of the individual beta cell.

Clearly, additional studies will be necessary to describe the limits of the compensatory response of the endocrine pancreas as well as to detail the influence of various external factors on these events.

ACKNOWLEDGMENTS

We are especially grateful to Dr. Jon Kosek for his help in assessing pathological changes in the tissues of this study, and to Helen Ho, Marjorie Johnson, Kris Nichols, and Lori Johnson, whose combined technical skills made this study possible.

This work was supported in part by grants from the National Institutes of Health AG-01237 and by the Research Services of the Veterans Administration.

REFERENCES

1. Reaven, E. P., G. Gold, and G. M. Reaven. 1979. Effect of age on glucose-stimulated insulin release by the beta cell of the rat. *J. Clin. Invest.* **64:** 591–599.
2. Reaven, E. P., G. Gold, and G. M. Reaven. 1980. Effect of age on leucine-induced insulin secretion by the beta cell. *J. Gerontol.* **35:** 324–328.
3. Wexler, B. C., and C. T. Fischer. 1963. Hyperplasia of the islets of Langerhans in breeder rats with arteriosclerosis. *Nature (Lond.).* 33–37.
4. Wexler, B. C., and C. T. Fischer. 1963. Abnormal glucose tolerance in repeatedly bred rats with arteriosclerosis. *Nature (Lond.).* 133–136.
5. Mondon, C. E., C. B. Dolkas, and G. M. Reaven. 1980. Site of enhanced insulin sensitivity in exercise-trained rats at rest. *Am. J. Physiol.* **239:** E169–E177.
6. Desbuquois, B., and G. D. Aurbach. 1971. Use of poly-

ethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassay. *J. Clin. Endocrinol. Metab.* **33:** 732–738.
7. Weibel, E. R. 1973. Stereological techniques for electron microscopic morphometry. *In* Principles and Techniques of Electron Microscopy. M. A. Hayat, editor. Van Nostrand Reinhold Co. **3:** 237–291.
8. Hadju, A., and G. Rona. 1967. Morphological observations on spontaneous pancreatic islet changes in rats. *Diabetes.* **16:** 108–110.
9. Hadju, A., F. Herr, and G. Rona. 1967. The functional significance of a spontaneous pancreatic islet change in aged rats. *Diabetologia.* **4:** 44–47.
10. Shino, A., T. Matsuo, H. Iwatsuka, and Z. Susuoki. 1973. Structural changes of pancreatic islets in a genetically obese rats. *Diabetologia.* **9:** 413–421.
11. Kolterman, O. G., G. M. Reaven, and J. M. Olefsky. 1978. Relationship between *in vivo* insulin resistance and decreased insulin receptors in obese man. *J. Clin. Endocrinol. Metab.* **48:** 487–494.
12. Hadden, D. R., D. A. D. Montgomery, R. J. Skeely, E. R. Trimble, J. A. Weaver, E. A. Wilson, and K. D. Buchanan. 1975. Maturity onset diabetes mellitus: response to intensive dietary management. *Br. Med. J.* **3:** 276–278.
13. Savage, P. J., L. J. Bennion, E. J. Flock, M. Nagulesparan, D. Motte, J. Roth, R. H. Unger, and P. H. Bennett. 1979. Diet-induced improvement of abnormalities in insulin and glucagon secretion and in insulin receptor binding in diabetes mellitus. *J. Clin. Endocrinol. Metab.* **48:** 999–1007.
14. Stanik, S., and R. Marcus. 1980. Insulin secretion improves following dietary control of plasma glucose in severely hyperglycemic obese patients. *Metabolism.* **29:** 346–350.
15. Bjorntorp, P., M. Faklen, G. Grimby, A. Gustafson, J. Holm, P. Renstrom, and T. Schersten. 1972. Carbohydrate and lipid metabolism in middle-aged physically well-trained men. *Metabolism.* **21:** 1037–1044.
16. Goulder, T. J., K. G. M. Alberti, and D. J. A. Jenkins. 1978. Effect of added fiber and metabolic responses to a mixed meal in normal and diabetic subjects. *Diabetes Care.* **1:** 351–356.