Decrease in Glucose-Stimulated Insulin Secretion With Aging Is Independent of Insulin Action

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While the incidence of diabetes increases with age, a decrease in β-cell function independent of age-related insulin resistance has not been conclusively determined. We studied insulin secretion (by hyperglycemic clamp) in 3-, 9-, and 20-month-old chronically catheterized, awake, Sprague Dawley (SD) rats ($n = 78$). Insulin action was modulated in a group of old rats by caloric restriction (CR) or by surgical removal of visceral fat (VF−). During the first 2 h of the clamp (11 mmol/l glucose), insulin secretion and insulin resistance ($S_{\text{i hyper clamp}}$) demonstrated the characteristic hyperbolic relationship. However, after hyperglycemia for an additional 2 h, the ability to maintain insulin secretion, commensurate with the degree of insulin resistance, was decreased in all aging rats ($P < 0.05$). Increasing plasma glucose levels to 18 mmol/l glucose, after clamp at 11 mmol/l, increased insulin secretion by approximately threefold in young rats, but failed to induce similar magnitude of response in the aging rats (~50%). However, elevation of plasma free fatty acid (FFA) levels by twofold (by intralipid infusion during 11 mmol/l glucose clamp) resulted in a robust, approximate twofold response in both young and old rats. Thus, prolonged stimulation by hyperglycemia unveiled a functional defect in insulin secretion with aging. This age-related defect is independent of insulin action and is specific to glucose and not FFAs. We suggest that prolonged hyperglycemic stimulation can be a tool to identify functional defects in insulin secretion, particularly in the context of the hyperbolic relationship with insulin action, in elderly subjects or those at risk for type 2 diabetes. *Diabetes* 53:441–446, 2004

Age is a significant risk for development of type 2 diabetes (1). This is evident in the significantly higher prevalence of impaired glucose tolerance test (GTT) and type 2 diabetes in Americans older than 65 years (2). As in the younger age-group, decreased physical activity and increased adiposity impair insulin sensitivity in the elderly. This is initially compensated with an increase in insulin secretion (1); however, the appearance of impaired GTT and type 2 diabetes suggests that adequate insulin secretion cannot be maintained. Thus, relative insulin secretory defects in the presence of insulin resistance may contribute to the increased incidence of age-related glucose intolerance and diabetes.

Many studies have utilized oral GTTs (OGTTs) to assess insulin response in aging; however, as the relative role of insulin resistance, β-cell function, and gastrointestinal and neural factors cannot be deduced from these studies, they do not provide clear evidence that aging per se decreases insulin secretion (3,4). Age-related decrease in insulin response has been demonstrated with intravenous GTT, suggesting impaired β-cell function (5,6). Recently, a clinical study utilizing a mixed meal and an intravenous injection of glucose demonstrated that age-related diminution in insulin secretion contributes to the glucose intolerance in the elderly (7). However, only few studies have utilized the hyperglycemic clamp, the gold standard technique, to assess glucose-mediated insulin secretion, and the results have not been consistent. While some studies have shown an impairment in β-cell function with aging (8), few have shown no differences in the insulin secretion between the young and old subjects (9,10). Thus far, while these studies have shown some age-related impairment in insulin secretion in human subjects, they have failed to account for the great variability in the metabolic profile and the role of insulin resistance on β-cell function in elderly subjects. As a result, a conclusive statement about the decline in β-cell mass or function with aging has yet to be made (3).

To overcome some of these difficulties and to suggest a novel approach to assess functional decline in insulin secretion, we studied the effects of aging on insulin secretion in a rodent model utilizing the hyperglycemic clamp technique. Rodent models are genetically homogeneous and can be subjected to similar controlled environment and levels of activity. Furthermore, anthropometric and metabolic parameters can be manipulated as they age. In addition, Sprague Dawley (SD) rats offer a unique advantage because they do not develop diabetes with aging. We used two additional models to specifically delineate the effect of aging on glucose-stimulated insulin secretion. First, we employed caloric restriction (CR) to allow us to study effects of age without the confounder of age-related obesity and insulin resistance (11,12). Second, we reversed insulin resistance, without limiting nutrients, by surgically removing visceral fat (VF) (13,14). We also continued the clamp for 4 h to study the relationship

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AUC, area under the curve; AUC_I, AUC for insulin; CR, caloric restriction; FFA, free fatty acid; FPIR, first-phase insulin response; GTT, glucose tolerance test; LBM, lean body mass; OGTT, oral GTT; VF, visceral fat; VF−, removal of VF.

TABLE 1
Body composition and basal metabolic characteristics of SD rats

	3-month-old	9-month-old	20-month-old	20-month-old VF−	20-month-old CR
n	6	6	9	6	6
Body weight (g)	322 ± 14.3*	583 ± 17.3	585 ± 28	572 ± 22	374 ± 5.2*
Fat mass (g)	26.6 ± 2.9*	170.1 ± 7.1	179.8 ± 9.8	166.8 ± 23.4	29.8 ± 6.2*
Total VF (g)	4.9 ± 0.3*	32.2 ± 5.3	30.9 ± 2.3	8.0 ± 1.0†	5.3 ± 0.6*
Insulin (ng/ml)	1.5 ± 0.3‡	3.1 ± 0.6	3.0 ± 0.5	1.4 ± 0.4‡	1.4 ± 0.2‡
Glucose (mmol/l)	8.1 ± 0.2	8.1 ± 0.2	8.0 ± 0.3	7.2 ± 0.3	7.2 ± 0.1
FFAs (mmol/l)	0.7 ± 0.1*	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.2	0.7 ± 0.1*

Data are means ± SE. *$P < 0.05$ vs. 9-month-old, 20-month-old, and 20-month-old VF− rats; †$P < 0.05$ vs. all other groups; ‡$P < 0.05$ vs. 9-month-old and 20-month-old rats.

between insulin secretion and insulin action across ages. In addition to glucose, we used intralipid infusions to study the effects of free fatty acids (FFAs) on insulin secretion in young and old rats.

RESEARCH DESIGN AND METHODS

Animals. Male SD ($n = 78$) (Charles River Laboratories, Wilmington, MA) were used for this study. Rats were housed in individual restrictive cages and were subjected to a standard light (6:00 A.M. to 6:00 P.M.)/dark (6:00 P.M. to 6:00 A.M.) cycle. Five groups of rats—3-month-old (human equivalent of young adult), 9-month-old (human equivalent of mature adult), and 20-month-old rats (human equivalent of ~70 years); 20-month-old caloric restricted (CR) rats; and 20-month-old rats that underwent surgical removal of VF at 5 months of age (VF−)—were studied (8–11). All rats, except 20-month-old CR rats, were fed ad libitum using regular rat chow that consisted of 64% carbohydrate, 30% protein, and 6% fat with a physiologic fuel value of 3.3 kcal/g chow. The 20-month-old CR rats were fed on 60% of the diet as the age-matched fed rats since 3-month-old of age. One week before the study, under isoflurane anesthesia, indwelling catheters were placed in the right intern jugular vein and in the left carotid artery (13–15). This method of anesthesia allows fast recovery and normal food consumption after 1 day. The venous catheter was extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch. Recovery was continued until body weight was within 3% of the preoperative weight (~4–6 days).

Body composition estimation. Lean body mass (LBM) and fat mass were calculated from the whole-body volume of distribution of water estimated by tritiated water bolus injection in each experimental rat (11–14). On the morning of the study, 20 μCi of ^3H_2O (NEN, Boston, MA) was injected intra-arterially. Steady state for ^3H_2O specific activity in rats was generally achieved within 30–45 min, and eight samples were collected between 1 and 1.5 h after injection. The distribution space of water was calculated by dividing the total radioactivity injected by the steady-state specific activity of plasma water, which was assumed to be 93% of the total plasma volume. LBM was calculated from the whole-body distribution space, divided by 0.73 (the percent of water content of the LBM). Fat mass was calculated as the difference between total body weight and LBM. Epididymal, perinephric, and mesenteric fat pads were dissected and weighed after completion of the study as an additional index of adiposity.

Hyperglycemic clamp study. Studies were performed in awake, unstressed, chronically catheterized rats (11–14) after an ~6-h period of fast. All rats were subjected to moderate hyperglycemia (~11 mmol/l) as previously described (17). Briefly, 25% glucose was infused intravenously to raise the plasma glucose concentration acutely to ~11 mmol/l. The glucose infusion rate was then varied to maintain the plasma glucose concentration at this level during the 4 h of the hyperglycemic clamp (study 1, $n = 33$). In a separate study, after the first 90 min of hyperglycemia at 11 mmol/l, the 3-month-old, 20-month-old, and 20-month-old CR rats were subjected to either hyperglycemic of 11 mmol/l ($n = 15$), 18 mmol/l ($n = 15$), or intralipid infusion during a 11-mmol/l clamp (10% intralipid at 1.5 ml/h) ($n = 15$) (study 2, $n = 45$) for an additional 90 min. Plasma samples for insulin and C-peptide were obtained at 10-min intervals throughout the study. Samples were also obtained for determination of FFA concentrations. Approximately 6.0 ml fresh blood (obtained by heart puncture from a littermate of the test animal) was infused at a constant rate throughout the study to prevent volume depletion and anemia. At the end of the clamp study, rats were sacrificed using 100 mg pentobarbital sodium/kg body wt i.v. The study protocol was reviewed and approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

Analytic procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA), and plasma insulin was measured by radioimmunoassay using rat insulin standards. C- peptide was analyzed using the Linco C-peptide assay kit (Linco Research, St. Charles, MO). Plasma nonesterified fatty acid concentrations were determined by an enzymatic method with an automated kit according to the manufacturer's specification (Waco Pure Chemical, Osaka, Japan).

Terminology and calculations. As a measure of insulin sensitivity, $S_{i\ hyper\ clamp}$ was calculated as the ratio of glucose infusion rate to steady-state plasma insulin levels (15). The area under the curve (AUC) of the first-phase insulin response was calculated by the trapezoid rule using 0-, 2-, 4-, 6-, 8-, and 10-min samples with the formula $\{(i\ 0 + i\ 2)/2 \times 0.5 + (i\ 2 + i\ 4)/2 \times 0.5 + (i\ 4 + i\ 6)/2 \times 0.5 + (i\ 6 + i\ 8)/2 \times 0.5 + (i\ 8 + i\ 10)/2 \times 0.5\}$. Change (Δ) in insulin was calculated as the difference between the insulin levels achieved during the last hour of the initial 120-min or 90-min and subsequent 2-h or 90-min clamp periods depending on the study.

Statistic analysis. All values shown are expressed as means ± SE. Statistic analyses were performed using ANOVA in multiple comparisons. When the main effect was significant, a post hoc test (Tukey's) was applied to determine individual differences between means. A P value <0.05 was considered to be statistically significant. All statistic analyses were performed using SPSS for Windows. Reciprocal transformation provided the best fit for association analysis between insulin levels and $S_{i\ hyper\ clamp}$ in various models. The hyperbolic curve with 95% CI ($\alpha = 0.05$) was created with data from the 3- and 9-month-old rats, and the average of the groups was plotted on that curve.

RESULTS

Preclamp basal metabolic characteristics. The preclamp basic metabolic characteristics of the rats from the various groups are summarized in Table 1. There was a progressive increase in the weight of the rat and fat mass with age ($P < 0.0001$), which was prevented by CR. CR led to a decrease in total fat mass and VF ($P < 0.0001$). Though the total fat mass in VF− rats was similar to age-matched controls, VF was only one-third of the fed rats ($P < 0.0001$). While preclamp basal glucose level was similar across all ages, insulin levels were higher in the 9-month-old and 20-month-old compared with young 3-month-old, 20-month-old CR, or 20-month-old VF− rats ($P < 0.05$).

Relationship between glucose-stimulated insulin secretion and insulin action during 2-h hyperglycemic clamp. Glucose was clamped at 11 mmol/l in all the groups. During first 2 h of hyperglycemic clamp, there was a progressive increase in insulin levels in all the groups, with highest insulin levels in 20-month-old rats (Fig. 1*A*). Insulin levels were lower in the 20-month-old VF− and CR groups compared with age-matched controls. C-peptide levels paralleled the changes in insulin with highest levels in the 20-month-old rats (3,457 ± 195, 3,488 ± 206, 4,708 ± 245, 4,093 ± 354, and 2,634 ± 429 pmol/l in 3-month-old, 9-month-old, 20-month-old, 20-month-old VF−, and 20-month-old CR rats, respectively, $P < 0.05$). First-phase insulin response (FPIR), as assessed by the area under the

TABLE 2
Metabolic characteristics during hyperglycemia clamp study

	3-month-old	9-month-old	20-month-old	20-month-old VF−	20-month-old CR
n	6	6	9	6	6
Glucose (mmol/l)					
70–120 min	11.0 ± 0.3	11.2 ± 0.1	11.1 ± 0.3	10.9 ± 0.2	11.3 ± 0.2
190–240 min	11.1 ± 0.2	10.9 ± 0.2	11.0 ± 0.1	11.1 ± 0.3	11.1 ± 0.3
Insulin (ng/ml)					
70–120 min	7.9 ± 1.0*	12.3 ± 1.1	15.1 ± 2.0	11.7 ± 0.6	8.3 ± 1.0*
190–240 min	27.8 ± 2.4	34.9 ± 1.8	27.4 ± 2.3	23.2 ± 3.4	13.5 ± 1.7†
FFAs (mmol/l)					
70–120 min	0.50 ± 0.1*	0.73 ± 0.1	0.72 ± 0.1	0.70 ± 0.1	0.45 ± 0.1*
190–240 min	0.48 ± 0.1*	0.62 ± 0.1	0.77 ± 0.1	0.67 ± 0.1	0.30 ± 0.1*
$S_{\text{i hyper clamp}}$					
70–120 min	5.3 ± 0.5*	2.6 ± 0.3	2.2 ± 0.3	3.1 ± 0.5	4.7 ± 0.3*
190–240 min	2.3 ± 0.2‡	1.0 ± 0.1	1.2 ± 0.1	1.6 ± 0.2	3.4 ± 0.5*
Glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$)					
70–120 min	40.6 ± 5.2‡	27.8 ± 2.1	30.4 ± 2.5	37.3 ± 5.8	36.2 ± 3.9
190–240 min	58.6 ± 5.7*	34.1 ± 2.5	30.1 ± 2.1	37.0 ± 4.2	46.7 ± 5.5‡

Data are means ± SE. *$P < 0.05$ vs. 9-month-old, 20-month-old, and 20-month-old VF− rats; †$P < 0.05$ vs. all other groups; ‡$P < 0.05$ vs. 9-month-old and 20-month-old rats.

curve for insulin (AUC_I), was also highest in the 20-month-old rats (11.79 ± 2.1, 16.94 ± 3, 21 ± 2.1, 15.31 ± 2, and 12.79 ± 1.3 in 3-month-old, 9-month-old, 20-month-old, 20-month-old VF−, and 20-month-old CR rats, respectively, $P < 0.05$). FPIR and insulin sensitivity, as assessed by $S_{\text{i hyper clamp}}$, demonstrate a hyperbolic relationship, with a decrease in insulin sensitivity accompanied by higher FPIR (Fig. 1*B*). A hyperbolic relationship can also be demonstrated across all ages between the insulin response during the clamp and insulin sensitivity ($S_{\text{i hyper clamp}}$) (Fig. 1*C*). As expected, the $S_{\text{i hyper clamp}}$ was highest in the 3-month-old and lowest in the 20-month-old rats. VF− and CR improved the insulin sensitivity by ~30% and twofold, respectively, compared with the 20-month-old rats ($P < 0.05$). The FFA levels were significantly suppressed with the clamp in all groups ($P < 0.05$) (Table 2).

Relationship between glucose-stimulated insulin secretion and insulin action during 4-h hyperglycemic clamp. To test if the relationship between insulin resistance and insulin secretion is altered with prolonged stimulation, we subjected the rats to an additional 2 h of hyperglycemic clamp at 11 mmol/l. As previously shown, with continued clamp at 11 mmol/l, insulin secretion continued to increase in all the groups (19,20). After an additional 2 h of hyperglycemic clamp, highest insulin levels were reached in 9-month-old rats, although 20-month-old rats started the clamp with highest insulin levels at 2 h (Fig. 2*A* and *B*). Similar trends were seen in the C-peptide levels among the various groups (6,215 ± 617, 6,553 ± 636, 6,710 ± 590, 5,226 ± 660, and 2,964 ± 317 pmol/l in 3-month-old, 9-month-old, 20-month-old, 20-month-old VF−, and 20-month-old CR rats, respectively, $P < 0.05$). The $S_{\text{i hyper clamp}}$ was highest in 3-month-old, approximately twofold higher than 9- and 20-month-old rats ($P < 0.05$). Interestingly, the highest $S_{\text{i hyper clamp}}$ and lowest insulin levels were seen in the 20-month-old CR group. When insulin levels were plotted against insulin sensitivity, the hyperbolic relationship was not maintained and the old rats "left" the hyperbola (Fig. 2*C*); the insulin levels were lower compared with the degree of insulin resistance in the 20-month-old ($P = 0.03$), VF− ($P < 0.005$), and CR ($P < 0.0001$) groups (Table 1).

Stimulated insulin secretion with 18 mmol/l glucose and FFAs. When 3-month-old, 20-month-old, and 20-month-old CR rats were subjected to an 18-mmol/l glucose clamp for 90–180 min, insulin secretion was similar in 3-month-old and 20-month-old rats. However, considering the higher insulin levels achieved during the first 90 min of the clamp by 20-month-old rats, the further increment with the 18-mmol/l clamp was significantly lower (from 13.7 ± 2.0 to 16.11 ± 1.9 in the 20-month-old rats and from 11.6 ± 2.4 to 13.5 ± 2.1 in 20-month-old CR rats compared with 12.6 ± 2.1 to 19.02 ± 2.2 in the 3-month-old rats). The increase in insulin with the 18-mmol/l clamp was much less in old rats, both fed and CR rats (50, 25, and 20% increase in 3-month-old, 20-month-old, and 20-month-old CR rats, respectively, $P < 0.05$). C-peptide levels showed a similar trend: 45, 26, and 28% increase (from 2,398 ± 311 to 4,348 ± 178, 3,252 ± 399 to 3,946 ± 287, and 2,245 ± 237 to 3,023 ± 71 pmol/l, $P < 0.05$) in 3-month-old, 20-month-old, and 20-month-old CR rats, respectively (Fig. 3).

Intralipid infusion in addition to the 11-mmol/l clamp increased FFA levels by 3-fold, 2.8-fold, and 2-fold in 20-month-old, 20-month-old CR, and 3-month-old rats, respectively (0.47 ± 0.19 to 1.21 ± 0.28, $P < 0.001$). Insulin secretion increased significantly in both young and old rats with the addition of intralipid: 12.6 ± 2.1 to 20.7 ± 3.3 in 3-month-old, 13.7 ± 2.0 to 27.2 ± 2.3 in 20-month-old, and 11.6 ± 2.4 to 22.4 ± 2.9 in 20-month-old CR rats (75, 90, and 100% in 3-month-old, 20-month-old, and 20-month-old CR rats, respectively, $P < 0.001$). The changes in C-peptide levels were parallel to changes in insulin (from 2,355 ± 126 to 4,946 ± 375, 3,148 ± 413 to 10,960 ± 1,352, and 2,552 ± 329 to 7,183 ± 937 pmol/l, a 2-fold, 3.5-fold, and 2.8-fold increase in 3-month-old, 20-month-old, and 20-month-old CR rats, respectively, $P < 0.001$). Stimulated insulin secretion with glucose and intralipid infusion was similar in the young, whereas there was a marked difference in the insulin levels between the two stimuli in the old rats ($P < 0.05$).

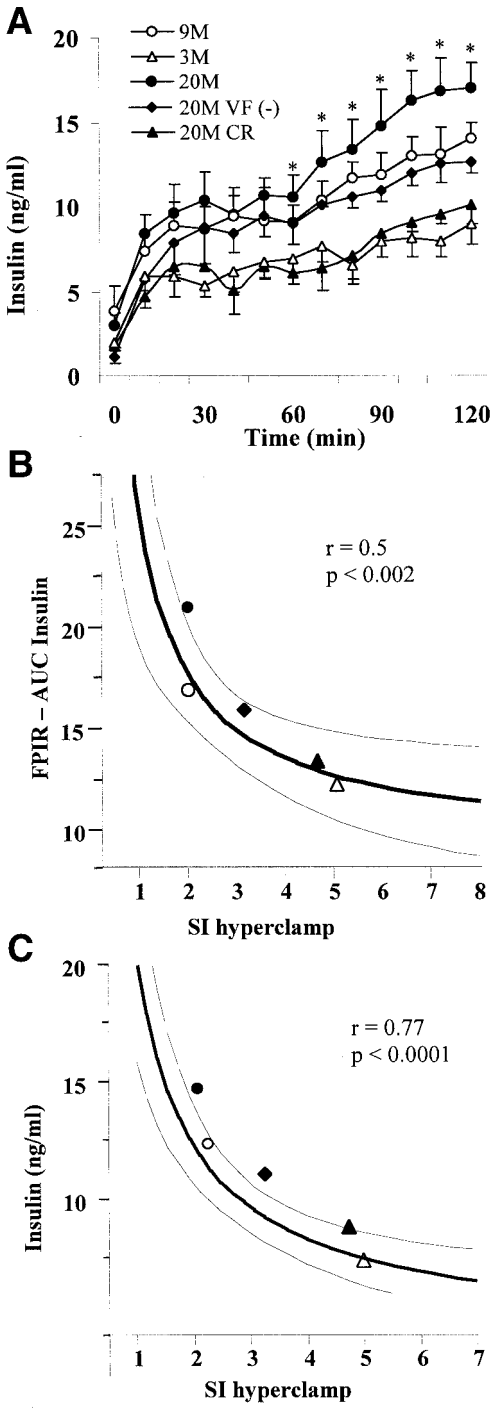

FIG. 1. Plasma insulin levels during a hyperglycemic clamp (0–120 min). *A*: Three-month-old (3M; $n = 6$), 9-month-old (9M; $n = 6$), 20-month-old (20M; $n = 9$), 20-month-old VF− (20M VF (-); $n = 6$), and 20-month-old CR rats (20M CR; $n = 6$) were studied under ~11-mmol/l hyperglycemic clamp conditions. The highest insulin levels were demonstrated in the 20-month-old rats ($P < 0.05$ vs. 3-month-old and 20-month-old CR rats). *B*: Hyperbolic curve with 95% CI was obtained by plotting the insulin levels during the first phase against insulin sensitivity in 27 individual rats of the 3-month-old and 9-month-old groups ($r = 0.5$, $P < 0.002$). *C*: Similarly, a hyperbolic curve was obtained by plotting insulin sensitivity against insulin levels during the second hour of the clamp ($r = 0.77$, $P < 0.0001$). Mean values of data from 3-month-old, 9-month-old, 20-month-old, 20-month-old VF−, and 20-month-old CR rats were then plotted on the hyperbolic curve (*B* and *C*).

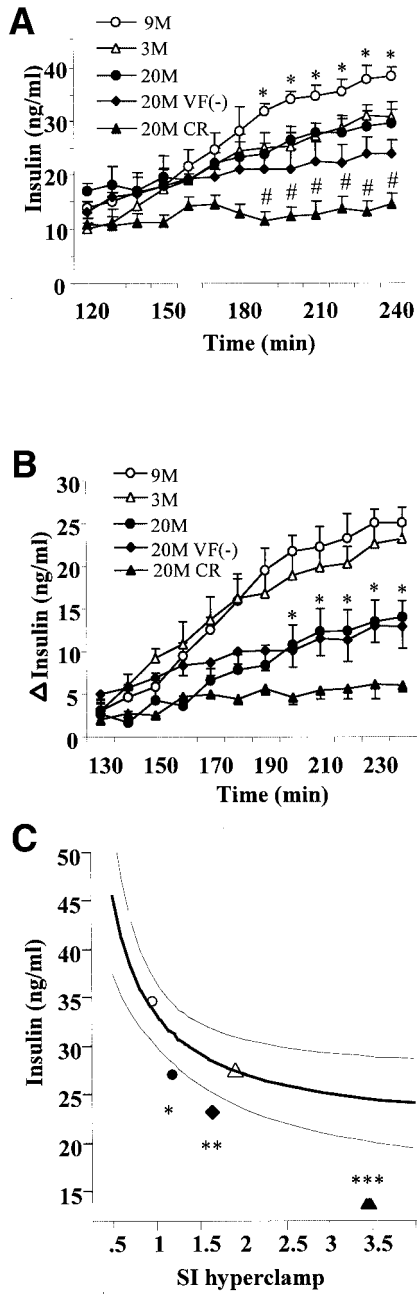

FIG. 2. Plasma insulin levels during prolonged hyperglycemic clamp (120–240 min). Plasma glucose was maintained at ~11 mmol/l for an additional 2 h of the hyperglycemic clamp (120–240 min) in all the groups. *A*: Insulin secretion is significantly different in the 9-month-old (*$P < 0.05$) and 20-month-old CR rats (#$P < 0.05$) groups compared with 3-month-old, 20-month-old, and 20-month-old VF− rats. *B*: Changes in insulin levels during the last hour of the clamp over the level at 120 min. The older rats (20-month-old, 20-month-old VF, and 20-month-old CR rats) demonstrate significantly lower Δ insulin when compared with 3-month-old and 9-month-old rats ($P < 0.05$). *C*: Hyperbolic curve was obtained initially by plotting the insulin levels during the last hour of the clamp against insulin sensitivity in 27 individual rats of the 3-month-old and 9-month-old groups ($r = 0.7$ and $P < 0.001$). Mean values of data from all the groups were then plotted on the curve. The 20-month-old (*$P < 0.05$), 20-month-old VF− (**$P = 0.005$), and 20-month-old CR rats (***$P < 0.0001$) had significantly lower insulin secretion commensurate to the degree of insulin resistance compared with the 3-month-old and 9-month-old groups. 9M, 9-month-old; 3M, 3-month-old; 20M, 20-month-old; 20M VF(-), 20-month-old VF−; 20M CR, 20-month-old CR rats.

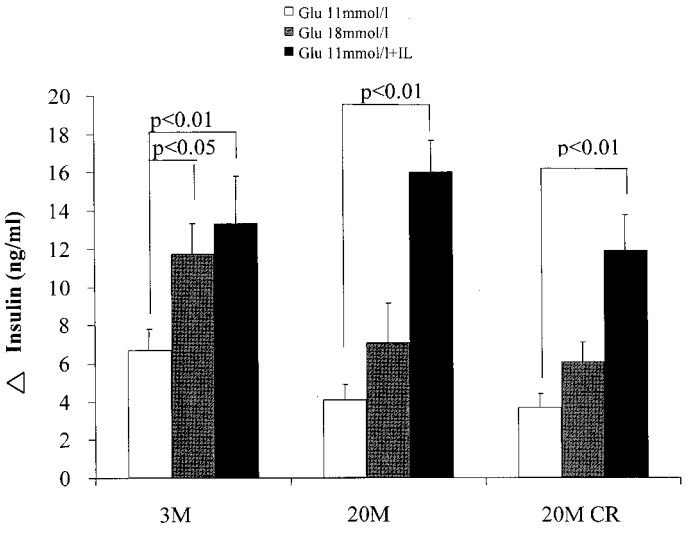

FIG. 3. Changes in plasma insulin levels during 11 mmol/l, 11 mmol/l with intralipid, and 18 mmol/l hyperglycemic clamp. Three-month-old (3M; $n = 15$), 20-month-old (20M; $n = 15$), and 20-month-old CR rats (20M CR; $n = 15$) were subjected to an 11-mmol/l clamp for 90 min and then the clamp conditions were changed to 18 mmol/l for an additional 90 min or intralipid was infused from 90 to 180 min. A similar increase in insulin secretion was achieved with the 18-mmol/l clamp ($P < 0.05$) and intralipid infusion ($P < 0.01$) in young rats. The old rats (20-month-old and 20-month-old CR rats) did not demonstrate a significant change in insulin secretion with 18 mmol/l, while the increase with intralipid infusion was robust ($P < 0.01$).

DISCUSSION

In this study we show that glucose-mediated insulin secretion is impaired independent of insulin resistance and nutritional intake in the old rats. While insulin action could predict insulin secretion during a short physiologic stimulation, a more prolonged stimulation in aging animals led to a decrease in insulin secretion. This defect seems to be specific for glucose and not to stimuli such as FFAs.

With increasing age, changes in anthropometric characteristics, such as an increase in fat mass and visceral fat, may lead to insulin resistance in both rodents and humans (19,20). Sedentary lifestyle, genetic predisposition, and presence of other diseases contribute further to the diminished insulin sensitivity in humans. The relationship between increasing age and the decline in insulin action has been demonstrated under euglycemic-hyperinsulinemic clamp conditions (21). This decrease in insulin sensitivity is associated with a compensatory increase in insulin secretion (3) under basal and certain stimulated conditions. The recently coined term "glucose allostasis" describes the slightly higher glucose levels, albeit within the normal range, in insulin-resistant states that continue to drive the β-cells to produce higher levels of insulin (22). During the first 2 h of hyperglycemic clamp, insulin secretion and insulin action demonstrated a hyperbolic relationship across all ages—a decrease in insulin sensitivity associated with higher insulin levels.

Although a hyperbolic relationship between insulin action and insulin secretion has been demonstrated in many studies in humans through the use of a variety of techniques (23), a causative role of insulin action in determining insulin secretion has not been established. Prevention of insulin resistance with CR and improvement in insulin sensitivity with removal of VF is associated with lower insulin levels and a marked improvement in insulin sensitivity. However, the model of VF removal is unique as these animals are similar in body weight to the ad libitum–fed animals and only differs significantly in the amount of VF (Table 1). Similarly, the food intake of the VF− rats was comparable to that of ad libitum–fed animals, while the food intake of CR animals was restricted. Through the demonstration of hyperbolic relationship between insulin action and insulin secretion in these two unique models and in ad libitum–fed 3-month-old, 9-month-old, and 20-month-old models, we demonstrate that insulin action, independent of nutrients, age, and fat mass, determines acute insulin secretion.

Prospective studies in Pima Indians have shown that subjects with increasing insulin resistance, when compensated, move on the hyperbolic curve, increasing insulin secretion capacity with time (24). Studies have also demonstrated that subjects who eventually developed type 2 diabetes with time had declines in insulin secretion and moved "off the curve" (24). In this study we demonstrate that in aging animals, a decline in insulin secretion can be demonstrated not in years but in just a few hours of stimulation. Stimulation, either by increasing the duration of the clamp to 4 h or by increasing the glucose stimuli to 18 mmol/l, unmasked the differences between the old and the young rats. With continued clamp for an additional 2 h, although all rats continued to increase the insulin secretion throughout the period of study as described earlier (17,25,26), the magnitude of increase in insulin levels was much lower in the old rats. It is rather striking that a relatively short period of hyperglycemia distinguished the old rats, with old rats demonstrating lower insulin secretion for the degree of insulin resistance compared with 3- and 9-month-old rats. Glucose-stimulated insulin secretion as assessed by an 18-mmol/l clamp is also decreased in old age. Because insulin clearance may be decreased in aging (27,28), the lower insulin levels demonstrated here may underestimate the degree of the defect in insulin secretion. In addition, parallel changes in C-peptide suggest a defect in insulin secretion. Inherent defects in insulin secretion with aging, when superimposed on increased insulin requirements, may contribute toward the higher risk for abnormal glucose tolerance in old age. This is corroborated by a study in elderly humans, using mixed-meal challenge and intravenous glucose infusion, that showed a decrease in both insulin secretion and insulin action with aging, suggesting that the effect on insulin secretion is independent of the effects on insulin action (7).

In light of a functional decrease in insulin secretion with aging, it is rather interesting that islet number, islet size, and secretory granules are actually increased in old SD rats compared with young animals (29,30). Despite the increased size of the islets, the release of insulin in response to glucose is decreased in old age in vitro (31–34). This functional defect is demonstrable in both sexes and across species of rats and is independent of body weight, although the pancreas of old males has higher islet tissue coupled with more impaired secretion (31). An impaired stimulation-secretion coupling has been demonstrated in the β-cells of aging rats in response to glucose and arginine (35).

Interestingly, the defect in insulin secretion in old age

appears to be specific to glucose. Additional stimulation with FFAs seems to be necessary to elicit a higher insulin response. FFAs are potent insulin secretagogues and augment insulin release only in the presence of glucose (36–38), and this effect may be mediated through an increase in cytoplasmic long-chain acyl CoA (39,40).

In conclusion, we demonstrate that there is a functional impairment of glucose-stimulated insulin secretion with old age. This defect in insulin secretion in aging appears to be specific to glucose. β-cells in old age seem particularly vulnerable and a few hours of hyperglycemia during the clamp appears sufficient to unmask significant defects in glucose-stimulated insulin secretion. Prolonged hyperglycemic stimulation may be a tool in identifying defects in insulin secretion in relation to insulin action in elderly subjects or those at risk for type 2 diabetes.

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