Lack of Deterioration of Insulin Action With Aging in the GK Rat: A Contrasted Adaptation as Compared With Nondiabetic Rats

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One of the main characteristics of non–insulin-dependent diabetes mellitus (NIDDM) is an alteration of tissue insulin sensitivity, which is also observed during the aging process in the nondiabetic. In this study, we evaluated the influence of age on insulin resistance in a genetic lean model of NIDDM, the Goto-Kakisaki (GK) rat, using the euglycemic-hyperinsulinemic clamp technique at 2, 12, and 18 months of age. In GK rats, basal hyperglycemia (11 mmol/L) and insulinemia, glucose intolerance, and the specific failure of the insulin response to glucose apparent at 2 months of age remained stable until 18 months. Whatever the age, the insulin-suppressive effect on glucose production was significantly less in GK rats than in Wistar rats. The insulin effect on whole-body glucose utilization was decreased at 2 months (15.8 ± 1.0 mg/min/kg *v* 23.5 ± 2.0, $P < .001$) and was only mildly aggravated between 2 and 18 months (10.3 ± 0.9 mg/min/kg, $P < .05$). By contrast, in Wistar control rats, basal insulinemia and the insulin response to glucose markedly increased between 2 and 18 months (2-month ΔI *v* 18-month ΔI, 1.4 ± 0.1 mU/ml · min *v* 2.9 ± 0.3, $P < .001$) and glucose tolerance remained normal. In 18-month-old Wistar rats, the insulin-stimulated glucose utilization rate (GUR) was found to be markedly decreased compared with that of 2-month-old Wistar rats (9.9 ± 0.8 mg/min/kg *v* 23.5 ± 2.0, $P < .001$), thus demonstrating an age-related decrease of insulin action. In conclusion, we find that there is no major alteration of insulin action due to aging in the GK rat, at variance with the pattern in nondiabetic rodents. It is speculated that such an adaptation in this lean model of NIDDM could be related to the limited capacity of these rats to expand their body weight with age, since it is recognized that body weight gain is largely responsible for the age-related impairment in peripheral insulin action in nondiabetic humans and nondiabetic animal models.

AGING in both humans and rodents is associated with an increase in fasting plasma insulin levels,[1-3] resistance to insulin-mediated glucose disposal,[4,5] and impaired glucose-induced insulin release.[6] However, the changes in carbohydrate metabolism are not entirely attributable to aging itself, but have been associated in part with alterations in diet, physical activity,[7] and/or fat distribution.[8-10] Moreover, in humans the association of aging and insulin resistance is confounded by other coexisting factors such as non–insulin-dependent diabetes mellitus (NIDDM), hypertension, and/or arteriosclerosis.[11] Although there are numerous studies describing metabolic disturbances that occur during NIDDM and aging considered as separate entities, little is known about possible interactions between these two conditions. On the basis of these considerations, we propose the use of a suitable animal model of NIDDM without obesity, the Goto-Kakisaki (GK) rat, to examine the interaction of aging and diabetes. This spontaneous-diabetes model was produced by selective breeding (with glucose intolerance as a selection index) repeated over many generations, starting from a nondiabetic Wistar colony.[12] All the offspring had a diabetic glucose tolerance test after the 10th generation, and the diabetic state became stable after the 30th generation.[12] This study was undertaken to determine whether defective insulin secretion and defective insulin action as they are expressed in the adult GK rat (2 months of age) would worsen with time (12 and 18 months of age). In addition, we wished to compare the age-related changes in insulin action and insulin secretion in GK rats with those occurring in nondiabetic rats (Wistar strain), using a colony of GK rats issued from F35 of the original colony.

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MATERIALS AND METHODS

Animals

Female GK and Wistar rats bred in our laboratory were weaned at 4 weeks of age and fed ad libitum with a commercial pelleted diet (diet 113; Usine d'Alimentation Rationnelle, Villemoison-sur-orge, France). The animals were maintained on a 12-hour light (7 AM to 7 PM)/dark (7 PM to 7 AM) cycle at a room temperature of 20°C. The phase of the ovarian cycle of the animals was not take into account at the time of the experiments. Glucose tolerance tests and euglycemic-hyperinsulinemic clamp studies were performed in Wistar and GK rats anesthetized with pentobarbital sodium intraperitoneally (4 mg/100 g body weight). Incidentally, comparison of our data obtained in anesthetized rats to those obtained by others in conscious chronically catheterized rats[10,13] indicates that the use of pentobarbital anesthesia does not mask the decline of insulin action during aging. Food intake and body weight were recorded every 2 days during a period of 1 month at the different ages studied.

Glucose Tolerance Tests

Intravenous glucose tolerance tests (0.5 g glucose/kg body weight) were performed at 11 AM in anesthetized rats fasted from 8 AM. Blood samples withdrawn from the tail vein were immediately centrifuged, and the plasma was stored at −20°C until glucose and insulin assays were performed.

Euglycemic-Hyperinsulinemic Clamp Studies

Tests were performed in Wistar and GK rats anesthetized and deprived of food for 3 to 4 hours, because in these postabsorptive conditions it has been shown that gut-derived glucose is negligible.[14,15] A tracheotomy was performed to avoid respiratory difficulties during anesthesia, and body temperature was maintained at 38°C with a heating lamp. A catheter was inserted into a carotid artery for blood sampling. Infusions (insulin, unlabeled glucose, and labeled glucose) were administered via the saphenous vein. Before all infusions and after a stabilization period (30 minutes) following the end of surgery, a blood

sample was collected for determination of basal blood glucose and plasma insulin.

Similar high plasma insulin values (170 μU/mL) were obtained by infusing insulin (0.2 U/h/kg) in Wistar and GK rats. Insulin (Actrapid porcine monocomponent; Novo Nordisk, Copenhagen, Denmark) was dissolved in 0.9% NaCl containing 0.2% bovine serum albumin (Sigma Chemical, St Louis, MO). To maintain plasma glucose at a similar level (120 to 130 mg/dL) in all groups of rats, a variable infusion of glucose was started 5 minutes after beginning the insulin infusion. Plasma glucose concentrations were measured every 5 minutes with a glucose analyzer (Beckman, Palo Alto, CA). Adjustments (left to the judgment of the investigator) in the exogenous glucose infusion rate were made to maintain euglycemia depending on the changes in plasma glucose observed. Blood samples were collected 45, 50, and 55 minutes (ie, once a steady state was achieved for both glycemia and glucose specific activity) after beginning the insulin infusion to determine blood glucose specific activity and plasma insulin levels.

Endogenous Glucose Production and Whole-Body Glucose Utilization

Endogenous glucose production and glucose turnover in the basal state and during hyperinsulinemic-euglycemic clamp studies were assessed with a primed continuous infusion of [3-^{3}H]glucose (New England Nuclear, Bad Homburg, Germany). Labeled glucose was administered intravenously as an initial priming dose (4 μCi), followed immediately by a continuous infusion at a rate of 0.2 μCi/min. A steady state of glucose specific activity was established by 40 minutes both in the basal state and in the clamp studies. The rate of glucose appearance (Ra) was then equal to the rate of glucose disappearance (Rd). These two parameters were calculated by dividing the [3-^{3}H]glucose infusion rate (dpm per minute) by the steady-state value of glucose specific activity (dpm per milligram). In the basal state, the rate of endogenous glucose production is equal to Ra. In the clamp studies, the rate of endogenous glucose production was calculated by subtracting the exogenous steady-state glucose infusion rate (SSGIR) from Ra. The rate of whole-body glucose utilization (GUR) was calculated as GUR = Rd, and the liver glucose production rate (GPR) as GPR = Ra − SSGIR.

In Vivo Insulin Action in Individual Tissues

Insulin action within individual tissues in vivo was studied in the basal state or during the euglycemic-hyperinsulinemic clamp using the nonmetabolizable glucose analog 2-deoxyglucose as described previously.[16] These experiments were performed in 2-month-old and 18-month-old rats used for the overall determination of glucose production and utilization in the basal state and during insulin infusion. 2-Deoxy-[1-^{14}C]glucose (Isotopchim, Ganagobie, France) was administered as a 15-μCi intravenous bolus through the saphenous vein under steady-state plasma glucose and insulin levels. Blood was sampled via the carotid artery for determination of 2-deoxy-[1-^{14}C]glucose specific radioactivity and blood glucose concentration. After the last blood sampling, the rat was killed, the tissues were immediately removed and immersed in 0.5 mL 1N NaOH, and 2-deoxy-[^{14}C]glucose-6-phosphate content was determined as described previously.[16] Because the main purpose of this study was to compare glucose utilization in diabetic and control rats, we estimated peripheral glucose utilization by calculating the simplified index of tissue glucose uptake, R'g (nanograms per minute per milligram tissue).[17]

Samples, Analytical Techniques, and Calculations

Plasma glucose was determined using a glucose analyzer (Beckman). Blood samples for measuring glucose or 2-deoxyglucose specific activity were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and immediately centrifuged. An aliquot of the supernatant was used for determination of glucose using a glucose oxidase method (Boehringer, Mannheim, Germany). For determination of blood [^{3}H]glucose, another aliquot of the supernatant was evaporated to dryness at 60°C to remove tritiated water. The dry residue was dissolved in 0.1 mL distilled water and counted with 3 mL Ready Solv-MP scintillation solution (Beckman). A third aliquot of the supernatant was counted for determination of blood 2-deoxy-[^{14}C]glucose. In glucose tolerance studies, plasma insulin level was measured by radioimmunoassay using a commercial kit (Sorin-Biomedica-INSIK-5, Anthony, France) with purified rat insulin (Novo Nordisk) as standard. In clamp studies, plasma immunoreactive insulin was estimated using purified rat (basal state) or porcine (clamp studies) insulin as standards (Novo Nordisk), with the antibody to mixed (porcine + bovine) insulin cross-reacting similarly with pork and rat insulin and porcine monoiodinated [^{125}I]insulin. Charcoal was used to separate free and bound hormone. The method allows determination of 2 μU/mL with a coefficient of variation within and between assays of 10%. Absolute insulin values in the same plasma obtained with this antibody are lower than those obtained with the antibody from the commercial kit.

Glucose tolerance was evaluated by the rate of glucose disappearance, K, calculated from the slope of the regression line, ln[gluc] = f(time), and the insulin response to glucose was estimated by the mean incremental insulin area (ΔI).

Statistical Analysis

Results are given as the mean ± SEM. Statistical analysis was performed using ANOVA followed by Scheffe's F test.

RESULTS

Characteristics of the Animals

Characteristics of the GK and Wistar rats at 2, 12, and 18 months of age are listed in Table 1. Diabetic GK rats had a significantly lower body weight than Wistar animals at the corresponding age ($P < .05$). The daily food intake of 2-month-old GK rats was significantly lower ($P < .001$) than that of 2-month-old Wistar rats. Nevertheless, the decrease in daily food intake observed during the aging process was of lesser magnitude in GK than in Wistar rats.

Whatever the age, GK rats exhibited a significantly higher plasma glucose concentration in the postabsorptive state than age-matched control Wistar rats. In GK and Wistar rats, plasma glucose was significantly decreased ($P < .001$) in 18-month-old animals compared with respective 2-month-old rats.

Plasma insulin concentration was similar in 2- and 12-month-old GK and Wistar rats. A marked increase ($P < .01$) of plasma insulin was observed in 18-month-old Wistar rats compared with 2-month-old Wistar rats, whereas plasma insulin was not significantly increased in 18-month-old GK rats compared with 2-month-old GK rats.

Glucose Tolerance and In Vivo Insulin Secretory Response in Wistar and GK Rats

After the intravenous glucose load, glucose tolerance estimated by the K index was markedly decreased in GK rats compared with respective control Wistar rats (Table 1).

Glucose tolerance did not deteriorate during the aging process in Wistar rats, whereas glucose intolerance in GK rats was slightly aggravated ($P < .05$) in 18-month-old animals.

The mean incremental insulin area (ΔI) was dramatically decreased in GK rats compared with age-matched Wistar rats (Table 1). However, in GK rats, the low insulin secretory

Table 1. Characteristics of Diabetic GK and Control Rats at 2, 12, and 18 Months

Group	No. of Rats	Body Weight (g)	Body Weight Increase (g)	Food Intake (g/d)	Plasma Glucose (mg/dL)	Plasma Insulin (μU/mL)	K (%/min)	ΔI (μU/mL · min)
Wistar								
2 mo	12	194 ± 5		17.6 ± 0.35	139 ± 3	150 ± 18	4.32 ± 0.15	1,393 ± 105
12 mo	15	302 ± 4	108 ± 4	16.04 ± 0.47	132 ± 3	120 ± 47	5.43 ± 0.22	911 ± 280
18 mo	24	392 ± 1	189 ± 11	13.77 ± 0.22¶	112 ± 2¶	265 ± 26‖	4.18 ± 0.26	2,914 ± 297‖
GK								
2 mo	12	162 ± 10*		15.54 ± 0.18‡	226 ± 7‡	172 ± 19	1.77 ± 0.08‡	402 ± 54‡
12 mo	16	275 ± 11*	113 ± 11	15.56 ± 0.83	250 ± 9‡	114 ± 18	1.63 ± 0.1‡	206 ± 38‡
18 mo	24	268 ± 1‡	106 ± 4‡	14.43 ± 0.22*‖	185 ± 6‡¶	202 ± 14*	1.46 ± 0.07‡§	345 ± 59‡

NOTE. Rats were in the postabsorptive state. Values are expressed as the mean ± SEM.
*$P < .05$, †$P < .01$, ‡$P < .001$: GK *v* Wistar.
§$P < .05$, ‖$P < .01$, ¶$P < .001$: 12/18 mo *v* 2 mo.

response to glucose remained stable during the aging process. By contrast, ΔI was markedly increased in 18-month-old Wistar rats compared with 2- and 12-month-old Wistar rats.

Endogenous Glucose Production

In the basal state, hepatic glucose production was significantly increased ($P < .001$) in GK rats compared with Wistar rats, whatever the age (Table 2). In the hyperinsulinemic state, the rate of endogenous glucose production remained markedly higher ($P < .05$) in GK rats at the three ages considered (Table 3 and Fig 1). By contrast, under the same conditions, hepatic glucose production was almost totally inhibited by insulin in 2-, 12-, and 18-month-old Wistar rats (Table 3 and Fig 1).

Overall Glucose Utilization

The basal GUR was significantly elevated ($P < .001$) in GK rats compared with respective Wistar rats, whatever the age (Table 2).

During the euglycemic-hyperinsulinemic clamp, the GUR was markedly lower in GK rats at 2 months of age than in age-matched Wistar rats. It was only mildly decreased with aging (Table 3 and Fig 1).

By contrast, in Wistar rats, the ability of insulin to stimulate glucose utilization markedly worsened during the aging process (GUR, 23.45 ± 2.06 mg/min/kg at 2 months *v* 9.86 ± 0.79 at 18 months, $P < .001$). Finally, the GUR at 18 months was found to be similar in GK rats and Wistar rats (Table 3 and Fig 1).

Table 2. Blood Glucose, Plasma Insulin, Glucose Infusion Rate, and Glucose Kinetics During Basal Studies in Diabetic GK and Control Rats

Group	No.	SSPI (μU/mL)	SSBG (mg/dL)	GUR/GPR mg/min	GUR/GPR mg/min/kg
2 months					
Wistar	6	59 ± 4	92 ± 4	2.08 ± 0.2	9.24 ± 0.7
GK	6	75 ± 8	185 ± 12‡	2.87 ± 0.29	16 ± 1.09‡
12 months					
Wistar	5	58 ± 10	89 ± 3	2.59 ± 0.5	9.14 ± 1.77
GK	5	58 ± 13	158 ± 7‡	3.23 ± 0.44	12.32 ± 0.44
18 months					
Wistar	5	119 ± 8	85 ± 2	1.92 ± 0.21	5.38 ± 0.21§
GK	5	76 ± 12†	189 ± 7‡	2.64 ± 0.12*	10.52 ± 0.5‡

NOTE. Rats were in the postabsorptive state. Values are expressed as the mean ± SEM.
Abbreviations: SSPI, steady-state plasma insulin; SSBG, steady-state blood glucose.
*$P < .05$, †$P < .01$, ‡$P < .001$: GK *v* Wistar.
§$P < .01$, 12/18 mo *v* 2 mo.

Glucose Utilization in Individual Tissues of 2- and 18-Month-Old GK and Wistar Rats

In the basal state, 2-deoxyglucose uptake was increased in insulin-sensitive tissues of 2- and 18-month-old GK rats compared with respective Wistar rats, except in heart and interscapular brown adipose tissue (Table 4).

During the euglycemic-hyperinsulinemic clamp, 2-deoxyglucose utilization was not decreased in skeletal muscles, diaphragm, and white adipose tissue in 2- and 18-month-old GK rats compared with age-matched Wistar rats (Table 5).

Insulin-stimulated 2-deoxyglucose uptake was markedly lower in heart and brown adipose tissue of 2-month-old GK rats versus Wistar rats ($P < .001$). However, this difference was not observed in 18-month-old GK rats compared with 18-month-old Wistar rats (Table 5).

During the aging process in Wistar rats, the ability of insulin to stimulate 2-deoxyglucose uptake was markedly decreased in heart and in brown and white adipose tissues. In old GK rats, a decrease was also observed in heart and white adipose tissue ($P < .01$). However, brown adipose tissue of GK rats was less resistant to insulin at 18 months than at 2 months of age ($P < .001$; Table 5).

DISCUSSION

As a reference for comparison to the GK rats, we first investigated the characteristics of female Wistar rats during aging from 2 to 18 months of age. Our data (Table 2) show that in nondiabetic Wistar rats, basal insulinemia and the insulin response to an intravenous glucose load were markedly increased at 18 months of age compared with levels in 2-month-old rats, whereas basal glycemia and glucose tolerance remained stable. Moreover, the GUR measured in the basal state or after insulin infusion (Table 3) was markedly decreased in 18-month-old normal Wistar rats compared with 2-month-old rats and became similar to the whole-body GUR of diabetic GK rats. We also found a decreased insulin-stimulated glucose uptake in the heart, paraovarian white adipose tissue, and

Table 3. Blood Glucose, Plasma Insulin, and Glucose Infusion Rate During Hyperinsulinemic-Euglycemic Clamps in Diabetic GK and Control Rats

Group	No.	BPI (μU/mL)	SSPI (μU/mL)	BBG (mg/dL)	SSBG (mg/dL)	SSGIR (mg/min)	GPR		GUR	
							mg/min	mg/min/kg	mg/min	mg/min/kg
2 months										
Wistar	5	74 ± 4	143 ± 5	115 ± 3	121 ± 4	4.77 ± 0.33	0.13 ± 0.29	0.55 ± 1.31	5.15 ± 0.43	23.45 ± 2.06
GK	6	52 ± 6	149 ± 9	197 ± 6‡	123 ± 3	0.87 ± 0.07‡	1.93 ± 0.25†	10.9 ± 1.29‡	2.78 ± 0.18‡	15.82 ± 1.02‡
12 months										
Wistar	6	88 ± 14	150 ± 17	99 ± 4	107 ± 5	4.89 ± 0.72	0.82 ± 0.48	2.88 ± 1.7	5.33 ± 0.76	18.83 ± 2.68
GK	6	56 ± 10	196 ± 29	184 ± 21†	120 ± 2*	1.29 ± 0.29†	2.06 ± 0.23*	7.85 ± 0.88*	3.29 ± 0.22	12.55 ± 0.84*
18 months										
Wistar	6	119 ± 8	204 ± 29	91 ± 7	93 ± 2	3.31 ± 0.33	0.17 ± 0.32	0.47 ± 0.84	3.48 ± 0.31	9.86 ± 0.79‖
GK	7	76 ± 12†	163 ± 11	190 ± 9‡	104 ± 3	1.8 ± 0.12‡	1.09 ± 0.11*	3.88 ± 0.47†	2.89 ± 0.19	10.27 ± 0.87§

NOTE. Rats were in the postabsorptive state. Values are expressed as the mean ± SEM.

Abbreviations: BPI, basal plasma insulin; SSPI, steady-state plasma insulin; BBG, basal blood glucose; SSBG, steady-state blood glucose; SSGIR, steady-state glucose infusion rate.

*$P < .05$, †$P < .01$, ‡$P < .001$: GK *v* Wistar.

§$P < .05$, ‖$P < .001$: 12/18 mo *v* 2 mo.

interscapular brown adipose tissue (Table 4) of 18-month-old Wistar rats compared with 2-month-old rats. These results confirm the appearance of peripheral insulin resistance in elderly nondiabetic rats, previously described by others.[18-20]

Our data also show that insulin normally suppressed hepatic glucose output in Wistar rats whatever the age. This demonstrates that peripheral insulin resistance is not associated with altered hepatic insulin action during aging. Moreover, our data clearly illustrate an adaptation of insulin secretion during the aging process in nondiabetic Wistar rats to maintain basal glycemia and glucose tolerance in the normal range.

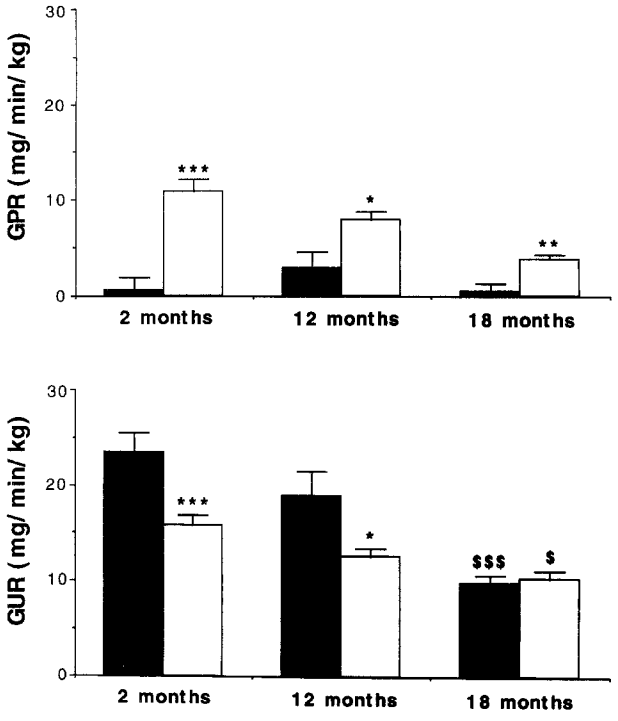

Fig 1. GPR and GUR during hyperinsulinemic-euglycemic clamps in diabetic GK (□) and control (■) rats according to age. Rats were in the post absorptive state. Height of bars represents the mean ± SEM for each group (n = 5 to 6). *$P < .05$, **$P < .01$, ***$P < .001$: GK *v* Wistar. §$P < .05$, §§§$P < .001$: 12/18 mo *v* 2 mo.

In fact, more detailed examination of pancreatic endocrine function by Reaven et al[21] has shown that islet hypertrophy develops with aging in the rat and that such a process enables the organism to secrete enough insulin to meet its needs despite the reduction of insulin secretory function per cell.[21] There is also evidence that aging in rats may be associated with a decrease of insulin degradation.[22] This phenomenon may also participate in the maintenance of higher plasma insulin levels in aged rats.

Our data using deoxyglucose uptake in Wistar rats indicate that the reduction of in vivo insulin-stimulated glucose utilization during aging is associated with a similar age-related loss in the ability of insulin to promote in vivo glucose uptake in the heart and white adipose tissue. Our conclusions related to white adipose tissue and obtained in vivo are entirely consistent with findings obtained in vitro in isolated fat cells.[19,23] Under the latter conditions, it was proposed that the impaired insulin-stimulated glucose transport was due to a depletion of the intracellular adipocyte pool of glucose transporter during aging.[19] Our observations related to insulin-stimulated deoxyglucose uptake in vivo by skeletal muscles are consistent with a previous report that the insulin-stimulated rate of glucose transport by Wistar soleus muscle incubated in vitro is not significantly altered during aging.[18] These conclusions do not imply that intracellular glucose metabolism is not impaired in skeletal muscle of aging rats, because defective glycolysis and/or glycogen synthesis in response to insulin have been reported.[10,18,24,25]

In 2-month-old female GK rats, basal plasma glucose levels were significantly increased and glucose intolerance was detectable after intravenous glucose administration. These defects are due, at least in part, to a marked abnormality in glucose-induced insulin secretion observed in this study, as well as some other in vitro studies,[26-28] but they could also be related to defective insulin action.

Our present data confirm our previous data[29] that basal hepatic glucose production is increased in 2-month-old GK rats.

Table 4. Index of Glucose Utilization in Extrahepatic Tissues During Basal Studies in GK and Wistar Rats

Group	No.	R'g (ng/min/mg tissue)							
		Soleus	EDL	Epitrochlearis	Diaphragm	Heart	iBAT	pWAT	iWAT
2 months									
Wistar	6	1.87 ± 0.24	1.92 ± 0.34	1.74 ± 0.22	4.72 ± 0.84	38.64 ± 8.31	9.08 ± 1.73	0.86 ± 0.1	1.19 ± 0.17
GK	6	3.25 ± 0.25†	4.14 ± 0.31‡	4.48 ± 0.55‡	10 ± 2.55	20.94 ± 4.09	10.03 ± 3.48	1.18 ± 0.23	2.16 ± 0.21†
18 months									
Wistar	5	1.6 ± 0.21	1.71 ± 0.33	1.34 ± 0.27	5.01 ± 1.65	16.79 ± 1.62§	9.51 ± 4.26	0.56 ± 0.09	0.95 ± 0.13
GK	5	3.04 ± 0.43*	3.84 ± 0.92	5.01 ± 0.51†	6.66 ± 0.84	13.28 ± 2.52	5.79 ± 0.91	1.7 ± 0.57	2.23 ± 0.23†

NOTE. Rats were in the postabsorptive state. Values are expressed as the mean ± SEM.

Abbreviations: R'g, index of glucose utilization; EDL, extensor digitorum longus; iBAT, interscapular brown adipose tissue; pWAT, paraovarian white adipose tissue; iWAT, inguinal white adipose tissue.

*$P < .05$, †$P < .01$, ‡$P < .001$: GK *v* Wistar.

§$P < .05$, 12/18 mo *v* 2 mo.

The clamp studies revealed the presence of a decreased insulin-suppressive effect on hepatic glucose production, again confirming that the liver of adult GK rats is indeed resistant to insulin action.[29,30] However, one cannot presently eliminate the possibility that abnormal levels of some blood-borne substances (eg, corticosterone and glucagon) may also contribute to hepatic insulin resistance in the GK rat.

In these GK rats, glucose uptake by the whole body in the basal state was increased compared with that in controls. Such was the tendency in most of the tissues tested individually. Because this was obtained in GK rats in the presence of high plasma glucose levels, it is difficult to conclude from this sole experiment whether peripheral insulin action is impaired in these diabetic rats. The clear answer was obtained from the hyperinsulinemic-euglycemic clamp experiments, which indicated that (1) the whole-body GUR was significantly decreased in 2-month-old GK rats and (2) most of the peripheral tissues (epitrochlearis, soleus, extensor digitorum longus, and diaphragm muscles and periovarian and inguinal white adipose tissues) were normally reactive to the effect of insulin compared with corresponding tissues in control rats. By contrast, heart and interscapular brown adipose tissue were resistant to insulin action. Therefore, there was no evidence for a clear-cut decreased muscle insulin action in adult GK rats. Our group and others have previously mentioned a minimal impairment of insulin-stimulated muscle glucose uptake in adult GK rats[29] or adult hybrid GK/Wistar rats,[31] but it was limited to epitrochlearis muscle only. Because the overall decreased insulin-induced glucose uptake in GK rats could not be supported by a reduced insulin-induced glucose uptake by the skeletal muscles, we suggest that it would not be related to a defective insulin action, but rather to an impaired proportion of white adipose tissue and/or muscle tissue in the GK rat compared with the Wistar rat. Preliminary data from our group indicate that the Lee index (body weight/body length^3) is significantly decreased in 2-month-old GK rats as compared with related Wistar rats.

The final objective of the present study was to document the effect of aging on the alterations of glucose metabolism in the GK rat. The current data provide evidence that no major additional deterioration of both basal hyperglycemia and glucose intolerance could be detected in older GK rats (12- or 18-month-old) compared with 2-month-old GK rats. None of the following parameters in GK rats were significantly affected by aging: basal plasma insulin level, insulin secretion in response to glucose in vivo, basal hepatic glucose production, insulin-suppressive effect on hepatic glucose production, and basal glucose utilization by the whole body. Only insulin-stimulated whole-body GURs and some individual tissue GURs (heart and paraovarian and inguinal white adipose tissues) were significantly decreased during aging. Such a pattern is strikingly different from the situation reported in the aging Wistar nondiabetic rats studied in parallel, in the sense that whereas both insulin secretion and insulin action are profoundly modified by age in the nondiabetic rat, they are only marginally affected in the GK rat. The deterioration of insulin sensitivity during the aging process in both nondiabetic humans and rats seems closely related to an increase in fat mass.[10,32,33] The limitation of body weight gain by long-term caloric restriction in aging rats[21,34-36] or monkeys[37] prevents the increase in plasma insulin levels and the development of insulin resistance of aging. Prevention of the increase in plasma insulin levels and insulin resistance of aging was also reported in different

Table 5. Index of Glucose Utilization in Extrahepatic Tissues During Euglycemic-Hyperinsulinemic Clamps in GK and Wistar Rats

Group	No.	R'g (ng/min/mg tissue)							
		Soleus	EDL	Epitrochlearis	Diaphragm	Heart	iBAT	pWAT	iWAT
2 months									
Wistar	5	4.09 ± 0.4	5.15 ± 1.11	7.27 ± 0.83	42.37 ± 7.8	224.47 ± 9.77	323.16 ± 56.83	7.86 ± 2.06	5.05 ± 0.5
GK	6	5.85 ± 0.66	8.13 ± 0.73*	6.64 ± 0.14	51.13 ± 5.93	153.4 ± 9.75†	15.33 ± 3.42†	7.36 ± 1.06	7.09 ± 1.1
18 months									
Wistar	6	5.09 ± 0.49	5.14 ± 0.31	5.87 ± 0.86	32.66 ± 3.12	92.52 ± 13.96‖	66.09 ± 12.41‖	1.12 ± 0.1§	2.15 ± 0.7‡
GK	7	4.96 ± 0.38	9.5 ± 2.53	6.48 ± 0.94	46.47 ± 6.07	73.87 ± 7.7‖	95.31 ± 13.49‖	1.58 ± 0.26‖	2.55 ± 0.44§

NOTE. Rats were in the postabsorptive state. Values are expressed as the mean ± SEM.

*$P < .05$, †$P < .001$: GK *v* Wistar.

‡$P < .05$, §$P < .01$, ‖$P < .001$: 12/18 mo *v* 2 mo.

protocols aimed to maintain the body weight of aging rats at the level of early adulthood by dietary restriction and/or exercise.[34-36] The weak increase in body weight observed in old GK rats suggests that growth of the total fat mass is limited in these rats, and could partly account for the lack of deterioration of insulin resistance with aging in these animals. Supporting this view, preliminary data from our group indicate that the Lee index significantly increases from 2 to 18 months in Wistar rats, whereas it is not modified during the same period in GK rats.

Finally, it is of interest to mention clinical observations that are relevant to our observations in the GK model. It has been reported that non-obese elderly subjects with NIDDM showed only minimally reduced insulin-mediated glucose disposal when compared with age-matched control subjects, despite a marked impairment in insulin release.[38] Moreover, when comparing elderly and middle-aged subjects, NIDDM patients appear to have a similar degree of insulin resistance, and the difference between control subjects and NIDDM patients was smaller in the elderly.[38] Also in humans, aging does not appear to intensify the peripheral insulin resistance of obese states when both coexist in the same individual.[39]

In conclusion, there is no major alteration of insulin action due to aging in the GK rat, at variance with the pattern shown in nondiabetic rodents. Such an adaptation in this model of NIDDM could be related to a limited capacity of these rats to expand their fat mass with age, since it is recognized that the relative contribution of fat mass to whole-body weight gain is largely responsible for the age-related impairment in peripheral insulin action in nondiabetic humans and nondiabetic animal models.[10,40] From this perspective, it would be particularly advantageous in the GK model to prospectively evaluate the changes in body composition, lean mass, and fat mass throughout life.

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