Evolution of Insulin Resistance in New Zealand Obese Mice

MARTA C. VERONI, JOSEPH PROIETTO, AND RICHARD G. LARKINS

The etiology of non-insulin-dependent diabetes mellitus (NIDDM) is not known. Hyperglycemia is due to increased hepatic glucose production (HGP), decreased glucose uptake, and impaired insulin secretion. It is unknown if these defects are coinherited or if one precedes and causes the others. The aim of this study was to determine the earliest defects in the evolution of the syndrome in the New Zealand obese (NZO) mouse, a polygenic model of NIDDM. NZO and control NZC mice were studied at 4–5 and 20 wk of age. Glucose turnover and glucose uptake in individual tissues were measured basally and during a hyperinsulinemic clamp. First-phase insulin secretion was measured after an intravenous glucose load. HGP was higher in the NZO mice both basally and during the clamp at both ages. At 4–5 wk of age, there was evidence of insulin insensitivity in brown adipose tissue, soleus, diaphragm, red quadriceps, and red gastrocnemius but not in heart, white quadriceps, and white gastrocnemius. In 20-wk-old mice, insulin responsiveness was decreased in white and brown adipose tissue and soleus muscle but not in heart, diaphragm, red and white quadriceps, and red and white gastrocnemius. First-phase insulin secretion (percentage rise above basal) 3 min after the glucose bolus was impaired in NZO mice at both ages. We conclude that hepatic glucose overproduction, brown adipose tissue and skeletal muscle insulin resistance, and impaired first-phase insulin secretion are all early abnormalities in the NZO mouse. *Diabetes* 40:1480–87, 1991

The etiology of non-insulin dependent diabetes mellitus (NIDDM) remains unknown. In established diabetes, increased hepatic glucose production (HGP), reduced glucose uptake into muscle and adipose tissue, and impaired glucose-stimulated insulin secretion combine to produce hyperglycemia (1). Although it is possible that these three abnormalities are coinherited, it is generally believed that one precedes and causes the others. Thus, impaired first-phase insulin secretion (2), peripheral insulin resistance (3), and, more recently, hepatic insulin resistance (4) have all been proposed as the primary defects in NIDDM. A proposed mechanism by which each of these defects could cause the others is glucose toxicity (5). There is now good evidence that hyperglycemia per se can induce insulin resistance (6,7) and a defect in insulin secretion (8,9). Thus, it has been demonstrated that insulin deficiency caused by partial pancreatectomy (6) or alloxan (10) results in peripheral insulin resistance. The development of insulin resistance in these circumstances can be prevented if the hyperglycemia is corrected, for example, by inducing renal glycosuria with phloridzin (6). It has also been shown that hyperglycemia resulting from chronic infusion of glucose will induce a defect in glucose- but not arginine-mediated insulin secretion (8). Thus, insulin resistance can lead to impaired insulin secretion, and impaired insulin secretion can cause insulin resistance, each by causing hyperglycemia.

Attempts have been made to investigate what may be the primary defect in human NIDDM. For example, relatives of patients with NIDDM have a defect in the first phase of insulin secretion (11). On the other hand, peripheral insulin resistance has been described in young diabetes-prone Pima Indians before the development of hyperglycemia (12). Young Australian aborigines who have both parents with NIDDM have both hepatic and peripheral insulin resistance at a time when they have normal fasting glucose and increased basal and stimulated insulin levels (13). The presence of each of these defects in young subjects at risk of developing NIDDM may indicate that all three abnormalities are inherited or may simply reflect the fact that, by the time the subjects reach early adulthood, the secondary defects have already appeared. Because there are obvious ethical

From the University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Parkville, Australia.

Address correspondence and reprint requests to Dr. J. Proietto, Department of Medicine, Royal Melbourne Hospital, Parkville 3050, Australia.

Received for publication 18 February 1991 and accepted in revised form 12 June 1991.

difficulties in performing studies in young children, animal models of NIDDM are useful when studying the evolution of the diabetic syndrome. However, care should be taken when extrapolating the data to the human condition.

The New Zealand obese (NZO) mouse is obese, hyperphagic, insulin resistant, and glucose intolerant. It was first described in 1953 (14) and was obtained by inbreeding, starting from a mixed mouse colony, and is thus considered a polygenic model of human NIDDM. Previous studies have shown that these mice have impaired insulin secretion (15,16) and action (17). The aim of this study was to determine what is the earliest defect(s) in the evolution of the syndrome in this mouse.

RESEARCH DESIGN AND METHODS

Male NZO and control NZC mice were purchased from colonies maintained by the Walter and Eliza Hall Institute (Parkville, Australia). These colonies were established in 1967 from mice obtained from the original New Zealand colony. At the time of transfer, NZC and NZO mice were at the 79th and 77th generation, respectively. Since then, the colonies have been maintained by inbreeding. The mice were transferred to the animal facility in the Department of Medicine, Royal Melbourne Hospital at 3 wk of age and were maintained under standard laboratory conditions with controlled temperature and a 12-h day/night cycle. Mice were fed standard laboratory chow (Barastoc, Pakenham, Australia) ad libitum. All experiments were performed in the morning on overnight-fasted mice. Mice were anesthetized with 60 mg/kg i.p. pentobarbital sodium (Nembutal, Boehringer Ingelheim, Artarmon, Australia). Two incisions were made longitudinally on either side of the neck and silastic catheters (0.012:0.025 ID/OD, Dow Corning, Midland, MI) were inserted into the right jugular vein for infusions and the left carotid artery for blood sampling. A tracheostomy was then performed to prevent upper airways obstruction. The animals were kept at 37°C with a heating lamp. Temperature was monitored with a rectal temperature probe. After surgery, the mice were rested for 30 min before commencing the studies. The depth of anesthesia was checked regularly, and extra pentobarbital sodium was given when required. Mice were studied at 4–5 or 20 wk of age.

Because of the small size of the animals, it was necessary to perform each set of studies on different groups of mice. For example, basal and insulin-stimulated glucose turnover were measured in four different groups of animals: young and old NZO and young and old NZC mice.

Measurement of basal and insulin-stimulated glucose turnover. Fasting HGP, glucose disappearance rate (R_d), and metabolic clearance rate (MCR) of glucose were measured with a primed continuous infusion of [6-^{3}H]glucose (0.14 μCi/min). The priming dose was given over the first 5 min at 3 times the subsequent flow rate. Blood samples (200 μl) were taken at 60, 65, and 70 min after the start of tracer infusion for measurement of glucose, [6-^{3}H]glucose specific activity, and insulin.

The effects of insulin on HGP and glucose disposal were measured with a hyperinsulinemic clamp (18). At the start of the study, primed continuous infusions of insulin and [6-^{3}H]glucose were commenced and maintained at constant rates for the duration of the experiment. Insulin was infused at 1 $mU \cdot kg^{-1} \cdot min^{-1}$ in the young mice and 3 $mU \cdot kg^{-1} \cdot min^{-1}$ in the older mice. Plasma glucose was maintained by infusion of 5% glucose at a variable rate, which was adjusted according to blood glucose analysis at 10- to 15-min intervals. The blood taken for each glucose measurement (25 μl) was replaced with an equal volume of normal saline. When steady state was reached (usually after 60–90 min) three blood samples (200 μl each) were taken at 5-min intervals for analysis as described above. At the end of each study, the infusion rate of the labeled (dpm/min) and unlabeled (μmol/min) glucose was accurately measured.

Measurement of basal and insulin-stimulated 2-deoxyglucose (2-DG) uptake in individual tissues. 2-DG uptake in individual tissues was measured basally and during a hyperinsulinemic clamp with a previously described method (19,20). A bolus of [^{14}C]-2-DG was injected 30 min after surgery in the basal group and when steady state was reached in the insulin-infused group, and blood samples (200 μl) were taken for estimation of [^{14}C]-2-DG, glucose, and insulin at 2, 5, 10, 15, and 30 min. After each sampling, the blood was rapidly spun and the plasma separated, and after resuspension, the erythrocytes were reinjected into the mice. At the end of this time, the animals were killed with an overdose of pentobarbital sodium and the following tissues were rapidly sampled: epididymal fat, brown adipose tissue, heart, diaphragm, soleus, red and white quadriceps, and red and white gastrocnemius. After removal, the tissues were frozen in liquid N_2 and stored at −70°C until assayed.

Measurement of insulin secretion in response to an intravenous glucose load. Overnight-fasted mice were anesthetized as described above, and a carotid catheter and a tracheostomy tube were inserted. The mice were allowed to recover for 30 min before the start of the test. A basal blood sample was taken (200 μl) and immediately spun. A bolus of 0.6 g/kg glucose was then infused over 1 min through the carotid catheter. The catheter was flushed with heparinized saline and further rinsed by the repeated withdrawal and reinjection of blood. Blood was sampled at 3, 6, 15, and 30 min for measurement of glucose and insulin. After each blood sample, the erythrocytes were reinfused into the animals.

Analytical procedures. Blood was kept on ice before centrifugation, and plasma was stored at −20°C.Glucose was measured with a YSI glucose analyzer (Yellow Springs, OH). Plasma insulin levels were measured by radioimmunoassay (Pharmacia, Uppsala, Sweeden) with a second antibody to separate free from bound insulin. To determine [6-^{3}H]-glucose specific activity, 25 μl of plasma was deproteinized with equal volumes of $Ba(OH)_2$ (0.3 M) and $ZnSO_4$ (0.3 M). After centrifugation, the supernatant was dehydrated to remove tritiated water and counted in a liquid-scintillation counter (Beckman LS 3810, Fullerton, CA) after the addition of 4 ml of water and 10 ml of liquid-scintillation cocktail (Ready Value, Beckman). Because of the small volume of plasma processed, it was considered impractical to pass individual samples through ion-exchange columns on a routine basis. To determine the radioactivity in the plasma existing as charged metabolites of glucose, the plasma remaining from the three samples from each mouse was pooled, and after deproteinization, half was passed down

an ion-exchange column (Bio-Rad Ag-2X-8, Richmond, CA), and the other half was counted without prior passage down the column. There was no difference in the number of counts retained by the column between the NZO and NZC mice at either age either in the basal or clamp studies, with the exception of the 4-wk-old mice during the clamp protocol. In this group, passing through the anion-exchange resin resulted in a 7.3 ± 0.7% retention from NZC mice versus 1.4 ± 0.9% from NZO mice ($P < 0.01$). This 6% difference in the retained counts would result in a 6% underestimation of R_a in the NZC group; however, this would not alter the conclusion reached.

To measure plasma [^{14}C]-2-DG, plasma was deproteinized as described above and spun, and an aliquot of the supernatant was counted in a β-counter after the addition of 4 ml of water and 10 ml of liquid-scintillation cocktail. To measure tissue [^{14}C]-2-DG uptake, each tissue was weighed and dissolved in 0.5 ml of 1 M NaOH kept in a shaking water bath at 60°C for 1 h. When the tissues had dissolved completely, the total volume was increased to 1 ml with 1 M NaOH. This was neutralized with 1 ml of 1 M HCl. Two 500-μl aliquots were taken. One was deproteinized with 500 μl of $Ba(OH)_2$ and 500 μl of $ZnSO_4$, and the other was deproteinized with 2 ml of 6% $HClO_4$. The solutions were centrifuged, and 1 and 2 ml of supernatant from each precipitation, respectively, was placed in scintillation vials and counted in a β-counter after the addition of water (to make up to 4 ml) and 10 ml of scintillant. The $HClO_4$ supernatant contains both phosphorylated and unphosphorylated 2-DG, whereas the $Ba(OH)_2$ and $ZnSO_4$ supernatant contains only the unphosphorylated form. Thus the difference in counts represents the phosphorylated 2-DG (20).

Calculations. Total glucose appearance (R_a; μmol/min) was determined at steady state as F/SA, where F is the infusion rate of [6-^{3}H]6 glucose (dpm/min) and SA is the steady-state specific activity (dpm/μmol). At steady state, plasma glucose is constant and $R_a = R_d$. Metabolic clearance of glucose (ml/min) was calculated as $R_d/[G]$ where [G] is the prevailing glucose concentration. After an overnight fast, R_a is assumed to equal HGP. In the insulin-clamp studies, HGP was calculated by subtracting the exogenous glucose infusion rate from the total rate of glucose appearance.

Tissue glucose uptake was calculated as described by Kraegen et al. (19) as

$$R_{g'} = \frac{C_p C_m \cdot (t)}{\int_0^t C_p \cdot dt}$$

where $R_{g'}$ (μmol · 100 g^{-1} · min^{-1}) is the tissue glucose metabolic index, C_p is the glucose concentration, $C_m \cdot (t)$ is the total [^{14}C]-2-DG-6-phosphate accumulated in the tissue over t and $\int_0^t C_p \cdot dt$ is the integrated value for the plasma [^{14}C]-2-DG levels over time t.

Comparisons between groups were made with Student's t test for unpaired samples.

RESULTS

Table 1 shows the mean values for body mass index (BMI; g/cm^2), fasting glucose, insulin, HGP, and MCR of glucose in the young (4- to 5-wk-old) and adult (20-wk-old) mice that underwent the basal glucose turnover study. The NZO mice had higher BMIs than the lean controls at both ages, but the difference was much greater at 20 wk of age, indicating rapid accumulation of fat in the first 20 wk of life. There was no difference in fasting glycemia in the young animals, but older NZO mice had mild hyperglycemia compared with NZC mice. Fasting insulin was significantly higher in the NZO mice at both ages. Despite the higher insulin level, HGP was higher in the obese mice at both ages. This was true regardless of whether the data were expressed per mouse (data not shown) or per BMI. Glucose clearance was not different between the two groups of mice at either age.

During the insulin infusion in the young mice, plasma glucose was clamped at ~10 mM (Table 2). With matched insulin and glucose concentrations, the NZO mice had a higher rate of HGP than the control mice, confirming hepatic insulin resistance. MCR of glucose was not different between the groups and did not rise above basal at these low insulin concentrations.

In the older animals, insulin was infused at a higher rate than in the younger mice (3 mU · kg^{-1} · min^{-1}). Insulin concentrations of ~700 pM were achieved in both groups of

TABLE 1
Basal glucose turnover in NZC and New Zealand obese (NZO) mice

	NZC	NZO	P
4–5 wk old			
n	9	11	
BMI (g/cm^2)	0.24 ± 0.01	0.28 ± 0.01	<0.001
Glucose (mM)	5.9 ± 0.4	6.9 ± 0.7	
Insulin (pM)	134 ± 17	227 ± 22	<0.05
HGP/BMI (μmol · cm^2 · g^{-1} · min^{-1})	4.9 ± 0.5	6.7 ± 0.8	<0.05
MCR/BMI (ml · cm^2 · g^{-1} min^{-1})	0.88 ± 0.08	0.99 ± 0.05	
20 wk old			
n	10	10	
BMI (g/cm^2)	0.28 ± 0.01	0.41 ± 0.01	<0.001
Glucose (mM)	5.1 ± 0.2	6.8 ± 0.6	<0.03
Insulin (pM)	147 ± 37	368 ± 79	<0.05
HGP/BMI (μmol · cm^2 · g^{-1} · min^{-1})	4.03 ± 0.33	5.77 ± 0.67	<0.05
MCR/BMI (ml · cm^2 · g^{-1} · min^{-1})	0.78 ± 0.05	0.85 ± 0.05	

Values are means ± SE except as noted. BMI, body mass index; HGP, hepatic glucose production; MCR, metabolic clearance rate of glucose. Statistical analysis was performed with Student's t test.

TABLE 2
Glucose turnover during hyperinsulinemic clamp in NZC and New Zealand obese (NZO) mice

	NZC	NZO	*P*
4–5 wk old			
n	18	15	
BMI (g/cm²)	0.22 ± 0.003	0.26 ± 0.003	<0.001
Glucose (mM)	10.2 ± 0.5	9.1 ± 0.6	
Insulin (pM)	204 ± 37	212 ± 15	
HGP/BMI (μmol · cm² · g^{-1} · min^{-1})	2.3 ± 0.5	3.9 ± 0.4	<0.03
MCR/BMI (ml · cm² · g^{-1} · min^{-1})	0.96 ± 0.06	0.93 ± 0.05	
20 wk old			
n	9	11	
BMI (g/cm²)	0.29 ± 0.01	0.39 ± 0.01	<0.001
Glucose (mM)	8.7 ± 0.3	9.9 ± 0.5	<0.02
Insulin (pM)	656 ± 151	708 ± 119	
HGP/BMI (μmol · cm² · g^{-1} · min^{-1})	−0.66 ± 1.2	2.63 ± 0.87	<0.05
MCR/BMI (ml · cm² · g^{-1} · min^{-1})	1.07 ± 0.11	1.08 ± 0.08	

Values are means ± SE except as noted. BMI, body mass index; HGP, hepatic glucose production; MCR, metabolic clearance rate of glucose. Statistical analysis was performed with Student's *t* test.

mice (Table 2). Plasma glucose was clamped at ~10 mM, although glucose was lower in NZC than in NZO mice. At these glucose and insulin concentrations, HGP was completely suppressed in NZC but not in NZO mice. MCR of glucose rose in both the NZC and NZO mice to the same extent, and there was no difference between the two groups. These data show that hepatic insulin resistance is an early feature of the NZO mouse.

In the second set of experiments, glucose uptake in individual tissues was measured basally and during mild hyperinsulinemia and hyperglycemia in NZO and NZC mice. Figure 1 shows the glucose and insulin values basally and during the clamp in the young mice. Basal glycemia was the same in NZO and NZC mice, but as with the previous group of young mice, basal insulin was higher in the NZO than in the NZC mice. During the clamp, insulin was infused to achieve insulin values of ~200 pM. Figure 2 illustrates the R_g' in 4- to 5-wk-old animals in white and brown adipose tissue and in heart and soleus muscles. Table 3 shows the data for diaphragm, red and white quadriceps, and red and white gastrocnemius muscles. At 4 wk of age, NZO mice had evidence of insulin resistance in brown adipose tissue, soleus, diaphragm, and the red quadriceps and gastrocnemius muscles but not in white adipose tissue, heart, or white quadriceps and gastrocnemius. Despite the normal insulin response of the heart in NZO mice, basal glucose uptake was lower in the obese animals than the lean. This was the only tissue that showed a decrease in basal uptake. In all other tissues, basal R_g' was the same in obese and lean animals.

Figure 3 shows the glucose and insulin levels in the 20-wk-old mice. Unlike the previous group of similarly aged mice, the NZO mice did not have basal hyperglycemia. However, as with the previous mice, there was significant basal hyperinsulinemia. During the clamp, insulin concentrations of ~600 pM were obtained, and glucose was clamped at the basal level in both groups of mice. At 20 wk of age, white and brown adipose tissues were insulin resistant as was the soleus muscle. However, at the insulin levels used in this study, no evidence for resistance in heart, diaphragm, red and white quadriceps, and red and white gastrocnemius could be found (Fig. 4; Table 4). Indeed, glucose uptake was higher in the diaphragm in the NZO mice both basally and during the insulin infusion. Similarly, basal uptake was higher in white adipose tisssue, heart, white quadriceps, and white gastrocnemius muscles.

In the third group of studies, insulin secretion in response to an intravenous glucose bolus was investigated. Figure 5 shows glucose and insulin levels basally and at 3, 6, 15, and 30 min after a bolus of glucose in 4- to 5-wk-old animals. Figure 6 shows the corresponding data in 20-wk-old animals. Insulin levels are given as both the absolute concentration and as a percentage of the basal insulin concentration. Intravenous glucose tolerance was normal in 4- to 5-wk-old mice, but in 20-wk-old mice, fasting glucose was higher in the NZO mice as was the glucose concentration at 3 and 6 min after the bolus of glucose. However, the rate of fall of glucose was the same in the NZO and NZC mice from 3 to 6 min and faster in the NZO mice for the subsequent 24 min. As previously shown in the other groups, at 20 wk of age, NZO mice have high fasting insulin levels. However, at 4–5 wk, the difference in fasting insulin levels did not reach statistical significance in this group of animals. After the glucose load, insulin concentration was not different between the lean and obese animals at 4–5 wk of age but was different in the older animals at 3, 6, and 30 min. However, the first-phase insulin secretion, expressed as percentage rise above basal 3 min after the glucose bolus, revealed a defect in NZO mice at both 4–5 and 20 wk of age.

DISCUSSION

The NZO mouse has many features of NIDDM, including obesity, insulin resistance, and abnormalities in insulin secretion. It is polygenic in origin, having been derived by repeated inbreeding starting from a mixed mouse colony. In this regard, it also resembles the human condition. Previous studies on the NZO mouse have shown evidence for insulin resistance in skeletal muscle (17,21,22), white adipose tissue (17,21,23,24), and the liver (25) as well as defects in glucose-stimulated insulin secretion (15,16,26–29).

In this study, an attempt was made to study the earliest abnormalities in the development of glucose intolerance in

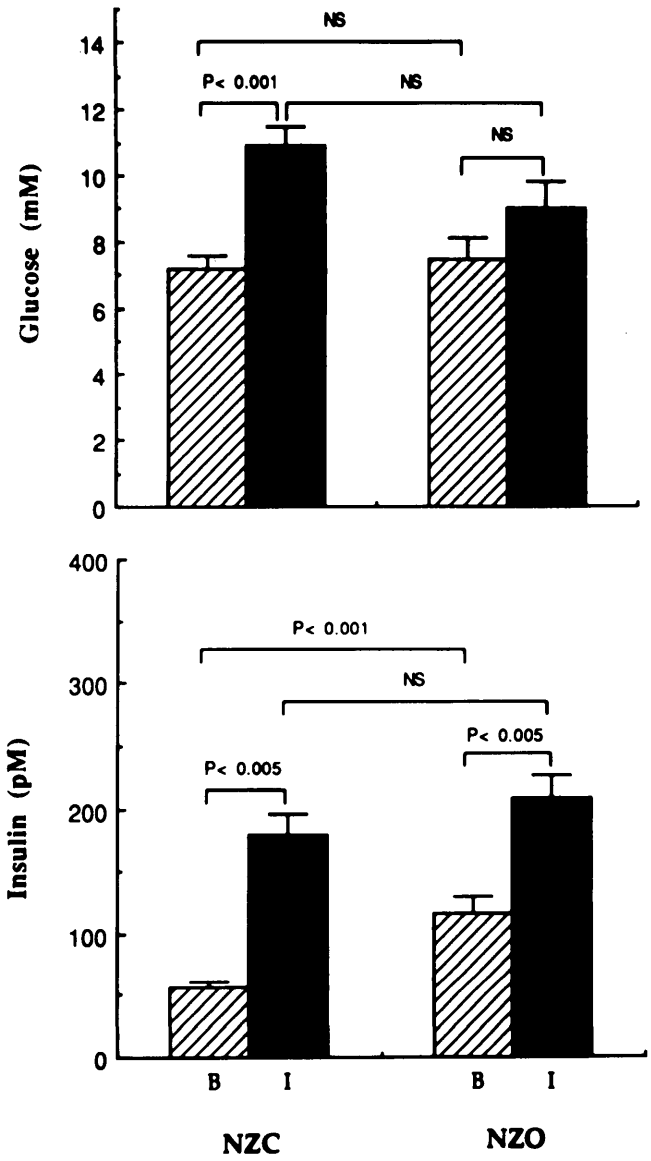

FIG. 1. Plasma glucose and insulin basally (B) and during insulin clamp (I) in 4- to 5-wk-old NZC and New Zealand obese (NZO) mice during 2-deoxy-glucose uptake study. Data are means ± SE. NZC, *n* = 9 basally and *n* = 11 during clamp; NZO, *n* = 9 basally and *n* = 12 during clamp.

the NZO mouse. Thus, not only was glucose uptake in individual tissue assessed in vivo, but for the first time, the evolution of hepatic insulin resistance and the development of defects in insulin secretion were investigated. The data from this study suggest that hepatic and skeletal muscle insulin resistance and impaired first-phase insulin secretion are all present as early as 4 wk of age. There were tissue differences in the pattern of insulin resistance. Thus, at 4 wk of age, no resistance could be demonstrated in white adipose tissue, whereas brown adipose tissue was profoundly insulin resistant. It is interesting to speculate on the role of this defect in brown adipose tissue in the development of obesity in these animals. Insulin resistance was not uniform in skeletal muscles, being evident in soleus, diaphragm, red quadriceps, and red gastrocnemius but not in heart and the fast-twitch white muscles. In the older mice, insulin action was assessed at a higher insulin concentration, and surprisingly, insulin unresponsiveness was only demonstrable in white and brown adipose tissue and soleus muscle. We cannot exclude the presence of impaired insulin action in other muscles at lower insulin concentrations. The higher 2-DG uptake by the diaphragms of the NZO mice (Table 3) was surprising and could possibly be due to increased work performed by the diaphragm in these very obese animals.

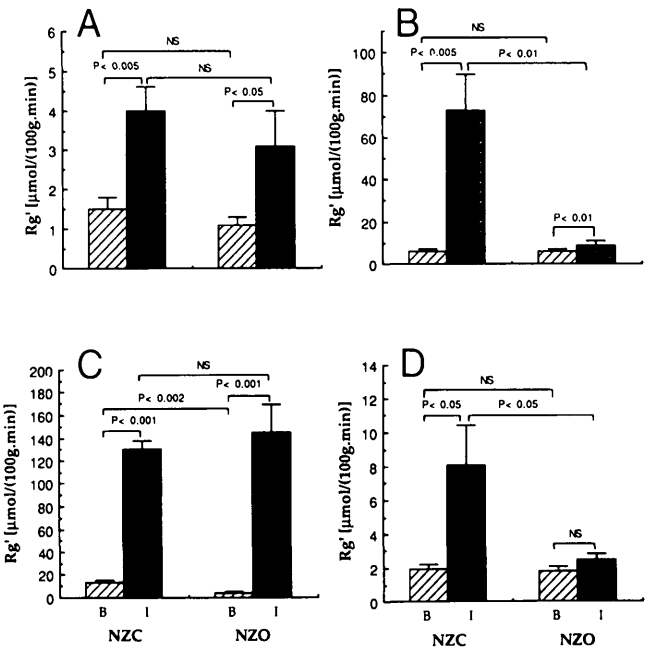

FIG. 2. Mean ± SE of glucose metabolic index (R_g' μmol · 100 $g^{-1} \cdot min^{-1}$) in 4- to 5-wk-old NZC and New Zealand obese (NZO) mice basally (B) and during insulin infusion (I). *A*, white adipose tissue; *B*, brown adipose tissue; *C*, heart; *D*, soleus.

The defect in the first-phase of insulin secretion has been well described in adult NZO mice (16). What we show herein for the first time is that this defect is present at 4–5 wk of age. In the older mice, both basal and stimulated insulin levels were significantly higher in the obese animals. The defect in first-phase insulin secretion is demonstrated only

TABLE 3
Glucose metabolic index in 4- to 5-wk-old NZC and New Zealand obese (NZO) mice

	NZC	NZO
Basal		
n	9	10
Diaphragm	3.4 ± 0.4	3.4 ± 0.9
Red quadriceps	2.3 ± 0.3	3.1 ± 0.5
White quadriceps	1.6 ± 0.1	2.4 ± 0.4
Red gastrocnemius	1.7 ± 0.2	2.3 ± 0.3
White gastrocnemius	1.3 ± 0.1	1.7 ± 0.3
Clamp		
n	11	12
Diaphragm	50.0 ± 3.7	33.5 ± 5.7*
Red quadriceps	10.8 ± 1.3	6.4 ± 0.6*
White quadriceps	4.6 ± 0.6	5.5 ± 0.6
Red gastrocnemius	10.3 ± 1.8	4.1 ± 0.4†
White gastrocnemius	3.2 ± 0.3	3.1 ± 0.4

Mean ± SE of glucose metabolic index (R_g'; μmol · 100 $g^{-1} \cdot min^{-1}$) in 4- to 5-wk-old NZO and NZC mice in diaphragm, red and white quadriceps, and red and white gastrocnemius muscles.
*$P < 0.05$, †$P < 0.01$, vs. NZC by Student's *t* test.

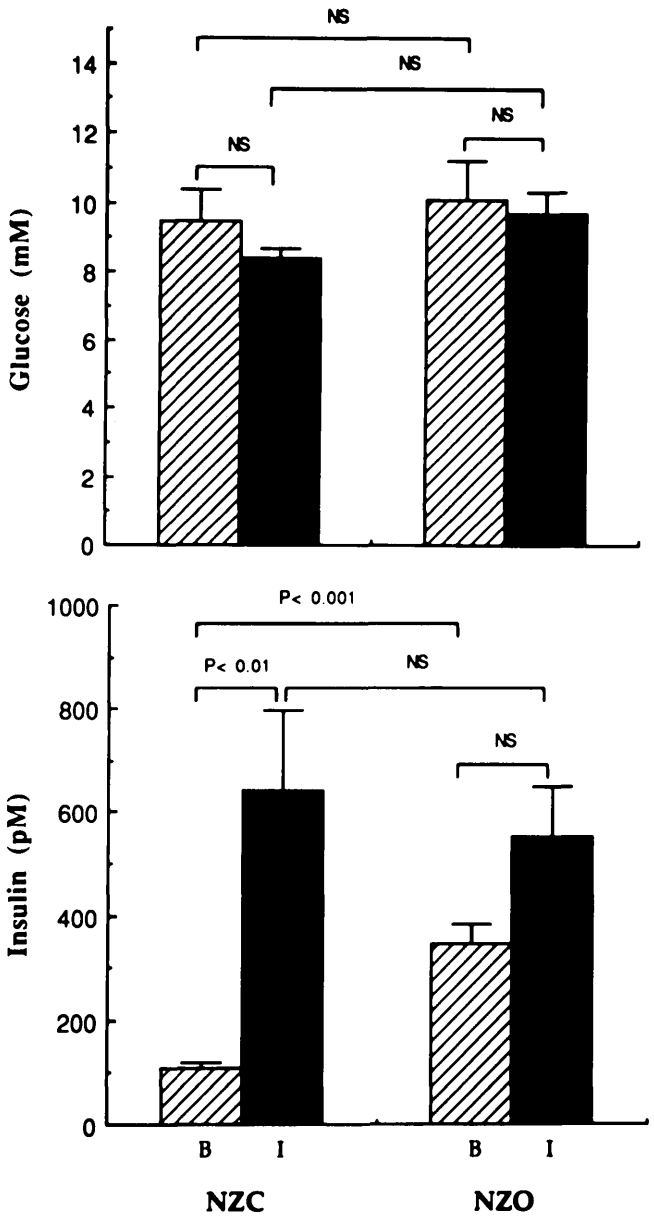

FIG. 3. Plasma glucose and insulin basally (B) and during insulin clamp (I) in 20-wk-old NZC and New Zealand obese (NZO) mice during 2-deoxyglucose uptake study. Data are means ± SE. NZC, *n* = 10 basally and *n* = 9 during clamp; NZO, *n* = 9 basally and *n* = 9 during clamp.

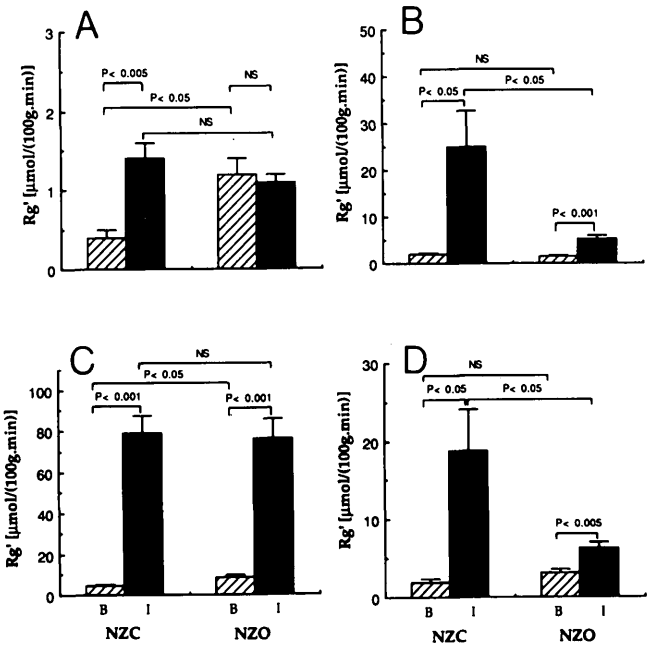

FIG. 4. Mean ± SE of glucose metabolic index (R_g'; µmol · 100 g^{-1} · min^{-1}) in 20-wk-old NZC and New Zealand obese (NZO) mice basally (B) and during insulin infusion (I). *A*, white adipose tissue; *B*, brown adipose tissue; *C*, heart; *D*, soleus.

when the data are expressed as percentage increase above basal. The conclusion that there is a defect in insulin secretion is only valid if it is assumed that the β-cells are not secreting at their full capacity in the basal state, i.e., that the higher basal insulin levels result from submaximal secretion from an expanded β-cell mass and not from maximal secretion from a reduced β-cell mass. There is evidence that β-cell mass is increased in the NZO mouse. For example, if insulin secretion is stimulated with arginine, there is substantial hypersecretion of insulin, suggesting an increased capacity (15). Furthermore, there is histological evidence that there is increased β-cell mass in NZO mice (14,30). Recent studies have shown that, in states of glucose intolerance, there is a reduction in the number of GLUT2 glucose transporters on β-cells (31,32). It is interesting to speculate whether the early defect in insulin secretion in NZO mice is also associated with a decrease in GLUT2 transporters.

In the adult mice, basal HGP was higher in the NZO compared with the NZC mice. During the clamp, the NZC mice suppressed completely, whereas the NZO mice continued to produce glucose, consistent with hepatic insulin resistance. This confirms the previous finding of Rudorff et al. (25), who showed that, when livers from adult mice were perfused in situ, hepatic glucose release could be modestly reduced by insulin in the control mice, but there was no decrease in

TABLE 4
Glucose metabolic index in 20-wk-old NZC and New Zealand obese (NZO) mice

	NZC	NZO
Basal		
n	10	9
Diaphragm	2.0 ± 0.4	5.3 ± 1.4*
Red quadriceps	1.8 ± 0.3	3.3 ± 0.7
White quadriceps	1.1 ± 0.1	2.8 ± 0.5†
Red gastrocnemius	1.4 ± 0.3	2.7 ± 0.8
White gastrocnemius	1.3 ± 0.2	3.2 ± 0.7*
Clamp		
n	9	9
Diaphragm	25.3 ± 3.7	44.1 ± 3.8‡
Red quadriceps	12.8 ± 2.8	8.4 ± 0.8
White quadriceps	9.9 ± 1.5	8.7 ± 0.8
Red gastrocnemius	6.5 ± 1.6	6.7 ± 0.6
White gastrocnemius	5.1 ± 0.9	6.2 ± 0.5

Mean ± SE glucose metabolic index (R_g'; µmol · 100 g^{-1} · min^{-1}) in 20-wk-old NZO and NZC mice in diaphragm, red and white quadriceps, and red and white gastrocnemius muscles.
*P = 0.05, †P < 0.01, ‡P < 0.005, vs. NZC by Student's t test.

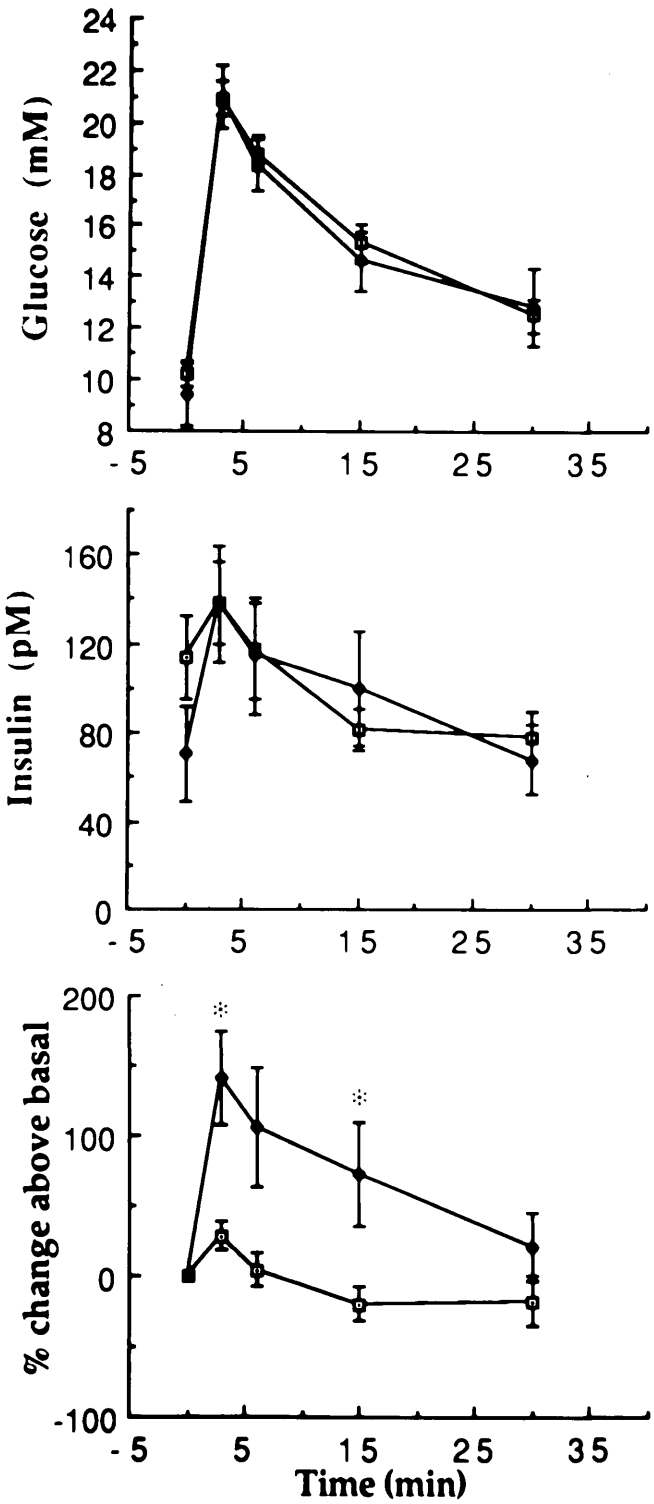

FIG. 5. Plasma glucose, insulin, and percent change in insulin above basal in 4- to 5-wk-old NZC (♦; n = 6) and New Zealand obese (NZO [□]; n = 8) mice during intravenous glucose tolerance test.

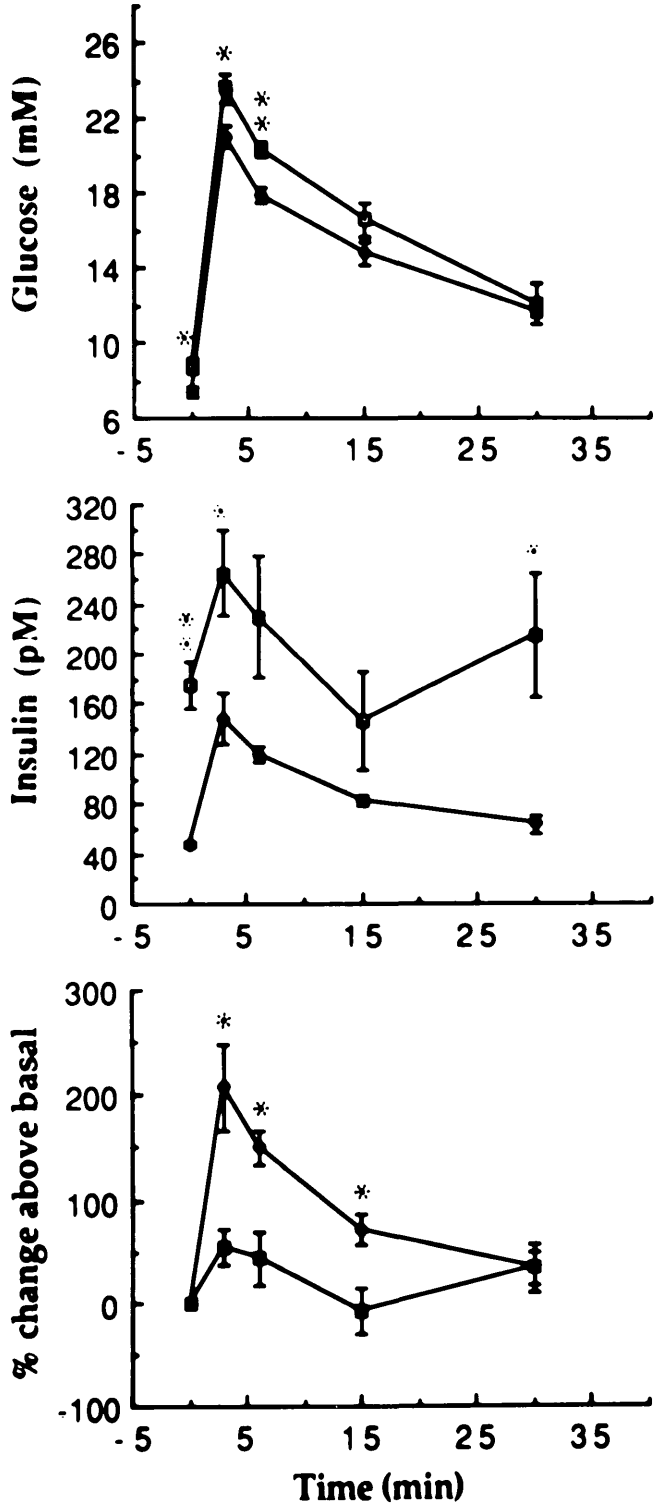

FIG. 6. Plasma glucose, insulin, and percent change in insulin above basal in 20-wk-old NZC (♦; n = 6) and New Zealand obese (NZO [□]; n = 7) mice during intravenous glucose tolerance test.

NZO mice. They concluded that adult NZO mice have hepatic insulin resistance. In this study, we showed that hepatic insulin resistance occurs early in these mice.

The aim of this study was to determine the initial event in the etiology of insulin resistance in the NZO mouse. It was thought that the syndrome could be caused by a single defect that secondarily induced the other two. The data presented herein clearly demonstrate that all three defects are present in the NZO mouse at 4–5 wk of age. This favors the view that all three defects are coinherited and are therefore present from birth. Given that increased HGP, reduced insulin-stimulated glucose uptake, and impaired first-phase insulin secretion cannot be explained by a single biochemical defect, it follows that the glucose intolerance in the NZO

mouse is probably due to at least two and possibly more unrelated inherited defects. For example, hepatic and muscle insulin resistance could be due to a defect in the insulin receptor. The defect in β-cell function must then be due to a separate unrelated abnormality resulting in impaired glucose recognition. Alternatively, there could be the same inherited defect of glucose transport and/or metabolism in peripheral tissues and β-cells resulting in insulin resistance in muscle and "glucose blindness" in β-cells and a separate defect in the regulation of hepatic glucose release.

However, although the data presented herein support the suggestion that there are multiple inherited defects in the NZO mouse, the possibility that there is a single primary defect cannot be definitively ruled out. An insulin secretion defect and peripheral insulin resistance can be rapidly induced by hyperglycemia (7,8). Thus, it is possible that, even at this very young age, secondary defects have already developed. There is some evidence for this view from data presented by Grundleger et al. (33) in *ob/ob* mice. These authors isolated soleus muscle from mice as young as 3 wk of age and measured 2-DG uptake in vitro. They found that there was no insulin resistance at 3 wk of age, but by 4 wk, impaired insulin response had already appeared, which became maximal by 6 wk.

In conclusion, hepatic glucose overproduction, muscle insulin resistance, and impaired first-phase insulin secretion are all early abnormalities in the NZO mouse.

ACKNOWLEDGMENTS

This work was supported by a program grant from the National Health and Medical Research Council of Australia. J.P. is a Wellcome Australia Senior Research Fellow.

REFERENCES

1. DeFronzo RA: Lilly Lecture 1987: the triumvirate: β-cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* 37:667–87, 1988
2. O'Rahilly SP, Nugent Z, Rudenski AS, Hosker JP, Burnett MA, Darling P, Turner RC: Beta cell dysfunction rather than insulin insensitivity is the primary defect in familial type 2 diabetes. *Lancet* 2:360–63, 1986
3. Swislocki ALM, Donner CC, Fraze E, Chen YDI, Reaven GM: Can insulin resistance exist as a primary defect in non-insulin-dependent diabetes mellitus? *J Clin Endocrinol Metab* 64:778–82, 1987
4. Proietto J: The aetiology of non-insulin dependent diabetes mellitus: is abnormal suppression of hepatic gluconeogenesis the primary event? *Med Hypotheses* 35:223–28, 1991
5. Unger RH, Grundy S: Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. *Diabetologia* 28:119–21, 1985
6. Rosetti L, Smith D, Shulman GI, Papachristou D, DeFronzo R: Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. *J Clin Invest* 79:1510–15, 1987
7. Garvey WT, Olefsky JM, Mathaei S, Marshall S: Glucose and insulin coregulate the glucose transport system in primary cultured adipocytes: a new mechanism of insulin resistance. *J Biol Chem* 262:189–97, 1987
8. Leahy JL, Cooper HE, Deal DA, Weir GC: Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion: a study in normal rats using chronic in vivo glucose infusions. *J Clin Invest* 77:908–15, 1986
9. Rosetti L, Shulman GI, Zawalich W, DeFronzo RA: Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomised rats. *J Clin Invest* 80:1037–44, 1987
10. Caruso G, Proietto J, Calenti A, Alford F: Insulin resistance in alloxan-diabetic dogs: evidence for reversal following insulin therapy. *Diabetologia* 25:273–79, 1983
11. Efendic E, Luft R: Aspects of the pathogenesis of type 2 diabetes. *Endocr Rev* 5:395–410, 1984
12. Lillioja S, Mott DM, Zawadzki JK, Young AA, Abbott WGH, Knowler WC, Bennett PH, Moll P, Bogardus C: In vivo insulin action is familial characteristic in nondiabetic Pima Indians. *Diabetes* 36:1329–35, 1987
13. Proietto J, Nankervis A, Traianedes K, Rosella J, O'Dea K: Identification of early metabolic defects in young people from a diabetes-prone Australian aboriginal community (Abstract). *Diabetes Res Clin Pract* 5:S623, 1988
14. Bielschowsky M, Bielschowsky F: A new strain of mice with hereditary obesity. *Proc Univ Otago Med Sch* 31:29–31, 1953
15. Larkins RG, Martin FIR: Selective defect in insulin release in one form of spontaneous laboratory diabetes. *Nature* (Lond) 235:86–88, 1972
16. Cameron DP, Opat F, Insch S: Studies of immunoreactive insulin secretion in NZO mice in vivo. *Diabetologia* 10:649–54, 1974
17. Stauffacher W, Crofford OB, Jeanrenaud B, Renold AE: Comparative studies of muscle and adipose tissue metabolism in lean and obese mice. *Ann NY Acad Sci* 131:528–40, 1965
18. De Fronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 273:E214–23, 1979
19. Kraegen EW, James DE, Jenkins AB, Chisholm DJ: Dose-response curves for in vivo insulin sensitivity in individual tissues in rats. *Am J Physiol* 248:E353–62, 1985
20. Ferre P, Leturque A, Burnol A-F, Penicaud L, Girard J: A method to quantify glucose utilization in vivo in skeletal muscle and white adipose tissue of the anaesthetised rat. *Biochem J* 228:103–10, 1985
21. Stauffacher W, Renold AE: Effect of insulin in vivo on diaphragm and adipose tissue of obese mice. *Am J Physiol* 216:98–105, 1969
22. Veroni MC, Larkins RG: Evolution of insulin resistance in isolated soleus muscle of the NZO mouse. *Horm Metab Res* 18:299–302, 1986
23. Upton JD, Sneyd JGT, Rennie PIC: Insulin resistance in the New Zealand obese mouse (NZO): lipolysis and lipogenesis in isolated adipocytes. *Arch Biochem Biophys* 197:139–48, 1979
24. Macaulay SL, Larkins RG: Impaired insulin action in adipocytes of New Zealand obese mice: a role for post-binding defects in pyruvate dehydrogenase and insulin mediator activity. *Metabolism* 37:958–65, 1988
25. Rudorff K-H, Huchzermeyer H, Windeck R, Staib W: Uber den Einflub von insulin auf die Alaningluconeogenese in der isoliert perfundierten Leber von New Zealand Obese Mice. *Eur J Biochem* 16:481–86, 1970
26. Larkins RG: Defective insulin secretion in the NZO mouse: in vitro studies. *Endocrinology* 93:1052–56, 1973
27. Larkins RG: Defective insulin secretory response to glucose in the New Zealand obese mouse: improvement with restricted diet. *Diabetes* 22:251–55, 1973
28. Larkins RG, Simenova L, Veroni MC: Glucose utilization in relation to insulin secretion in NZO and C57B1 mouse islets. *Endocrinology* 107:1634–38, 1980
29. Re CA, Veroni MC, Larkins RG: Effect of islet activating protein on glucose tolerance, insulin secretion, and insulin responsiveness in the NZO mouse. *Diabetologia* 26:304–309, 1984
30. Sneyd JGT: Pancreatic and serum insulin in the New Zealand strain of obese mice. *J Endocrinol* 28:163–72, 1964
31. Johnson JH, Ogawa A, Chen L, Orci L, Newgard CB, Alam T, Unger RH: Underexpression of β cell high K_m glucose transporters in noninsulin-dependent diabetes. *Science* 250:546–49, 1990
32. Unger RH: Diabetic hyperglycemia: link to impaired glucose transport in pancreatic β cells. *Science* 251:1200–205, 1991
33. Grundleger ML, Godbole VY, Thenen SW: Age-dependent development of insulin resistance of soleus muscle in genetically obese (*ob/ob*) mice. *Am J Physiol* 239:E363–71, 1980