Diet-Induced Glucose Intolerance in Mice With Decreased β-Cell ATP-Sensitive K⁺ Channels

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ATP-sensitive K⁺ channels (K<sub>ATP</sub> channels) control electrical activity in β-cells and therefore are key players in excitation-secretion coupling. Partial suppression of β-cell K<sub>ATP</sub> channels in transgenic (AAA) mice causes hypersecretion of insulin and enhanced glucose tolerance, whereas complete suppression of these channels in Kir6.2 knockout (KO) mice leads to hyperexcitability, but mild glucose intolerance. To test the interplay of hyperexcitability and dietary stress, we subjected AAA and KO mice to a high-fat diet. After 3 months on the diet, both AAA and KO mice converted to an undersecreting and markedly glucose-intolerant phenotype. Although Kir6.2 is expressed in multiple tissues, its primary functional consequence in both AAA and KO mice is enhanced β-cell electrical activity. The results of our study provide evidence that, when combined with dietary stress, this hyperexcitability is a causal diabetic factor. We propose an “inverse U” model for the response to enhanced β-cell excitability: the expected initial hypersecretion can progress to undersecretion and glucose-intolerance, either spontaneously or in response to dietary stress. Diabetes 53:3159–3167, 2004

Type 2 diabetes arises from the interaction of multiple environmental and genetic factors. A high-fat diet and the consequent obesity are primary causal factors in the development of hyperinsulinemia and the insulin resistance that precedes type 2 diabetes in genetically predisposed individuals (1–4). Increased levels of cytoplasmic ATP/ADP (resulting from β-cell glucose metabolism) cause closure of ATP-sensitive K⁺ channels (K<sub>ATP</sub> channels), depolarization, and voltage-dependent Ca<sup>2⁺</sup> entry, which triggers insulin secretion (5). Persistent hyperinsulinemic hypoglycemia of infancy (PHHI), most frequently caused by loss-of-function mutations in one of the β-cell K<sub>ATP</sub> channel subunits (SUR1, Kir6.2) (6), illustrates the importance of electrical control. In β-cells from some PHHI patients, a complete lack of K<sub>ATP</sub> channel activity has been reported (7), but the electrophysiological phenotype of many PHHI mutations (6,8,9) suggests that K<sub>ATP</sub> channels may not always be completely absent (10); in fact, active K<sub>ATP</sub> channels have been reported in β-cells from some PHHI patients (11).

Mice lacking Kir6.2 or SUR1 (12–14), as well as mice expressing β-cell dominant-negative Kir6.2 mutant transgenes (Kir6.2[AAA] or Kir6.2[GI32S]) (15,16) have been generated. As is seen in human PHHI, reduced or absent K<sub>ATP</sub> channel activity should result in constitutive depolarization, elevated intracellular Ca<sup>2⁺</sup>, and hypersecretion of insulin (9). SUR1 and Kir6.2 knockout (KO) mice (12–14), which lack K<sub>ATP</sub> channels in multiple tissues, and Kir6.2[GI32S] mice, which specifically lack β-cell K<sub>ATP</sub> channels (16), show a complex phenotype. Transient hyperinsulinemia and hypoglycemia are present in these mice as neonates, but original studies have reported reduced glucose-stimulated insulin secretion (GSIS) and glucose intolerance in the mice as adults (12–14). More recent studies have indicated that KO mice can maintain and even hypersecrete insulin at low glucose (17,18) in the presence of leucine and glutamine, or cholinergic stimulation, reiterating findings in some PHHI cases (19) where a high-protein meal can induce hypoglycemia. β-cell–specific Kir6.2[AAA] dominant-negative mice demonstrating an apparently complete loss of K<sub>ATP</sub> channels in ~70% of β-cells and normal channel density elsewhere (15), exhibit enhanced GSIS, with elevated circulating insulin levels that persist through adulthood.

Many PHHI patients eventually cross over to a diabetic phenotype. This development may be generally attributable to the near total pancreatectomy required to treat the neonatal symptoms (20), but some nonsurgically treated PHHI patients also become diabetic (9,21). It is conceivable that β-cell death or other islet remodeling may occur in these patients, although this interpretation has been questioned (19), as the progression to diabetes is clearly not inevitable. There have been no studies to date examining the response of K<sub>ATP</sub> channel–deficient mice to a high-fat diet or examining the interplay of these genetic and environmental stresses. In the present study, we examined the hypothesis that hyperexcitability and hypersecretion in AAA and Kir6.2<sup>−/−</sup> mice is protective against glucose intolerance induced by a high-fat diet, as is the case for uncoupling protein-2 knockout mice (22). Our results ran counter to this hypothesis, and instead demonstrated that both AAA (K<sub>ATP</sub> channel–suppressed) and

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DMEM, Dulbecco’s modified Eagle’s medium; GFP, green fluorescent protein; GSIS, glucose-stimulated insulin secretion; IPGTT, intraperitoneal glucose tolerance test; K<sub>ATP</sub> channel, ATP-sensitive K⁺ channel; PHHI, persistent hyperinsulinemic hypoglycemia of infancy.

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Kir6.2<sup>−/−</sup> (K<sub>ATP</sub> channel—absent) mice progress to a reversibly undersecreting, diabetic phenotype. The data are consistent with an “inverse U” model (4) of response to β-cell hyperexcitability and provide evidence for a cooperative role of genetic and environmental factors in this response.

**RESEARCH DESIGN AND METHODS**

**Generation of transgenic mice.** Hemizygous Kir6.2[AAA] mice (23) from generations >F5 (backcrossed to C57BL/6) were bred to Kir6.2<sup>−/−</sup> mice (14). Heterozygous Kir6.2<sup>−/−</sup>[AAA] mice (from F1) were bred back to KO mice to generate the following genotypes: Kir6.2<sup>−/−</sup> (Ctrl), Kir6.2<sup>−/−</sup>[AAA] (A), Kir6.2<sup>−/−</sup> (KO), and Kir6.2<sup>−/−</sup>[AAA] (KO[AAA]) mice (Fig. 1A).

Littermates from these backcrosses were used in the study. Mice were typed using primers against the green fluorescent protein (GFP) tag (23), or neomycin-resistant gene for transmission of Kir6.2[AAA] and Kir6.2 gene disruption, respectively (14). Immediately after they were weaned (day 21), mice were placed on a normal diet (21% of calories provided by fat; Pico-Vac Mouse Diet 20; Roche) or a high-fat diet (42% of calories provided by fat; Harlan Teklad, Madison, WI) for 3–4 months.

**Insulin release experiments.** After being incubated overnight in low-glucose (5 mmol/l) Dulbecco’s modified Eagle’s medium (DMEM), islets (10 per well in 12-well plates) were preincubated in glucose-free DMEM plus 3 mmol/l glucose, then incubated in DMEM plus glucose, as indicated. Islets were incubated for 60 min at 37°C. The medium was removed and assayed for glucose content using the Glucometer Elite XL (Bayer, Elkhart, IN). Insulin was measured using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL).

**Blood glucose and plasma insulin measurements.** Tail blood was assayed for glucose content using the Glucometer Elite XL (Bayer, Elkhart, IN). Insulin was measured using the rat insulin radioimmunoassay kit according to the manufacturer’s procedures (Linco, St. Charles, MO). Intraperitoneal glucose tolerance tests (IPGTTs) were administered (after a 16-h fast) 4, 8, and 12 weeks after mice were weaned onto a normal or high-fat diet. Animals were injected intraperitoneally with glucose (1.5 g/kg body wt). Plasma insulin was measured using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL).

**Islet isolation.** All experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by the Washington University Animal Studies Committee. Mice were anesthetized with methoxyflurane (Metofane; 0.2 ml) in an anesthetizing chamber and killed by cervical dislocation. Their pancreases were removed and injected with Hank’s balanced salt solution (Sigma, St. Louis, MO) containing collagenase (0.5 mg/ml; pH 7.4) (23). Collagenase type XI was obtained from Sigma. Pancreases were digested for 8 min at 37°C; hand shaken, and washed three times in cold Hank’s solution (15). Islets were isolated by hand with the aid of a dissecting microscope and pooled.

**Immunohistological analysis.** Pancreases were fixed in Streck tissue fixatives (Streck Laboratories, Omaha, NE) overnight and embedded in paraffin for serial sectioning (5-μm thick sections). Hematoxylin-eosin staining was carried out as previously described (23). For immunofluorescence, sections were incubated overnight at 4°C with guinea pig anti-insulin or anti-glucagon primary antibodies (1:250 or 1:500, respectively; Linco). Primary antibodies were incubated for 60 min at 37°C. The medium was removed and assayed for glucose content using the Glucometer Elite XL (Bayer, Elkhart, IN). Insulin was measured using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL).
were detected by incubating sections for 1.5 h at 25°C with an anti–guinea pig secondary antibody conjugated with the AlexaTM 488 fluorescent dye (Molecular Probes, Eugene, OR).

**Statistics.** Data are presented as means ± SE. Differences between the normal and high-fat diets for the same genotype were compared using a nonpaired Student’s t test. Differences among the four genotypes were tested using ANOVA and a post hoc Duncan’s test. P < 0.05 was considered significant. Nonsignificant differences are not indicated.

**RESULTS**

**Abrogation of β-cell Kir6.2[AAA] phenotype in the Kir6.2 knockout background: hypersecretion in Kir6.2[AAA] is a consequence of incomplete suppression of ATP-sensitive potassium channel.** Mice expressing dominant-negative Kir6.2[AAA] (15) under rat insulin promoter-1 control are expected to show a primarily β-cell–specific expression of the transgene. These mice hypersecrete insulin as adults, in contrast to Kir6.2 and SUR1 KO animals (12–14). We previously postulated that hypersecretion depends on the incomplete suppression of β-cell K<sub>ATP</sub> channel activity in the former (15). One critical test is to express the Kir6.2[AAA] transgene in Kir6.2<sup>-/-</sup> mice. With no K<sub>ATP</sub> channel activity to suppress, the phenotype should reiterate that of Kir6.2<sup>-/-</sup> mice. We directly tested this prediction by comparing littermates from backcrossed animals (Fig. 1A; RESEARCH DESIGN AND METHODS). Although 4 month-old AAA mice showed enhanced glucose tolerance and elevated GSIS (Figs. 2E and 5A), KO[AAA] and KO mice were indistinguishable, with both genotypes showing reduced glucose tolerance and only baseline insulin secretion. With a normal diet (Fig. 1C), plasma insulin was elevated in AAA compared with control mice, whereas basal plasma insulin levels were maintained in both KO and KO[AAA] mice. It thus may be concluded that hypersecretion in Kir6.2[AAA] mice is indeed a consequence of incomplete suppression of K<sub>ATP</sub> channels.

**High-fat feeding causes relative insulin resistance and converts AAA mice from hyperinsulinemic to relatively hypoinsulinemic phenotype.** It is conceivable that the hyperexcitability in K<sub>ATP</sub> channel–suppressed mice could underlie a hypersecretory response that may be protective against stress-induced β-cell failure. On the other hand, the glucose intolerance that develops in mice completely lacking K<sub>ATP</sub> channels (14) may indicate that the hyperexcitability underlies a susceptibility to β-cell failure. Because a major environmental factor in the development of glucose intolerance is diet, we examined the consequences of long-term fat feeding on the above four genotypes. Litters expressing all four genotypes (control, AAA, KO, and KO[AAA]) were weaned onto a normal or high-fat diet (see RESEARCH DESIGN AND METHODS) and maintained on these diets for 3–4 months.

All high-fat diet mice became progressively heavier than their normal diet littermates, but without obvious differences between genotypes (Fig. 1B). In mice on a normal diet under fed conditions, hyperinsulinism was evident in the AAA group and glucose was elevated in the KO and KO[AAA] groups; these observations were consistent with the previously described phenotypes of Kir6.2<sup>-/-</sup> (14) and Kir6.2[AAA] mice (15). Mice maintained on a high-fat diet for 3 months showed striking differences in plasma insulin and glucose. Fed blood glucose was maintained at a constant level (~8 mmol/l) in control mice only, which achieved this with an approximate threefold increase in plasma insulin (Fig. 1C and D). AAA mice showed a trend toward higher blood glucose, KO and KO[AAA] mice showed significantly increased blood glucose relative to control mice (Fig. 1C, right), and all three failed to elevate plasma insulin (Fig. 1C, left). This failure to hypersecrete in the face of peripheral insulin resistance (see below) in each of these K<sub>ATP</sub> channel–reduced or –absent mice was marked, with AAA mice dramatically crossing over from hyperinsulinemia to relative hypoinsulinemia (Fig. 1C, left). As noted below, this resulted from a switch from a hypersecreting to an undersecreting β-cell phenotype within a short time of fat feeding.

After an overnight fast, blood glucose was not different between genotypes in the normal or high-fat diet groups, but all genotypes were significantly elevated on the high-fat diet (Fig. 1D). To characterize physiological responses to glucose load, mice underwent an IPGTT after an overnight fast. In the normal diet mice, blood glucose was higher at every time point in KO and KO[AAA] groups than in the control group, but was lower at every time point in the AAA group (Fig. 2A, C, and E); these observations are consistent with previous findings (14,15). With a high-fat diet, KO and KO[AAA] mice showed markedly decreased glucose tolerance by 3 months (Fig. 2B, D, and F). Despite the initially hyperinsulinemic phenotype, AAA mice on the high-fat diet showed a dramatic change in their response to glucose loading, becoming progressively and profoundly glucose intolerant. Within 12 weeks of being on the high-fat diet, AAA mice were as glucose intolerant as the KO and KO[AAA] mice (Fig. 2F).

Glucose-dependent insulin secretion in vivo was assessed in plasma insulin 30 min after glucose challenge. Insulin secretion was augmented in control mice on the high-fat diet, but mice in the AAA, KO, and KO[AAA] groups showed augmentation in plasma insulin (Fig. 3). One striking observation was that the marked increase in plasma insulin observed in the normal diet AAA group was abolished after the high-fat diet. Consistent with absent insulin responses, glucose was significantly elevated 30 min after glucose administration in KO and KO[AAA] mice on either the normal or high-fat diet, as well as in AAA mice on the high-fat diet (Fig. 3). The glucose-lowering effect of intraperitoneal insulin was not significantly different between control and AAA mice on the normal diet, and was reduced in both after 3 months on the high-fat diet (Fig. 4), a result consistent with the expected insulin-desensitizing effect of high blood lipids (3). Although KO and KO[AAA] animals showed an insulin sensitivity similar to that of control mice on the normal diet, they both became significantly more insulin-sensitive (24) after the high-fat diet (Fig. 4).

**High-fat feeding switches AAA islets from hypersecretion to undersecretion.** GSIS was assessed in islets isolated from normal and high-fat diet mice. Basal insulin secretion from KO and KO[AAA] islets from normal diet mice was lower than that from control islets, and these KO islets showed no GSIS, as previously described (14) (Fig. 5A). Isolated islets from normal diet AAA mice displayed increased GSIS (Fig. 5A), likely accounting for the significantly elevated serum insulin levels and enhanced glucose tolerance in these mice (15). However, consistent with the
marked loss of glucose tolerance described above, the GSIS of islets from high-fat diet AAA mice was significantly reduced compared with in control mice (Fig. 5B). After 16 weeks on the high-fat diet, the secretory capacity of AAA islets was not significantly different from that of KO and KO(AAA) islets (Fig. 5B).

The insulin content of isolated islets was measured in parallel with insulin secretion from non-glucose-challenged islets (50–100 islets, in replicates of 10, from each animal) (Fig. 5C). With the normal diet, the insulin content was significantly higher in AAA (15) relative to control and KO islets. With the high-fat diet, insulin content of AAA islets decreased to a level similar to that in control and KO islets (Fig. 5C).

**Reversibility of undersecretory phenotype after diet reversal.** Mice were maintained on a high-fat diet for up to 6 months. As shown in Fig. 6A, the glucose-intolerant phenotype persisted throughout this period. When the mice were switched back to normal diet for 3 months, there was a gradual reversal of the glucose intolerance in all genotypes (Fig. 6B and C), with a more dramatic reversal in AAA compared with KO or KO(AAA) mice.

**Morphology of islets is not affected by high-fat diet.** Immunohistochemical analyses were performed on sectioned pancreases from mice age 3–4 months. Hematoxylin-eosin staining revealed normal islet appearance in the control, AAA, and KO genotypes on either diet, and immunostaining for

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**FIG. 2.** IPGTT after normal (ND; A, C, and E) or high-fat diet (HFD; B, D, and F) for 4, 8, and 12 weeks, shown as blood glucose versus time postinjection of 1.5 g/kg glucose. Data represent means ± SE, n = 5–6. *P < 0.05 for AAA, KO, or KO(AAA) vs. control (Ctrl); ‡P < 0.05 for control vs. AAA, KO, and KO(AAA).
insulin and glucagon confirmed the relatively normal distribution of insulin-containing β-cells and glucagon-containing α-cells (Fig. 7A and B). Significant redistribution of α-cells and qualitative loss of β-cell mass (as defined by reduced immunofluorescence) was not observed, although a slight tendency for α-cell infiltration may be evident in KO mice on a normal or high-fat diet, as previously reported (14). To examine the expression of the Kir6.2[AAA]-GFP protein, islets were isolated from AAA mice after 3 months on a normal or high-fat diet. As shown in Fig. 7C, there was no obvious difference in the level or pattern of expression between diets. Green fluorescence within each islet indicated incomplete penetrance of the Kir6.2[AAA]-GFP transgene. As we reported previously, ∼70% of β-cells express the GFP-tagged transgene at visibly high levels, but ∼30% showed no detectable fluorescence, with the same pattern being observed with a normal or high-fat diet.

**DISCUSSION**

Incomplete suppression of K<sub>ATP</sub> channels causes hypersecretion, complete suppression causes undersecretion. The significance of membrane potential control of insulin secretion is underlined by the finding that most cases of PHHII result from a defect in K<sub>ATP</sub> channels (9). Mice expressing the dominant-negative Kir6.2[AAA] transgene primarily in β-cells demonstrate enhanced GSIS and a hyperinsulinism that persists through adulthood (15). In these mice, K<sub>ATP</sub> channels are absent from ∼70% of β-cells, but are present at normal levels elsewhere (15). The initial studies of mice completely lacking K<sub>ATP</sub> channels reported hypersecretion of insulin after birth, but a rapid loss of secretory capacity (12–14, 16). More recent studies (17, 18, 25) have produced contradictory results, even demonstrating a basal hypersecretion and main-
tained elevation in high glucose. A full explanation for these divergent results is yet to be found, but there is now clear evidence that glutamine and other amino acids can potently stimulate secretion in these KO islets (18,25). In the present study, KO islets in normal diet clearly under-secrete compared with littermate controls, and both KO and AAA mice progress to a further loss of secretory capacity on a high-fat diet. We suggest that in both cases, the combination of dietary stress and the preexisting hyperexcitability combine to drive a progression to under-secretion, an idea that has been previously suggested (4,26). In contrast, control islets respond to the diet-induced insulin insensitivity by hypersecreting, remaining below the threshold combination that drives the KATP channel−deficient islets to the undersecreting phenotype.

As discussed below, we suggest that genetic suppression of KATP channels leads to enhanced excitability and insulin secretion, but with potentially different time courses and consequences depending on severity. With complete loss of KATP channel activity (i.e., in Kir6.2−/− and SUR−/− mice), hypersecretion occurs immediately and progresses to a relative undersecretion (13,14). Although recent studies question this outcome for SUR1−/− islets (above; 25), there is clearly undersecretion and glucose insensitivity in Kir6.2−/− mice (14), whereas partial loss of KATP channels (in Kir6.2[AAA] mice) leads to later onset maintained hypersecretion (15) and, without

FIG. 6. IPGTT after reversion from high-fat diet (HFD) for 24 weeks (A) to normal diet (ND) for 4 (B) or 12 (C) weeks. Blood glucose versus time after injection of 1.5 g/kg glucose. Data represent means ± SE, n = 5–6; *P < 0.05 for control vs. KO and KO(AAA); †P < 0.05 for control vs. AAA, KO, and KO(AAA).

FIG. 7. A and B, top: Representative hematoxylin-eosin staining of pancreatic sections from control, AAA, or KO mice on normal (A) or high-fat (B) diet (12–16 weeks). Bottom: Representative immunofluorescence of consecutive sections of control, AAA, or KO pancreas on normal (A) or high-fat (B) diet incubated with anti-insulin or anti-glucagon antibodies. C: Confocal fluorescent images of isolated AAA islets at low magnification (left) and individual islets at higher magnification (right).
dietary stress, fails to progress to undersecretion. One important caveat to the latter interpretation is the possibility that the Kir6.2[AAA] phenotype could be due to some aberrant protein expression per se, rather than to suppression of $K_{\text{ATP}}$ channels. In the present study, we thus directly tested these possibilities by crossing the Kir6.2[AAA] transgene onto the Kir6.2$^{-/-}$ background. The phenotypes of KO(AAA) and littermate KO mice are indistinguishable. Because there is no $K_{\text{ATP}}$ channel activity to suppress in the KO background, we can reasonably conclude that hypersecretion in AAA mice is indeed a result of the only partial suppression of $K_{\text{ATP}}$ channel activity.

**Glucose homeostasis in response to a high-fat diet.**

Having bred the Kir6.2[AAA] transgene onto the Kir6.2$^{-/-}$ background, we had available littermate mice that show three distinct phenotypes—normal insulin secretion (control), hypersecretion of insulin (AAA), or undersecretion of insulin (KO, KO[AAA])—on a normal diet, solely due to differences in their Kir6.2 genotype. These mice then allowed us to test the consequences of dietary or other stresses on these differing secretory phenotypes. A high-fat diet generally results in peripheral insulin resistance and diet-induced hyperinsulinemia as a compensatory response (27). It has been demonstrated, both in vivo (28,29) and in vitro (30–32), that fatty acids can acutely augment GSIS. When subjected to a high-fat diet, control mice maintained normal blood glucose, with a compensatory increase in plasma insulin. In sharp contrast, AAA mice (which otherwise show hyperinsulinemia and euglycemia that persist to adulthood) (15) became severely hypoinsulinemic and hyperglycemic on a high-fat diet. KO and KO(AAA) animals, already slightly glucose intolerant on a normal diet, rapidly became more severely intolerant on a high-fat diet. Previous studies (24) have indicated the relative insulin hypersensitivity of Kir6.2$^{-/-}$ mice on a normal diet, but this was not observed in the present study (Fig. 4A). However, after a high-fat diet, these animals maintained greater insulin sensitivity than littermate control or AAA mice (Fig. 4B). Therefore, the high-fat diet–induced glucose intolerance in KO as well as in AAA animals can be explained by a reduced secretory capacity, rather than exacerbated insulin insensitivity.

**Progression from β-cell hypersecretion to undersecretion: a causal diabetic progression?** Decreased insulin secretion in type 2 diabetes may be preceded by stages of hypersecretion, and hyperinsulinemia can be present in nonaffected close relatives of type 2 diabetic individuals (33–35). A Finnish study demonstrated increased insulin secretion in subjects with impaired glucose tolerance, followed by a progressive decline in insulin levels as subjects became frankly diabetic (36). Similarly, members of a family with defective $K_{\text{ATP}}$ channel activity showed hyperinsulinemia when they were young, but developed diabetes with normal insulin sensitivity as adults (37). A mutation in the glucokinase gene that leads to enhanced β-cell glucokinase activity and elevated ATP:ADP ratio (with consequent suppression of $K_{\text{ATP}}$ activity) (38) has been described in another PHHII family (39). One interesting result was that one affected member subsequently developed diabetes, a finding consistent with the notion that β-cell hyperexcitability, and the resultant hyperinsulinemia, can exacerbate or precipitate diabetes.

The striking conversion of the AAA phenotype on a high-fat diet illustrated a detrimental effect of a long-term high-fat diet on β-cell function, reminiscent of the impaired glucose tolerance and loss of insulin response that follows early hypersecretion in type 2 diabetes (40–42). In type 2 diabetes, there is typically a gradual loss of secretory function. In many patients, sulfonylureas can control glycemia for an extended period, but over the long term (months to years), sulfonylurea therapies typically fail (43). Animal studies also provide clear evidence that long-term sulfonylurea treatment leads to impaired glucose tolerance and GSIS (44–46). Intriguing results from one clinical study (47) have indicated that nonobese type 2 diabetic patients with secondary failure to sulfonylureas regained significant β-cell secretory activity after 3 months of intensive insulin therapy. In these patients, therefore, control of blood glucose with insulin restored secretory capacity, a result paralleling our observation that removal of a high-fat diet leads to recovery of secretion and glycemic control in AAA and KO mice.

When combined with previous results from $K_{\text{ATP}}$ channel KO and transgenic animals, a consistent picture emerges: primary hypersecretion resulting from enhanced β-cell excitability (due to genetic depletion of $K_{\text{ATP}}$ channels or chronic sulfonylurea treatment) progresses to loss of glucose sensitivity and undersecretion, either spontaneously (13,14,16) or in response to dietary stress (this study). AAA mice, harboring a predisposing genetic defect, but not otherwise progressing to a diabetic state (15), show rapid progression to marked glucose intolerance when exposed to a high-fat diet. Those mice provide a dramatic demonstration of how a single β-cell defect can cause hyperexcitability and hypersecretion in normal conditions, but loss of secretory capacity, diabetes, and hyperglycemia when stressed (48).

At this juncture, we can only speculate on the underlying mechanism of the loss of secretory capacity. Hematoxycin-eosin staining of sectioned pancreases indicated normal islet size and distribution in all genotypes on both the normal and the high-fat diet (Fig. 7). The glucose insensitivity was at least partially reversible after reversion to a normal diet (Fig. 6), which may indicate a metabolic, rather than structural, change. Immunostaining for insulin and glucagon revealed abnormal appearance of α-cells among the β-cell core in some cases, as previously observed in adult Kir6.2$^{-/-}$ islets (14), but no consistent changes in cell distribution in any genotype were seen after the high-fat diet (Fig. 7). Total insulin content was reduced in KO/KO(AAA) and particularly in AAA mice on a high-fat diet (Fig. 5C). If it is assumed that releasable insulin reflects the total content, then the loss of total content is likely to be a major contributor to the decreased insulin secretion from the AAA genotype in high-fat diet.

**“Inverse U” model for β-cell response to hyperexcitability.** The present results, showing marked loss of glucose tolerance in KO and AAA mice after a high-fat diet, provide a model for understanding the progression from hyperinsulinemia to hyperglycemia. We suggest that this “inverse U” model (4) (Fig. 8) might be a general β-cell response to hyperexcitability, whether generated by alter-
progression to undersecretion ensues. by diet-induced elevation of metabolic substrates, a rapid 25). AAA mice do not normally reach this trigger (15), but secretion and are glucose intolerant (12–14; however, see reached spontaneously, so that adult mice have only basal 100% decrease of K_{ATP}). Hypersercreting 100% decrease of K_{ATP} hypotheses (solid line) because of maximally enhanced excitability and also progress to undersecretion after a high-fat diet (dashed line).

FIG. 8. Proposed “inverse-U” model for response to β-cell hyperexcitability. Control islets (red) secrete normally, but after a high-fat diet (dashed line) progress to hypersecretion. During neonatal development, AAA mice (~70% decrease of K_{ATP}; green) (15) progress spontaneously (solid line) to a hypersecreting relationship. High-fat diet causes further enhancement of excitability, beyond the threshold that drives AAA islets “over the top” (dashed line) to undersecretion. KO islets (100% decrease of K_{ATP}) hypersecreta as neonates (solid line) because of maximally enhanced excitability and also progress to undersecretion after a high-fat diet (dashed line).

ations of K^{+} conductance or other electrical players. Initially, hyperexcitability will lead to enhanced insulin secretion at any blood glucose (49). The enhancement may be graded with the degree of hyperexcitability and likely be maximal for K_{ATP} channel–KO animals. Accordingly, hypersecretion is evident in neonatal K_{ATP} channel–KO mice (13) as well as in adult AAA (15) and perhaps even adult SUR1^{-/-} (25) mice. In normal mice, K_{ATP} activity is actually of lesser importance to the control of secretion in fetal and possibly early neonatal animals (50), and we hypothesize that in AAA mice (with only a partial loss of K_{ATP} channels), hypersecretion becomes evident only during the neonatal period (15,23). We propose that above a certain threshold, enhanced excitability triggers consequent failure of glucose sensing and secretion (48). The mechanisms for this are yet to be fully defined, although elevated intracellular [Ca^{2+}] (14,15) is an obvious candidate. In K_{ATP} channel–KO animals, this trigger may be reached spontaneously, so that adult mice have only basal secretion and are glucose intolerant (12–14; however, see 25). AAA mice do not normally reach this trigger (15), but when excitability is further enhanced, in the present case by diet-induced elevation of metabolic substrates, a rapid progression to undersecretion ensues.

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