

β -Cell Glucokinase Deficiency and Hyperglycemia Are Associated With Reduced Islet Amyloid Deposition in a Mouse Model of Type 2 Diabetes

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Type 2 diabetes is characterized by impaired β -cell function, hyperglycemia, and islet amyloid deposition. The primary constituent of islet amyloid is the 37-amino acid β -cell product called islet amyloid polypeptide (IAPP) or amylin. To study mechanisms of islet amyloid formation, we developed a transgenic mouse model that produces and secretes the amyloidogenic human IAPP (hIAPP) molecule and have shown that 81% of male transgenic mice develop islet amyloid after 14 months on a high-fat diet. To test whether impaired β -cell function and hyperglycemia could enhance islet amyloid formation, we cross-bred our hIAPP transgenic mice with β -cell glucokinase-knockout mice (GKKO) that have impaired glucose-mediated insulin secretion and fasting hyperglycemia. The resulting new (hIAPP \times GKKO) line of mice had higher basal plasma glucose concentrations than the hIAPP transgenic mice at 3, 6, and 12 months of age ($P < 0.05$), as did GKKO mice compared with hIAPP transgenic mice at 6 and 12 months of age ($P < 0.05$). Basal plasma immunoreactive insulin (IRI) levels were lower in hIAPP \times GKKO mice than in hIAPP transgenic mice at 6 months of age ($P < 0.05$). The area under the glucose curve in response to an intraperitoneal glucose challenge (1 g/kg body weight) was larger in hIAPP \times GKKO mice than in hIAPP transgenic mice at 3, 6, and 12 months of age ($P < 0.005$) and in GKKO mice compared with hIAPP transgenic mice at 6 and 12 months of age ($P < 0.005$). The area under the IRI curve was lower in hIAPP \times GKKO mice at 6 and 12 months of age ($P < 0.05$) than in hIAPP transgenic mice and in GKKO mice compared with hIAPP transgenic mice at 12 months of age ($P < 0.05$). Despite the presence of hyperglycemia, hIAPP \times GKKO mice had a lower incidence (4 of 17 vs. 12 of 19, $P < 0.05$) and amount (0.40 ± 0.24 vs. 1.2 ± 0.3 arbitrary units, $P < 0.05$) of islet amyloid than hIAPP transgenic mice had. As expected, no islet amyloid was

observed in GKKO mice lacking the hIAPP transgene (0 of 13). There was no difference in pancreatic content of IRI and hIAPP among the three groups of mice. Thus, despite the presence of impaired islet function and hyperglycemia, hIAPP \times GKKO mice had a decreased incidence and quantity of islet amyloid. Therefore, our data suggest that impaired β -cell glucose metabolism or hyperglycemia are not likely to contribute to islet amyloid formation in diabetes. Furthermore, this finding may explain the lack of progression of glycemia in patients with maturity-onset diabetes of the young. *Diabetes* 49:2056–2062, 2000

Islet amyloid deposition is a pathological feature of the pancreas in type 2 diabetes that is associated with reduced β -cell mass and insulin secretion (1). The unique peptide component of islet amyloid is islet amyloid polypeptide (IAPP) or amylin (2,3), a normal secretory product of the islet β -cell released with insulin in response to nutrient stimuli such as glucose and amino acids (4,5). The human IAPP (hIAPP) molecule contains a specific sequence of amino acids rendering it capable of forming amyloid fibrils (6). This amyloidogenic sequence is present in a number of species that develop islet amyloid (e.g., humans, primates, and cats) but is absent in rodents, thus explaining the lack of islet amyloid in diabetic rats and mice. Although this sequence is essential, it is not the sole factor responsible for islet amyloid formation, since most humans do not develop amyloid during their lifetimes (1).

To study factors that may be important in the development of islet amyloid in type 2 diabetes, we produced a transgenic mouse that expresses hIAPP in its pancreatic β -cells (7). These mice maintain normal β -cell secretory function, releasing hIAPP in a regulated manner in parallel with insulin (8). Furthermore, some two-thirds of these male transgenic mice developed islet amyloid after 12–16 months on a high-fat diet (9). Interestingly, the mice that developed islet amyloid also tended to be hyperglycemic, whereas those mice that lacked islet amyloid tended to be normoglycemic, suggesting that islet amyloid may contribute to hyperglycemia and/or that hyperglycemia might stimulate islet amyloidogenesis (9). This observation suggests a relationship between islet amyloidogenesis and hyperglycemia, which could be both cause and effect.

On the basis of these observations as well as the knowledge that glucose is a regulator of IAPP biosynthesis (10), we proposed that a hyperglycemic environment such as that

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CV, coefficient of variation; hIAPP, human IAPP; hIAPP-LI, human IAPP-like immunoreactivity; IAPP, islet amyloid polypeptide; IAPP-LI, IAPP-like immunoreactivity; IRI, immunoreactive insulin; MODY, maturity-onset diabetes of the young; MODY2, MODY resulting from defective β -cell glucokinase gene.

present in type 2 diabetes may facilitate and enhance islet amyloid formation. Furthermore, because type 2 diabetes is associated with decreased β -cell function (11), we believed that an impairment in β -cell function may further exacerbate islet amyloidogenesis. To test this hypothesis, we crossed our hIAPP transgenic mice that develop islet amyloid with a recently developed mouse model of hyperglycemia that lacks one allele of the β -cell glucokinase gene (12). These glucokinase-knockout (GKKO) mice, developed as a model of one of the genetic variants of human maturity-onset diabetes of the young (MODY) known as MODY2, display fasting hyperglycemia and impaired β -cell function manifest as reduced insulin secretion in response to glucose. On the basis of the characteristics of the two lines of mice, hIAPP transgenic and GKKO, the resultant line of mice (hIAPP \times GKKO) would be predicted to have elevated plasma glucose levels, impaired β -cell function, and a propensity to develop islet amyloid. This new mouse model would then serve as a useful model to investigate the potential importance of defective β -cell glucose metabolism and some aspects of hyperglycemia in islet amyloidogenesis.

RESEARCH DESIGN AND METHODS

Generation of hIAPP \times GKKO mice. To generate the animals used in this study, we bred transgenic mice expressing the hIAPP gene driven by the rodent insulin 2 promoter in their islet β -cells (7) with mice having one disrupted allele of the β -cell-specific glucokinase gene as a result of homologous recombination (12). The heterozygous hIAPP transgenic mice have been maintained on a C57BL/6 \times DBA/2 hybrid background while the heterozygous GKKO mice were on an ICR \times 129 hybrid background. Thus, to reduce effects of genetic background variation, littermates (hIAPP transgenic, GKKO, and hIAPP \times GKKO) were used throughout this study. Only male mice were used in the studies. Animals were reared in the Animal Research Facility at the Veterans Affairs Puget Sound Health Care System on a 12-h light/dark cycle at 22°C and fed a diet containing an increased quantity of fat (9% wt/wt) with water provided ad libitum, unless otherwise stated. The presence of the hIAPP transgene and the disrupted glucokinase gene in the offspring was determined by the polymerase chain reaction of tail DNA (13) using transgene-specific primers. For genotyping of the hIAPP gene, the sense primer was 5'-CTG AAG CTG GTA TTT CTC A-3' and the antisense primer was 5'-AGA TGA GAG AAT GGC ACC AAA-3'. The reaction mixture also included primers for the β 2-microglobulin gene, which was used as an indication of successful amplification. The sense primer for β 2-microglobulin was 5'-CAC CGG AGA ATG GGA AGC CGA A-3' and the antisense primer was TCC ACA CAG ATG GAG CGT CCA G-3'. The hIAPP allele resulted in a 182-bp and β 2-microglobulin allele in a 295-bp band. For genotyping of the GK gene, the sense primer was 5'-CAC CTG GTT GGA ACA GAA GC-3', and the antisense primers were 5'-ATG TGG AAT GTG TGC GAG GC-3' derived from a neomycin resistance gene driven by a pgk-1 promoter, and 5'-TTG GTG GCC TCC ATC CTG GC-3' derived from the GK gene (14). The wild-type allele resulted in a 450-bp band, and the recombinant allele resulted in a 250-bp band. The thermal cycle reaction for both consisted of 94°C for 30 s, followed by 35 cycles of 60°C (30 s) and 72°C (1 min).

Intraperitoneal glucose tolerance test. At 3, 6, and 12 months of age, mice were fasted overnight (18 h) and were anesthetized with an intraperitoneal injection (100 mg/kg body wt) of sodium pentobarbital (Nembutal; Abbott Laboratories). A glucose bolus (1 g/kg body wt) was injected intraperitoneally 40 min after the induction of anesthesia. Blood was drawn into heparinized capillary tubes from the retro-orbital sinus before and 15, 30, 60, and 120 min after glucose administration. The plasma was separated by centrifugation and stored at -20°C until assayed for glucose and immunoreactive insulin (IRI).

Plasma and tissue sampling at the time when the animals were killed. Two weeks after the intraperitoneal glucose tolerance test, animals were fasted for 4 h and then anesthetized as described above. Forty minutes after the induction of anesthesia, blood was collected from the orbital sinus using heparinized capillary tubes, centrifuged, and plasma separated and stored at -20°C until assayed for glucose, IRI, and IAPP-like immunoreactivity (IAPP-LI). The animals were then killed by cervical dislocation and the pancreases excised. Half the pancreas was fixed for histological analysis and the other half was frozen for peptide analysis.

Pancreas extraction procedure. When the animals were killed, a portion of the pancreas was taken and frozen in liquid nitrogen and stored at -70°C.

The tissue was then homogenized in 50% isopropanol/1% trifluoroacetic acid and incubated at 4°C for 4–8 h. After a freeze-thaw cycle at -70°C, the homogenate was centrifuged at 13,000 rpm for 15 min and the supernatant was used to measure IRI and IAPP content.

Histochemical analysis. For light microscopy, pancreases were fixed in 0.1 mol/l phosphate buffer (pH 7.4) containing 4% paraformaldehyde, rinsed in 0.1 mol/l phosphate buffer (pH 7.4), and embedded in paraffin. Sections (5 μ m) were deparaffinized and rehydrated before staining.

For thioflavin S staining, sections were incubated with 0.5% thioflavin S for 1 min, rinsed briefly 3 times in 70% ethanol, washed for 5 min in water, and mounted (9). Sections were independently scored by three observers who were blinded to the genotype of the mice using an arbitrary scale of 0–3, with 0 indicating the absence of amyloid, 1 indicating a single speck or multiple specks, 2 indicating that 10–40% of the islet was comprised of amyloid, and 3 indicating that >40% of the islet was comprised of amyloid.

Assays. Plasma glucose was assayed using the glucose oxidase method. IRI was measured by a modification of the double-antibody radioimmunoassay method of Morgan and Lazarow (15), using rat insulin as the standard. The assay has an intra-assay coefficient of variation (CV) of 6.4% and an interassay CV of 12%. Mouse IAPP was measured with a radioimmunoassay using an in-house antibody (8342), high-performance liquid chromatography-purified ¹²⁵I-labeled rat IAPP, and rat IAPP as standard (Peninsula, Belmont, CA). The 8342 antibody recognizes mouse and rat IAPP but not hIAPP (5). The assay has an intra-assay CV of 9% and an interassay CV of 12%. hIAPP was assayed using an enzyme immunoassay, using F024 as the capture antibody and F002 as the detection antibody (Amylin Pharmaceuticals, San Diego, CA.) (16). F002 specifically recognizes human and not mouse IAPP, such that samples that do not contain hIAPP read zero in this assay. The CVs for this assay are <10% for intra-assay and <15% interassay variation (16).

Calculations and statistical analysis. The areas under the curve for the glucose and insulin responses during the intraperitoneal glucose tolerance tests were calculated using the trapezoidal method. One-way analysis of variance and the Mann-Whitney *U* test were used to statistically analyze the data. A *P* value of ≤ 0.05 was considered significant.

RESULTS

Intraperitoneal glucose tolerance tests

Three months of age. Body weights were similar among the three groups of mice (Table 1). Basal plasma glucose concentrations after an overnight fast were higher in hIAPP \times GKKO mice ($n = 17$) (Table 1) than in hIAPP transgenic mice ($n = 19$). Administration of intraperitoneal glucose increased plasma glucose concentrations in all groups of mice (Fig. 1). The hIAPP \times GKKO mice were the most glucose intolerant, displaying higher plasma glucose levels at all time points compared with hIAPP transgenic mice ($P < 0.01$). Consequently, the area under the glucose curve was significantly greater in hIAPP \times GKKO mice than in hIAPP transgenic mice (Fig. 2, $P < 0.005$).

Overnight-fasted plasma IRI concentrations were not significantly different among the three groups of mice (Table 1). In response to glucose, plasma IRI levels increased in all three groups (Fig. 1). The hIAPP \times GKKO mice appeared to secrete less insulin over the course of the challenge than hIAPP transgenic and GKKO mice did, although statistical significance was only reached at 120 min compared with GKKO mice (Fig. 1, $P < 0.005$). The mean area under the insulin curve, shown in Fig. 2, was lower in hIAPP \times GKKO mice than in GKKO mice ($P = 0.05$). Interestingly, despite a 50% decrease in β -cell glucokinase levels, GKKO mice displayed plasma IRI concentrations similar to those of hIAPP transgenic mice at this age.

Six months of age. At 6 months of age, the three groups of mice had similar body weights (Table 1). Basal plasma glucose concentrations were higher in hIAPP \times GKKO ($n = 17$) and GKKO mice ($n = 13$) than in hIAPP transgenic mice ($n = 19$) (Table 1). After the glucose bolus, both hIAPP \times GKKO and GKKO mice were glucose intolerant and exhibited a greater

TABLE 1

Body weight and plasma glucose and IRI concentrations in overnight-fasted hIAPP transgenic, GKKO, and hIAPP×GKKO mice at 3, 6, and 12 months of age

	hIAPP	GKKO	hIAPP×GKKO
<i>n</i>	19	13	17
3 months			
Body weight (g)	39.5 ± 2.2	41.6 ± 2.6	37.1 ± 1.9
Plasma glucose (mmol/l)	10.0 ± 0.6	10.6 ± 0.7	12.9 ± 0.9*
Plasma IRI (pmol/l)	171.6 ± 49.0	31.8 ± 9.9	70.1 ± 25.2
6 months			
Body weight (g)	46.2 ± 2.5	49.0 ± 1.9	45.9 ± 1.8
Plasma glucose (mmol/l)	9.5 ± 0.5	12.1 ± 0.7*	13.3 ± 1.1†
Plasma IRI (pmol/l)	223.3 ± 64.7	107.8 ± 18.3	73.6 ± 8.0*
12 months			
Body weight (g)	55.1 ± 2.7	56.8 ± 2.6	56.7 ± 2.6
Plasma glucose (mmol/l)	9.4 ± 0.7	12.8 ± 1.5*	14.3 ± 0.9†
Plasma IRI (pmol/l)	635.5 ± 241.4	243.3 ± 103.6	265.8 ± 51.2

Data are means ± SE, unless otherwise stated. **P* < 0.05, †*P* < 0.005 vs. hIAPP transgenic mice.

plasma glucose excursion that was significant at all points (Fig. 1, *P* < 0.05), except at 60 min for GKKO mice compared with hIAPP transgenic mice. Accordingly, the area under the glucose curve was higher in hIAPP×GKKO and GKKO mice than in hIAPP transgenic mice (Fig. 2, *P* < 0.005).

Plasma IRI concentrations under basal conditions were significantly lower in hIAPP×GKKO mice than in hIAPP transgenic mice (Table 1). After the glucose bolus, hIAPP×GKKO mice tended to have lower plasma IRI concentrations that were significant at 30 min (*P* < 0.05) than

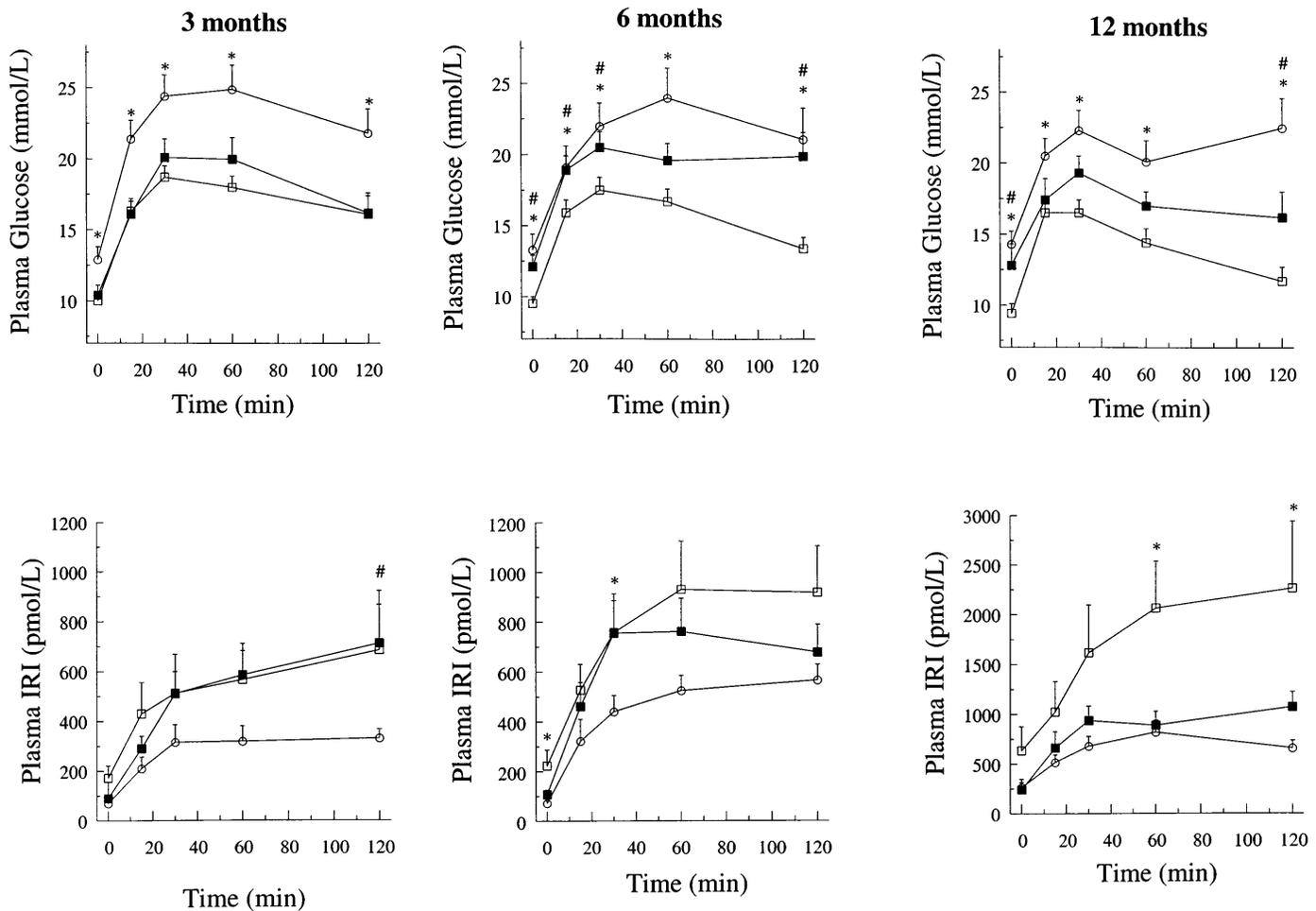


FIG. 1. Plasma glucose and IRI concentrations during an intraperitoneal glucose tolerance test (1 g/kg body wt) in hIAPP transgenic (□), GKKO (■), and hIAPP×GKKO (○) mice at 3, 6, and 12 months of age. **P* < 0.05 hIAPP×GKKO compared with hIAPP transgenic mice; #*P* < 0.05 GKKO compared with hIAPP transgenic mice.

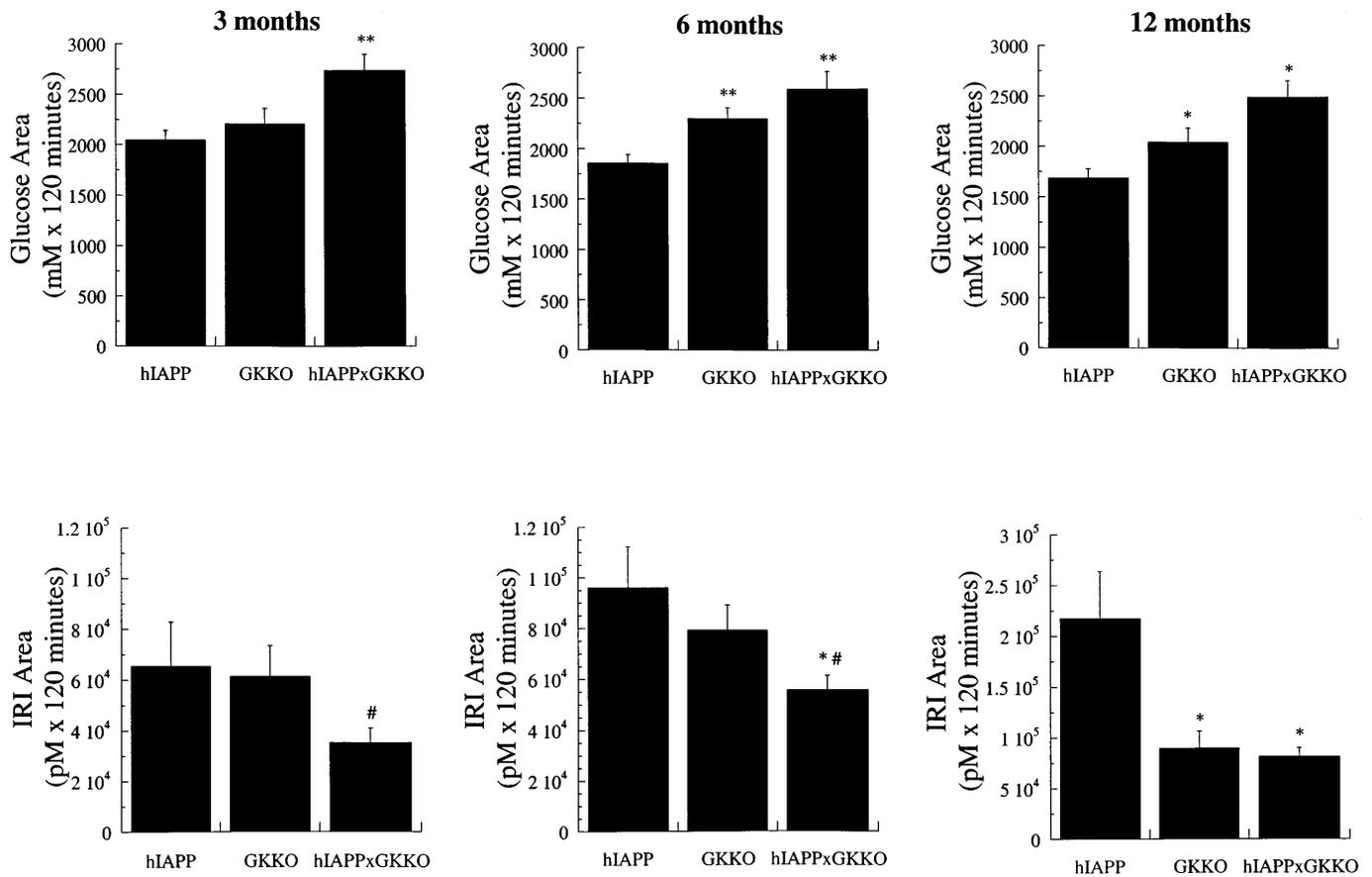


FIG. 2. Area under the glucose and IRI curves in response to intraperitoneal administration of glucose (1 g/kg body wt) in hIAPP transgenic, GKKO, and hIAPP×GKKO mice at 3, 6, and 12 months of age. * $P < 0.05$, ** $P < 0.005$ compared with hIAPP transgenic mice; # $P < 0.05$ GKKO compared with hIAPP×GKKO mice.

hIAPP transgenic mice (Fig. 1). Furthermore, the area under the insulin curve, shown in Fig. 2, was lower in hIAPP×GKKO mice than in both hIAPP transgenic ($P < 0.05$) and GKKO mice ($P < 0.05$). Similar to the result at 3 months of age, despite a tendency toward lower levels at the later time points, there was no significant difference in plasma IRI concentrations or area under the insulin curve when comparing GKKO and hIAPP transgenic mice.

Twelve months of age. Body weights were similar among the three groups of mice (Table 1). Basal plasma glucose concentrations were higher in both hIAPP×GKKO ($n = 17$) and GKKO ($n = 13$) mice than in hIAPP transgenic mice ($n = 19$) (Table 1). After the glucose bolus, plasma glucose levels were significantly higher in hIAPP×GKKO mice than in hIAPP transgenic mice (Fig. 1, $P < 0.05$ at all time points). Although there was a trend for plasma glucose concentrations to be higher in GKKO mice than in hIAPP transgenic mice, this was significant only for the 120-min time point ($P < 0.05$). The area under the glucose curve was higher in both hIAPP×GKKO ($P < 0.05$) and GKKO ($P < 0.05$) mice than in hIAPP transgenic mice (Fig. 2).

In spite of basal hyperglycemia in GKKO and hIAPP×GKKO mice, basal plasma IRI concentrations were not different among the three groups of mice (Table 1). After the glucose bolus, plasma IRI levels tended to be lower in both hIAPP×GKKO and GKKO mice than in hIAPP transgenic mice, but significantly so only at the 60- and 120-min time

points for hIAPP×GKKO mice (Fig. 1, $P < 0.05$). However, the area under the insulin curve was significantly decreased in both hIAPP×GKKO ($P < 0.05$) and GKKO ($P < 0.05$) mice in comparison with hIAPP transgenic mice (Fig. 2). Thus, both hIAPP×GKKO and GKKO mice exhibited impaired glucose-induced insulin secretion.

Body weight, plasma, and pancreatic peptide concentrations at the time when the animals were killed. Body weights and 4-h fasted plasma concentrations of glucose, IRI, and human IAPP-like immunoreactivity (hIAPP-LI) at 12 months of age are shown in Table 2. As expected, body weight did not differ among the three groups, with all mice tending to be obese.

Plasma glucose concentrations were significantly increased in GKKO and hIAPP×GKKO mice compared with those in hIAPP transgenic mice ($P < 0.001$). Despite a tendency for hIAPP×GKKO mice to have lower plasma IRI concentrations than hIAPP transgenic mice have ($P = 0.16$), there were no differences among the three mouse groups.

As expected, plasma hIAPP-LI was not detected in GKKO mice. Again, although there was a trend for hIAPP×GKKO mice to have a lower mean value for plasma hIAPP-LI levels than hIAPP transgenic mice had, this was not statistically significant ($P = 0.32$). In keeping with the co-release of IAPP and IRI, plasma hIAPP-LI and IRI were correlated in both hIAPP transgenic ($r = 0.79$, $P < 0.005$) and hIAPP×GKKO ($r = 0.75$, $P < 0.005$) mice.

TABLE 2

Body weight and plasma glucose, IRI, and hIAPP-LI concentrations in 4-h fasted hIAPP transgenic, GKKO, and hIAPP×GKKO mice at 12 months of age

	hIAPP	GKKO	hIAPP×GKKO
<i>n</i>	19	13	17
Body weight (g)	55.9 ± 3.2	56.1 ± 2.2	56.9 ± 2.6
Plasma glucose (mmol/l)	7.8 ± 0.5	11.5 ± 0.9*	13.9 ± 0.9*
Plasma IRI (pmol/l)	1,478.3 ± 490.1	1,036.3 ± 360.2	764.5 ± 158.3
Plasma hIAPP-LI (pmol/l)	60.3 ± 19.8	ND	39.8 ± 6.4

Data are means ± SE, unless otherwise stated. ND, not detectable. **P* < 0.001 vs. hIAPP transgenic mice.

Table 3 shows the pancreatic content of IRI, mouse IAPP-LI, and hIAPP-LI for hIAPP transgenic, GKKO, and hIAPP×GKKO mice. There was no difference in the pancreatic content of any of these peptides among the three groups of mice with the exception of hIAPP-LI, which, as expected, was not detected in pancreases from GKKO mice.

Frequency and severity of islet amyloid deposits. Islet amyloid, as detected with thioflavin S staining, was not observed in pancreatic sections from GKKO mice (0 of 13), which is in keeping with the fact these mice produce only non-amyloidogenic mouse IAPP. In contrast, when the animals were 12 months old, islet amyloid deposits were observed in 63% (12 of 19) of the hIAPP transgenic mice but only 23% (4 of 17) of the hIAPP×GKKO mice (*P* < 0.05). Further, as shown in Fig. 3, evaluation of the degree of islet amyloidogenesis demonstrated that hIAPP transgenic mice had a significantly greater degree of islet amyloid deposition than hIAPP×GKKO mice did (1.20 ± 0.30 vs. 0.40 ± 0.24, *P* < 0.05).

DISCUSSION

Hyperglycemia is the clinical hallmark of type 2 diabetes and results from impaired islet function. Islet amyloid is a pathological feature of the pancreas in type 2 diabetes, replacing β-cell mass and thereby contributing to impaired islet function. Thus, islet amyloid appears to be an important determinant of the severity of islet dysfunction (17). The primary constituent of these islet amyloid deposits is the 37-amino acid β-cell peptide, IAPP, which is normally cosecreted with insulin in response to glucose and nonglucose stimuli (5,18). However, under certain as-yet-undetermined conditions, IAPP forms amyloid deposits in type 2 diabetes.

Because of the lack of a suitable small-animal model of type 2 diabetes, we developed transgenic mice that express and produce the amyloidogenic human form of IAPP in the β-cells of their pancreases (7). In our initial report of islet amyloid formation in these hIAPP transgenic mice, we observed an association between hyperglycemia and amyloid deposition when mice were fed a relatively high-fat diet (9). Thus,

all transgenic hIAPP mice that were hyperglycemic displayed islet amyloid deposits whereas not all normoglycemic mice had islet amyloid. Because it is known that consumption of diets high in fat impairs β-cell function and is associated with the development of hyperglycemia (19,20), we hypothesized that β-cell dysfunction may in fact accelerate islet amyloid deposition in hIAPP transgenic mice.

To test this hypothesis, we cross-bred our hIAPP transgenic mice with transgenic mice that have reduced β-cell glucokinase activity (12). These mice were chosen because glucokinase catalyzes a rate-determining step in glycolysis and is widely believed to be the β-cell glucose-sensor for insulin secretion (21). In keeping with this critical role of glucokinase, these β-cell glucokinase-deficient animals have a defect in glucose-mediated insulin secretion with blunted first- and second-phase responses (12,22). The progeny of this crossbreeding resulted in a new line of double-transgenic mice (hIAPP×GKKO) harboring the hIAPP gene and half of the complement of β-cell glucokinase. These hIAPP×GKKO mice were used in this investigation to determine whether β-cell glucokinase deficiency and hyperglycemia per se accelerate islet amyloid formation. However, since glucokinase deficiency is not a feature of all forms of diabetes, it is unlikely that mice lacking glucokinase will develop features that necessarily apply to all forms of diabetes.

As expected, islet amyloid was not detected in GKKO mice, because they did not produce an amyloidogenic form of IAPP. However, despite having β-cell dysfunction, fasting hyperglycemia, and markedly impaired glucose tolerance, hIAPP×GKKO mice did not show increased amyloidogenesis compared with hIAPP transgenic mice. In fact, these double-transgenic mice had a significant decrease in both the incidence and quantity of islet amyloid compared with hIAPP transgenic mice.

Because glucose is known to be a regulator of both the IAPP and insulin genes, we sought to determine whether the reduction in β-cell glucokinase was associated with a reduction in pancreatic IAPP content and/or secretion and thus could

TABLE 3

Pancreatic content of IRI, mouse IAPP-LI, and hIAPP-LI in hIAPP transgenic, GKKO, and hIAPP×GKKO mice at 12 months of age

	hIAPP	GKKO	hIAPP×GKKO
IRI (pmol · l ⁻¹ · μg ⁻¹ protein)	1,529.3 ± 260.9	1,308.8 ± 253.4	1,235.9 ± 168.5
mIAPP-LI (pmol · l ⁻¹ · μg ⁻¹ protein)	44.3 ± 6.1	31.8 ± 6.6	32.1 ± 4.2
hIAPP-LI (pmol · l ⁻¹ · μg ⁻¹ protein)	26.7 ± 5.0	ND	27.2 ± 5.1

Data are means ± SE. ND, not detectable.

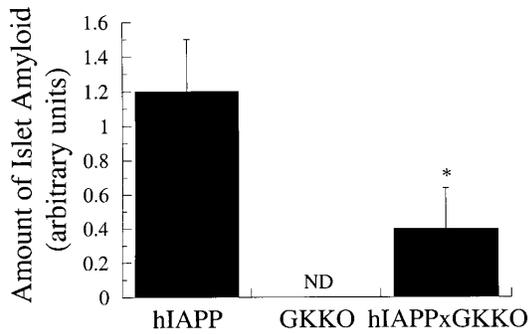


FIG. 3. Amount of islet amyloid deposition in hIAPP transgenic, GKKO, and hIAPPxGKKO mice at 12 months of age. Sections were independently scored by three observers who were blinded to the genotype of the mice using an arbitrary scale of 0–3, with 0 indicating the absence of amyloid; 1 indicating a single speck or multiple specks; 2 indicating that 10–40% of the islet was comprised of amyloid; and 3 indicating that >40% of the islet comprised amyloid. * $P < 0.05$ compared with hIAPP transgenic mice. ND, not detected.

explain the reduction in amyloid formation. We determined the pancreatic levels of hIAPP, mouse IAPP, and insulin and found that the pancreatic content of these peptides was not different among the three groups of mice, suggesting that increased flux due to the elevated glucose levels maintained peptide production at similar levels. It is of interest that Sreenan et al. (23) found similar islet insulin content in wild-type mice and another line of GKKO mice lacking both β -cell and hepatic glucokinase, suggesting that the presence of one allele of β -cell glucokinase in the presence of hyperglycemia is sufficient for normal production of islet hormones. Whether this degree of hyperglycemia for 12 months in the presence of a normal complement of β -cell glucokinase would have resulted in greater peptide content is unclear, but quite likely. Along the same lines, the similar insulin and mIAPP content in the hIAPP transgenic mice that develop amyloid as compared with that of the other two groups could be interpreted to mean that the degree of amyloidosis that we observed does not impair production of these peptides. We believe that it is difficult to draw this conclusion, however, since the other two groups lacked a β -cell glucokinase allele and could be expected to have a greater peptide content if they had both alleles. Nevertheless, what the peptide content data do clearly indicate is that the decrease in amyloid deposition in hIAPPxGKKO mice does not appear to be due to decreased production of hIAPP.

Our work in our hIAPP transgenic mice fed an increased amount of dietary fat and that from biopsy samples in people with type 2 diabetes (P. Westermark, A. Clark, personal communication) suggests that islet amyloid is largely extracellular and that IAPP needs to be secreted by the β -cell for amyloidogenesis to occur. Thus, the reduction in islet amyloidogenesis in hIAPPxGKKO mice might be explained by an insufficient rate of hIAPP secretion into the extracellular space. The hIAPPxGKKO mice have diminished insulin secretion in response to intraperitoneal glucose administration. Because insulin and IAPP are packaged in the same secretory granules and cosecreted by the β -cell in response to various stimuli, the strong correlation between hIAPP and IRI observed in the present study makes it reasonable to assume that hIAPP secretion is also depressed in hIAPPxGKKO mice. This assumption is also supported by a tendency for a

decrease in fasting plasma hIAPP-LI concentrations at 12 months of age in hIAPPxGKKO mice compared with hIAPP transgenic mice. Furthermore, two recent studies in which markedly increased hIAPP secretion was produced by crossing hIAPP transgenic mice with models of insulin resistance ($A^{vy/a}$, ob/ob) also led to islet amyloid deposition (24,25). Thus, it appears that hIAPP secretion under the right conditions contributes to the rate of islet amyloid formation.

Although it appears that the rate of IAPP secretion may be a determinant, it is important to recognize that simple overproduction of IAPP does not appear to be sufficient for amyloid deposition. We have found that increased IAPP release as a result of breeding our hIAPP transgenic mice to homozygosity or inducing insulin resistance with nicotinic acid for 12 months does not lead to appreciable islet amyloid formation when mice consume a diet with low dietary fat (26). In addition, others have shown that transgenic overexpression of hIAPP resulting in an up to 15-fold increase in plasma IAPP levels did not cause islet amyloid formation (27). Recently, it has been suggested that the contents of the secretory granule may play a role in determining the likelihood of hIAPP forming fibrils (28–30). Since mouse IAPP lacks an amyloidogenic sequence and amyloid is not observed in mice and rats that develop diabetes (6), it is possible that there is an interaction between mouse IAPP and hIAPP that diminishes the amyloidogenic process and thus the quantity of amyloid. However, because mouse IAPP was present in both transgenic models that produced hIAPP, it is not likely to have been the factor responsible for the differences in the severity of islet amyloid formation observed in this study.

It is interesting to note that in both GKKO and hIAPPxGKKO mice, insulin secretory capacity remained remarkably steady over the first 12 months of age. This result is despite the presence of hyperglycemia, which would be predicted to worsen β -cell function via glucotoxicity (31,32). This phenotype is also seen in patients with MODY as a result of defective β -cell glucokinase gene (MODY2) who do not progress to worsening glycemic control, as happens in patients with type 2 diabetes. The explanation for this may be that like our new mouse line (hIAPPxGKKO) presented in this study, patients with MODY2 are protected from the development of islet amyloid. This idea is supported by a report from Clark et al. (33) describing a patient with MODY2 in whom they did not find evidence of islet amyloid. Thus, the lack of islet amyloid in MODY2 would protect β -cells from dysfunction and/or death, leading to a relatively stable syndrome.

In conclusion, despite the presence of impaired β -cell function and hyperglycemia in mice expressing the amyloidogenic hIAPP gene but lacking one allele of the β -cell glucokinase gene, a decrease in both the incidence and amount of islet amyloid was observed in comparison with hIAPP transgenic mice. This finding may explain why patients with MODY2 do not display progressively worsening glycemia as patients with type 2 diabetes do.

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