Islet Amyloid–Associated Diabetes in Obese A^{vy}/a Mice Expressing Human Islet Amyloid Polypeptide

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We have previously shown that hemizygous transgenic mice expressing human islet amyloid polypeptide (hIAPP) in pancreatic β-cells have no diabetic phenotype, whereas in the homozygous state, they developed severe, early-onset hyperglycemia associated with impaired insulin secretion and β-cell death. We investigated the possibility that when the hemizygous mice are crossed onto an obese, insulin-resistant strain such as agouti viable yellow (A^{vy}/a), they would exhibit a phenotype more akin to human type 2 diabetes. The hIAPP-expressing A^{vy} males (TG-Y) displayed fasting hyperglycemia at 90 days of age and by 1 year progressed to severe hyperglycemia relative to their nontransgenic counterparts. Plasma insulin concentrations and pancreatic insulin content dropped 10- to 20-fold, suggesting severe impairment of β-cell function. Histopathological findings revealed β-cell degeneration and loss consistent with the drop in the plasma insulin concentration. In addition, large deposits of IAPP amyloid were present in TG-Y islets. We conclude that in transgenic mice expressing hIAPP, insulin resistance can induce overt, slow-onset diabetes associated with islet amyloid and decreased β-cell mass. *Diabetes* 47:743–750, 1998

Type 2 diabetes in humans is characterized by both insulin resistance (1,2) and failure of β-cells to compensate with increased insulin secretion (3). Several studies have found an association of this compensatory failure with deposition of islet amyloid in nonhuman primates (4) and in humans (5–7). This islet amyloid is derived from islet amyloid polypeptide (IAPP) (8), also referred to as amylin (9). Interest in the potential pathogenic role of IAPP in the development of type 2 diabetes has increased recently with the reports that certain strains of mice transgenic for human IAPP (hIAPP) develop hyperglycemia (10–12), although a causal relationship between the appearance of islet amyloid and the onset of diabetes has yet to be demonstrated conclusively.

Type 2 diabetes is invariably associated with chronic insulin resistance attributable to one or more predisposing causes, including obesity (13) and glucocorticoid excess (14). However, under conditions of high insulin demand, overt hyperglycemia does not generally occur until a significant percentage of the pancreatic β-cells is compromised. Induced insulin resistance can result in increased expression of insulin and IAPP (15,16). Because nondiabetic humans express and secrete IAPP from β-cells without developing islet amyloid or associated cytotoxicity, it is possible that there is a threshold above which expression of IAPP leads to deposition of IAPP in an aggregated fibrillar form that in turn leads to cytotoxicity. In support of this concept is the observation in one strain of mice transgenic for hIAPP that the hemizygous animals displayed a normal phenotype, but the homozygous animals with the anticipated twofold increase in expression of IAPP did develop diabetes (10). It is clear that the predisposition to diabetes in humans does not relate to IAPP gene copy number, but we and others have hypothesized that toxicity to β-cells may be mediated through increased expression of IAPP in the presence of insulin resistance (11). We previously demonstrated that induction of severe insulin resistance with exogenous growth hormone and glucocorticoid treatment in hemizygous mice transgenic for hIAPP caused islet amyloid formation associated with β-cell loss, and the animals developed hyperglycemia (11). However, we could not rule out the possibility that this effect was achieved as a consequence of direct actions of dexamethasone and/or growth hormone on the islet rather than a secondary effect of the induction of insulin resistance. Because the most common risk factor for type 2 diabetes in humans is obesity, in the present studies we sought to develop a model characterized by obesity and hemizygous expression of hIAPP (neither of which alone results in overt hyperglycemia) to test the hypothesis that insulin resistance related to the development of obesity induces islet amyloidosis and β-cell destruction in mice transgenic for hIAPP. This was achieved by cross-breeding the homozygous form of these transgenic mice with a well-established mouse model of insulin resistance, the agouti viable yellow mouse (A^{vy}/a). The A^{vy}/a mouse possesses a mutation of the agouti gene that causes ubiquitous expression of the encoded 131 amino acid protein (17). The phenotypic consequences of this ectopic expression include the appearance of obesity, concomitant induction of insulin resistance, and in some genetic backgrounds, progression to mild hyperglycemia (18,19). The A^{vy} allele of the agouti locus is dominant

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ANOVA, analysis of variance; hIAPP, human islet amyloid polypeptide; IAPP, islet amyloid polypeptide; NTG-A, +/+, A/a mice; NTG-Y, +/+, A^{vy}/A mice; TG-A, hIAPP/+, A/a mice; TG-Y, hIAPP/+, A^{vy}/A mice.

to both the A and a alleles. It also results in an obvious phenotypic change to a yellow coat color. To test the hypothesis that mice transgenic hemizygous for hIAPP develop diabetes associated with islet amyloid under conditions of insulin resistance, we crossed the hIAPP homozygotes to agouti viable yellow mice and compared the phenotypes of the yellow coat–colored offspring (A^{vy}/A; herein referred to as TG-Y) to their agouti coat–colored counterparts (A/a; herein referred to as TG-A).

RESEARCH DESIGN AND METHODS

Development and characterization of transgenic mice. Derivation of the RHF transgenic line containing the RIPHAT transgene was previously described (11). Homozygous RHF males (FVB/N strain, A/A genotype at the agouti locus) were crossed to A^{vy}/a (C57BL/6 strain) females for generation of the hybrid offspring. This allowed us to examine the impact of the hIAPP hemizygous state under conditions of insulin resistance (A^{vy}/A). We studied the phenotype of 14 male and 6 female hIAPP/+, A^{vy}/A mice (designated TG-Y) from 50 to 290 days of age. To control for the impact of the agouti background on the hIAPP hemizygous state, we examined 11 male and 9 female hIAPP/+, A/a (designated TG-A) mice during the same interval. The corresponding nontransgenic lines were generated by crosses of A^{vy}/a to nontransgenic FVB/N mice to generate mice with the following genotype: +/+, A^{vy}/A (designated NTG-Y; n = 8 males and 5 females) and +/+, a/a (designated NTG-A; n = 17 males and 6 females). The phenotypic characterization consisted of biweekly blood sampling and weighing. In addition, samples of pancreases (n = 8 for each of the four male subgroups) were isolated at 290 days of age for morphological examination. Another set (n = 6 from each of the four subgroups) of animals was killed at 1 year of age to obtain blood glucose, plasma insulin, and plasma IAPP concentrations. The pancreases from this group were used for determining insulin and IAPP content. Northern analysis was carried out as described (11).

Animal maintenance and techniques. Animal maintenance and studies were carried out in accordance with the Pfizer institutional animal care and use committee guidelines. Mice were fed standard rodent chow (Purina) and maintained on a 12-h light/dark cycle. In vivo blood samples were obtained from retro-orbital sinus. Animals were killed to obtain pancreas samples for light and electron microscopy as previously described (10). At the time of death, sufficient blood was obtained to measure plasma insulin and IAPP concentrations as described below.

Insulin and IAPP assays. Plasma insulin (Binax, Portland, ME) and IAPP (Peninsula Laboratories, Belmont, CA) were measured by radioimmunoassay using commercially available kits. Insulin assays were conducted without prior processing of the plasma. Assays for IAPP used plasma extracted according to the procedure specified by the manufacturer. Pancreatic concentrations of insulin and IAPP were measured in tissue extracts. These were prepared by promptly freezing excised pancreases in liquid nitrogen; homogenizing in ice-cold acid ethanol (0.2 mol/l HCl, 75% ethanol); extracting this homogenate by overnight mixing at 4°C; centrifuging to sediment the solids; and then neutralizing the supernatant by diluting 1:10 with 0.13 mol/l borate buffer, pH 8.0.

Morphological techniques. Tissues were processed and stained as described before (10). The combined insulin and Congo Red stain was performed by immunohistochemistry for insulin, followed by counterstain with Congo Red and hematoxilin. Thioflavin T staining of islet sections was carried out as described by Bancroft and Cook (20). Morphometric analysis was carried out as previously described (10). In brief, islet and β-cell areas were measured in at least 20 islets on paraffin sections immunohistochemically stained for insulin and in adjacent sections stained for both glucagon and stomatostatin. The islet area and combined α- and δ-cell areas were determined.

Statistical analysis. The blood glucose and body weight data at each time point in Figs. 2 and 3 are displayed as means ± SE. The significance of the individual values was ascertained by analysis of variance (ANOVA) for repeated measures using the StatView Analysis program (Abacus Concepts, Berkeley, CA). ANOVA using the same analysis package was carried out on the insulin and IAPP plasma concentration and the pancreatic content results in Tables 1 and 2 . For Table 3, ANOVA analysis followed by Tukey's procedure for multiple comparisons was performed on islet and β-cell area and on the percentage islet area occupied by β-cells or by α- and δ-cells.

RESULTS

Transgene expression levels on the A^{vy}/A background. To determine if the levels of hIAPP transgene expression in an inbred FVB/N and the hybrid FVB/N-C57BL/6 background were comparable, Northern analysis was carried out on fresh pancreatic tissue from each subgroup. The results are shown in Fig. 1. As expected, no transgene expression was detected in the NTG-Y and NTG-A mice. The levels of expression in TG-Y and TG-A mice were comparable to the original hIAPP hemizygous transgenic mice that did not spontaneously progress to overt diabetes (11). Expression levels were comparable in male and females in each subgroup (data not shown).

Metabolic phenotype. In male mice at 7 weeks of age, the fasting blood glucose concentration was not significantly different between TG-Y and TG-A (Fig. 2*A*). With aging, the blood glucose concentrations progressively increased in the TG-Y mice so that they became diabetic (i.e., blood glucose concentrations >7 mmol/l) between 80 and 100 days of age. In contrast, the fasting blood glucose concentrations remained constant in the three other groups, although those of the TG-A animals were subtly higher than those of the nontransgenic controls. In female mice, the TG-Y subgroup showed elevated fasting hyperglycemia (6 mmol/l), which remained stable from 70 to 290 days of age but did not reach the concentrations associated with diabetes (Fig. 2*B*). As expected, both male and female agouti viable yellow mice (NTG-Y) gained weight faster than did their agouti counterparts (NTG-A; Fig. 3*A*). Interestingly, the transgenic state for hIAPP seemed to enhance this weight gain, an effect that was more apparent in female than in male TG-Y mice (Fig. 3*B*). This is consistent with our observations in mice overproducing murine IAPP in which the increased IAPP concentration was associated with weight gain (W.C.S., J.J., J.C.P., M.D.C., R. Nelson, J. Stock, P.C.B., R.W.S., unpublished observations).

To further characterize the mechanism of the hyperglycemia observed in the male TG-Y mice, we examined their fed blood glucose, plasma insulin, and IAPP concentrations and

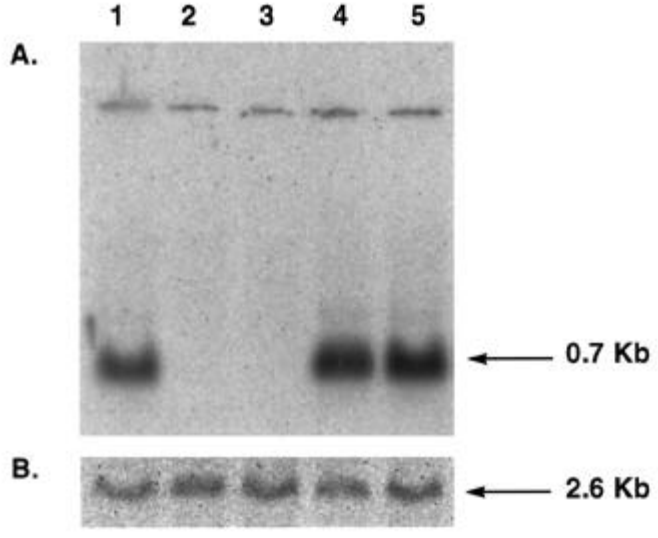

FIG. 1. Northern blot analysis. Total pancreatic RNA from 2- to 3-month-old male mice were prepared, electrophoresed, and blotted as described (11). The blot was hybridized to the transgene-specific (10) human glyceraldehyde-3-phosphate dehydrogenase RNA termination region (*A*) and subsequently stripped and reprobed to murine ribophorin cDNA (*B*). *Lane 1*, hemizygous RIPHAT male transgenic (RHFe); *lane 2*, NTG-A; *lane 3*, NTG-Y; *lane 4*, TG-A; *lane 5*, TG-Y.

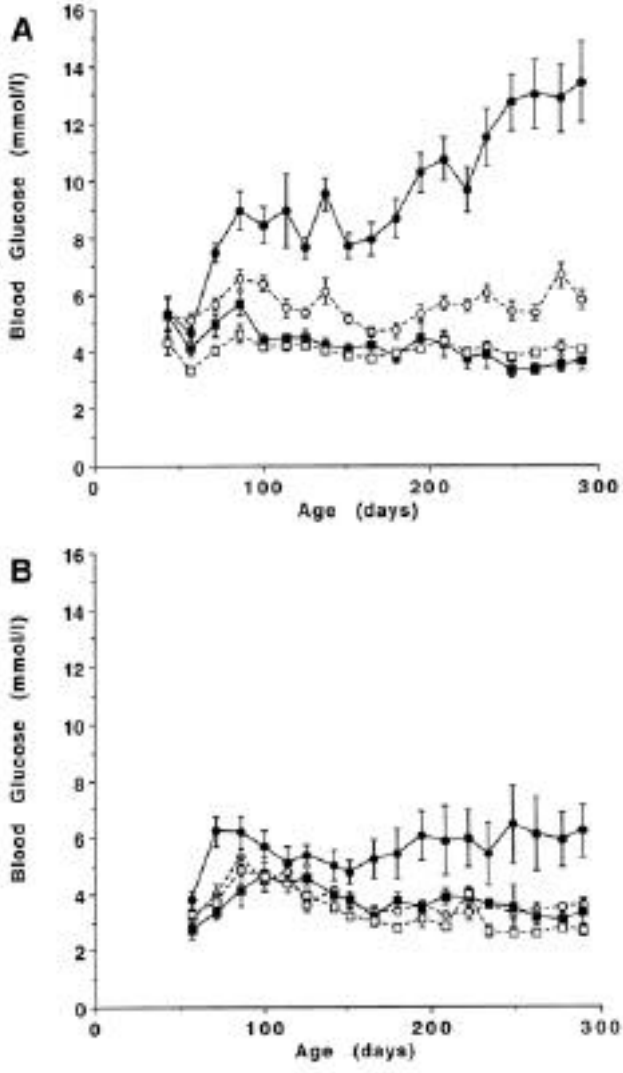

FIG. 2. Fasting blood glucose concentration versus age. *A*: In males, the glucose concentration of the TG-Y mice (●) was comparable to the other groups (○, TG-A; ■, NTG-Y; □, NTG-A) at 50 days of age but increased progressively thereafter. TG-A mice showed mild but significant ($P < 0.01$) and persistent hyperglycemia. *B*: In females, the pattern of blood glucose concentrations with age was similar to the male counterparts. However, the hyperglycemia of the TG-Y females, although significant ($P < 0.01$), was much less pronounced. The symbols for the four groups are identical to those in *A*.

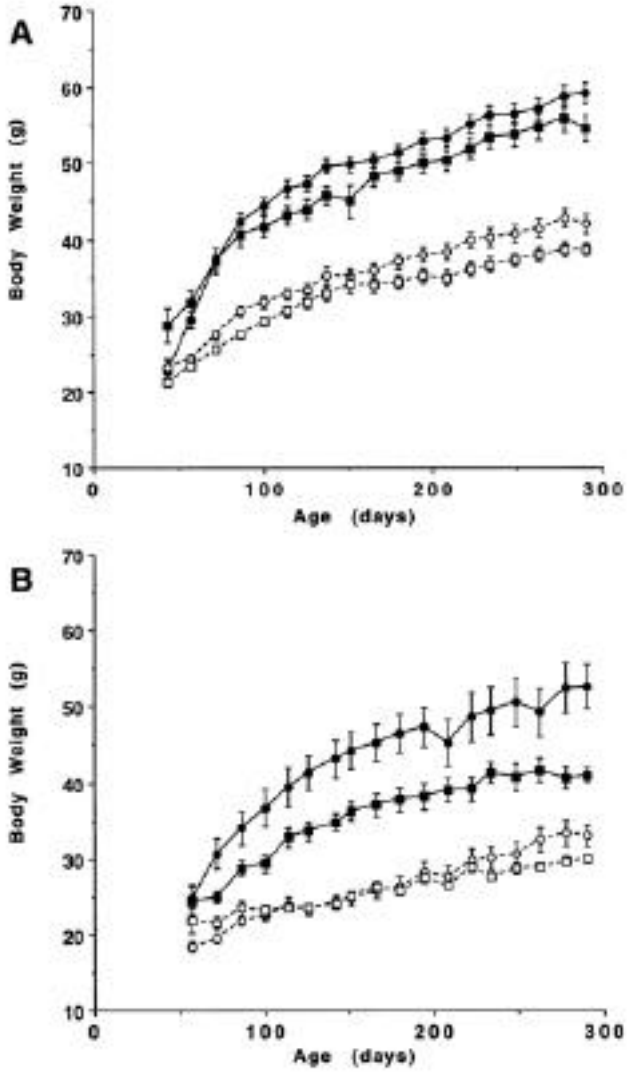

FIG. 3. Body weight versus age. *A*: For males, ●, TG-Y; ○, TG-A; ■, NTG-Y; □, NTG-A. *B*: For females, the same symbols as above are used. Insulin resistance (A^{vy} vs. wild-type agouti, i.e., NTG-Y vs. NTG-A) was associated with increased weight gain. The transgenic state was also significantly associated with increased weight gain in either the insulin-resistant ($P < 0.05$; TG-Y) or insulin-sensitive groups ($P < 0.05$; TG-A).

the corresponding pancreatic insulin and IAPP content at 1 year of age (Tables 1 and 2). The fed blood glucose concentrations were markedly raised in the TG-Y mice versus the other groups. The plasma insulin concentrations were markedly raised in the obese NTG-Y animals, consistent with the insulin resistance characteristic of obesity. In comparison, the mean plasma insulin concentration in the TG-Y mice was markedly lower (4.2% of NTG-Y value). The plasma insulin concentrations were higher in the NTG-A versus the TG-A mice, despite modestly higher glucose concentrations in the latter.

The corresponding pancreatic insulin contents showed a pattern similar to the plasma insulin concentrations (Table 2). Thus, the insulin content was markedly depleted (>12-fold depletion) in the TG-Y versus the NTG-Y mice. The markedly raised insulin content in the NTG-Y relative to NTG-A was consistent with the pronounced islet hyperplasia present in these mice in response to chronic insulin resistance (vide infra).

The plasma IAPP concentrations were raised in the TG-Y relative to the NTG-Y mice, consistent with the expression of the transgene (Table 1). The circulating concentrations in the TG-Y were, however, comparable to those in the TG-A mice. The pancreatic IAPP contents of the four subgroups were consistent with their respective pancreatic insulin contents (Table 2).

Light microscopic morphology. The islets in the NTG-Y mice showed marked hyperplasia in comparison to the NTG-A (Fig. 4*A* vs. *B* and Table 3), confirming the histological findings of earlier studies (21,22). This increase in islet size in response to the insulin-resistant phenotype was due to increased β-cell area (Table 3). In contrast, the islets in the TG-Y mice were characterized by markedly decreased insulin immunoreactive islet area in comparison to NTG-Y (Fig. 4*C* and Table 3). Moreover, these islets accumulated islet amy-

TABLE 1
Mean fed blood glucose concentrations, plasma insulin, and IAPP concentrations at 1 year of age

	Glucose (mmol/l)	Insulin (pmol/l)	IAPP (pmol/l)
TG-Y	24.1 ± 3.3*	152.6 ± 43.2	51.1 ± 4.9‡
TG-A	9.8 ± 1.1	264.9 ± 52.7	50.4 ± 6.1‡
NTG-Y	6.7 ± 0.4	3,616 ± 821†	9.6 ± 3.3
NTG-A	7.5 ± 0.4	1,264 ± 613	3.7 ± 0.8

Data are means ± SE for five mice per group. *Significant differences ($P < 0.01$) between TG-Y and the other three groups, †between NTG-Y and the rest, and ‡between the transgenic and nontransgenic mice are shown.

TABLE 2
Pancreatic content of insulin and IAPP at 1 year of age in the fed state

	Insulin (pmol/mg)	IAPP (pmol/mg)
TG-Y	213.6 ± 74*	20.6 ± 2.6†
TG-A	957.6 ± 160	58.1 ± 13.2
NTG-Y	2,749 ± 347	115.2 ± 13.9‡
NTG-A	1,863 ± 373	76.2 ± 15.0

Data are means ± SE, and are given in picomoles per milligram total tissue protein content for five mice per group. *Significant difference ($P < 0.01$) in insulin content between TG-Y and the two sets of nontransgenic mice, †significant difference ($P < 0.05$) in IAPP content between TG-Y and all other groups, and ‡significant difference between NTG-Y and the other three groups are shown.

loid that stained positively for IAPP by immunohistochemistry and fluoresced under UV light when stained with thioflavin T (Fig. 4*E* and *F*). In TG-Y mice, the islet amyloid was clearly present by 4 months of age (data not shown) but was more extensive by 10 months. There was a corresponding 72% decrease in the proportion of the islet area occupied by β-cells (Table 3). Of interest, pancreatic tissue from TG-A mice showed slight islet amyloidosis present by 10 months of age. However, in contrast to the TG-Y mice, there was not a marked decrease in islet area occupied by β-cells (Table 3). Of the 32 pancreases examined at 41 weeks of age ($n = 8$ for each of four groups), amyloidosis was observed in all 8 TG-Y samples, but none was observed in the NTG-Y or NTG-A sections. Five of eight individuals in the 41-week-old TG-A group displayed slight islet amyloidosis. Consistent with our previous observations in RHF homozygous mice (10), α- and δ-cells encroached from the peripheral distribution on islet cross-section to within the center of the TG-A islets.

Ultrastructural morphology. At 10 months of age, β-cells of the NTG-Y mice were packed with insulin secretory vesicles and exhibited normal cellular morphology, as characterized by the well-defined mitochondria (Fig. 5*A*). In TG-Y mice of the same age, large deposits of IAPP immunoreactive amyloid were present, containing fibrils with a diameter of 8–10 nm (Fig. 5*C*). As observed in hemizygous hIAPP transgenic mice treated with dexamethasone and growth hormone (11) and in other transgenic models (12), IAPP immunoreactive deposits also appeared in the perivascular interstitial space (Fig. 5*B*). The cell membrane was observed to directly border the IAPP immunoreactive deposits without necessarily causing β-cell degeneration (Fig. 5*D*), although both healthy and degenerating β-cells were observed in TG-Y islets.

DISCUSSION

In the present study, we sought to test the hypothesis that mice hemizygous transgenic for hIAPP develop diabetes under conditions of insulin resistance that progresses as the animals age and become obese. We used a well-established murine model of obesity and insulin resistance, the agouti viable yellow (A^{vy}/a) mouse (17), crossbred with a previously established homozygous transgenic mouse model expressing hIAPP because neither the A^{vy}/a nor the hemizygous hIAPP transgenic mouse develops overt, sustained diabetes in its own right. We report here that the resulting mouse hybrid develops diabetes with a gradual onset characterized by islet morphology similar to that present in humans with type 2 diabetes (7,23). These studies support the hypothesis and further focus interest on the potential role of hIAPP and islet amyloid formation in β-cell destruction in type 2 diabetes.

In a previous study using hIAPP hemizygous mice, we reported the concurrent development of hyperglycemia, islet amyloid, and β-cell degeneration, but only after 4 weeks of growth hormone and dexamethasone treatment selected to induce insulin resistance (11). Because untreated hemizygous mice did not develop this phenotype, we proposed that cytotoxicity to β-cells induced by the hIAPP transgene depends on the expression level of this gene. Because the known risk factors for type 2 diabetes have obesity and insulin resistance (vide supra) in common, this would be consistent with manifestation of the type 2 diabetes phenotype in humans. Although it is possible that dexamethasone and growth hormone had direct actions on the β-cells, the present studies are consistent with an essential contribution of insulin resistance to development of hIAPP-mediated β-cell destruction.

Interestingly, different hIAPP transgenic mouse models (10,12,24) exhibit markedly different rates of development of diabetes, if any, as well as variation in the extent and form of aggregates of IAPP present in the islets. The homozygous male hIAPP transgenic mice developed diabetes within 4–8 weeks (10), whereas the present TG-Y male mice developed diabetes over ~6 months. In both models, the development of diabetes was associated with β-cell loss and decreasing plasma insulin concentrations that tracked with the development of hyperglycemia, but there was a striking difference in the pattern of IAPP aggregates present. In the homozygous hIAPP transgenic mice, we observed small intracellular amorphous IAPP aggregates present at the time of maximal cell destruction, whereas amyloid deposits were not observed until after most cell destruction had occurred. In the present studies, classic large amyloid deposits that resembled those seen in humans with type 2 diabetes (23,6) were observed (Figs. 4 and 5) in male transgenic mice. The development of these amyloid deposits paralleled the development of hyperglycemia, consistent with the hypothesis that large extracellular amyloid deposits contribute to β-cell loss, as was proposed in a recent report of another transgenic mouse model that displays amyloid deposits (12).

Considering that amyloid deposits were also present in the TG-A mouse strain that developed only mild hypergly-

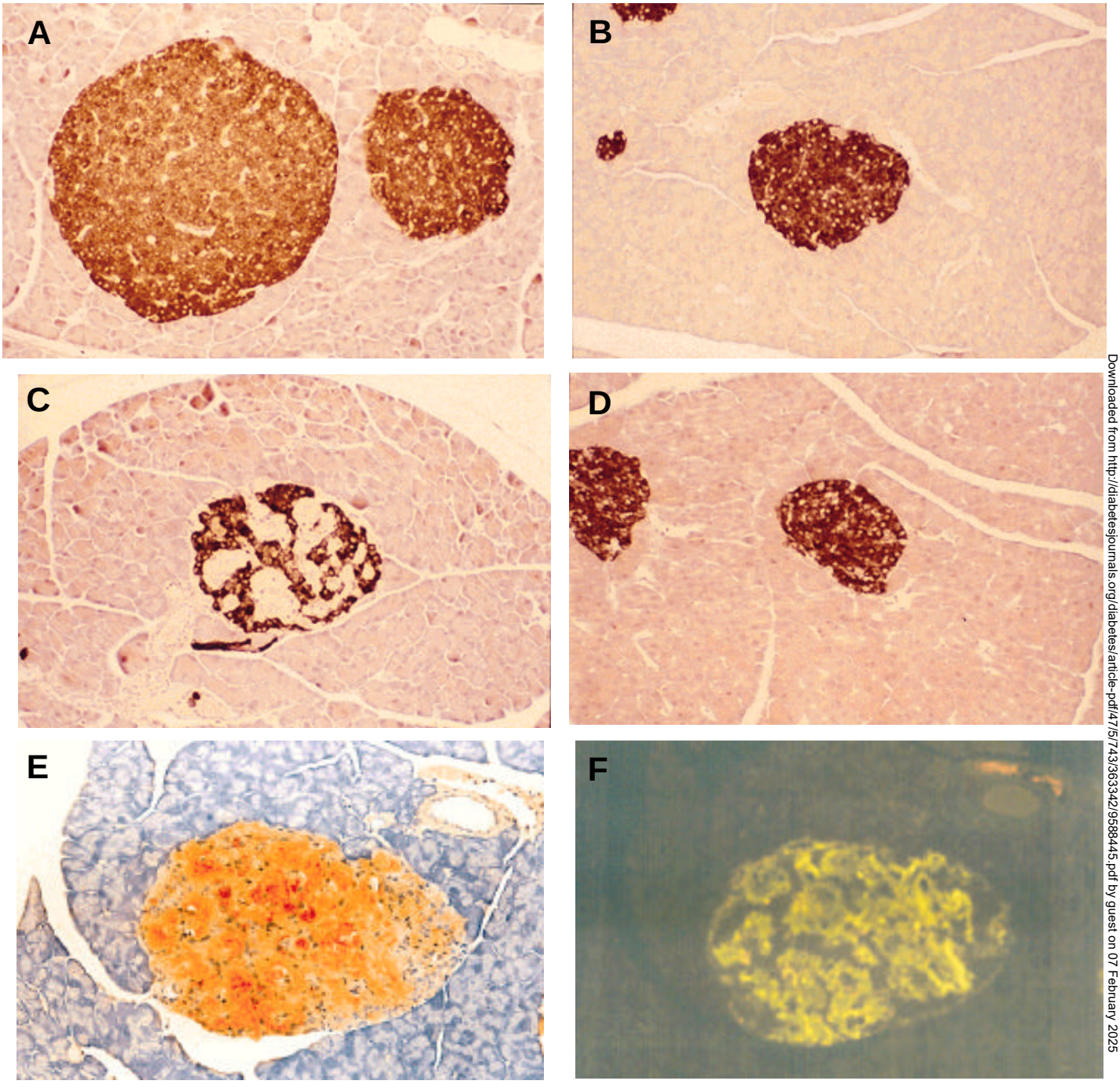

FIG. 4. Islet morphology (light microscopy). *A-D*: Representative islets immunostained for insulin and counterstained with Congo Red and hemotoxylin from NTG-Y (*A*), NTG-A (*B*), TG-Y (*C*), and TG-A (*D*) male mice at 41 weeks of age. The NTG-Y insulin-resistant genotype resulted in islet hyperplasia versus its insulin-sensitive counterpart NTG-A (*A* vs. *B*). In the insulin-resistant genotype, the hIAPP transgene induced marked islet amyloid and reduced β-cell mass (*C*), while, in contrast, the same transgene in the insulin-sensitive genotype led to infrequent small amyloid deposits without the reduced β-cell mass. *E*: TG-Y islet, immunostained for IAPP showing immunoreactivity of both the remaining β-cells and the amyloid deposit. *F*: Amyloid deposits in TG-Y islets of 41-week-old mice stained with thioflavin T. The fluorescence indicates the presence of β pleated sheet fibrils. Magnification: *A–D* = ×100; *E*, *F* = ×200.

cemia and not diabetes, even at 1 year of age, it is unlikely that amyloid deposition is a direct consequence of hyperglycemia. Another possible interpretation of these model-specific differences is that a common toxic precursor species containing hIAPP mediates cell destruction before being further aggregated into large extracellular deposits. A similar phenomenon has been suggested for the neurotoxicity of prions in the transmissible spongiform encephalopathies (25). If these processes occur independently but in parallel, the question arises as to why there is minimal islet amyloid in the homozygous hIAPP transgenic mice that developed diabetes rapidly versus the current model which developed diabetes

TABLE 3
Mean cross-sectional islet and β-cell area and the mean percentage of islet area occupied by β-cells or by α- and δ-cells at 10 months of age

	Islet area (10^4 μm^2)	β-cell area (10^4 μm^2)	β-cell area (% of islet area)	α- and δ-cell area (% of islet area)
TG-Y	9.4 ± 6.7*	2.4 ± 1.9	26 ± 13†	5.0 ± 3.4§
TG-A	1.3 ± 1.2	1.2 ± 1.1	84 ± 13	5.5 ± 4.5
NTG-Y	7.7 ± 9.0*	7.5 ± 8.8†	93 ± 8‡	0.4 ± 0.7†
NTG-A	1.9 ± 3.2	1.6 ± 2.9	81 ± 15	8.4 ± 5.1

Data are means ± SD. Significant differences: *P < 0.01 vs. TG-A and NTG-A; †P < 0.01 vs. each of the other three groups; ‡P < 0.05 vs. TG-A, P < 0.01 vs. NTG-A; §P < 0.5 vs. NTG-A.

slowly. We propose one model that could account for these different observations. If hIAPP expression is very high, then the production of the small toxic hIAPP aggregates occurs rapidly, producing a toxic burden that overwhelms most of the β-cells. If the expression of hIAPP is low, as in the hemizygous hIAPP transgenic model (11), neither toxic species nor amyloid develops. Expression of hIAPP intermediate to these extremes generates a low burden of toxic species that gradually inflicts β-cell loss. During the intermediate rates of hIAPP expression, the absolute number of toxic species being produced at any time is low, but because the process continues for a long period, the deposition of these species onto an extracellular amyloid deposit progresses accordingly. If this model for amyloidogenesis and β-cell destruction is valid, it would also explain the apparent discrepancy between the extent of islet amyloid and the diabetic phenotype in human type 2 diabetes, because the implication is that amyloidogenesis and β-cell destruction are associated by a common process but can occur at different rates.

Several other lines of evidence support this model. First, in the transgenic mice described in the present study (Fig. 5) as well as in islets of human patients with type 2 diabetes, the surviving β-cells were often embedded in, or surrounded by, large extracellular aggregates, but they showed no morphological appearances of cytotoxicity, suggesting that large extracellular aggregates of IAPP-derived amyloid do not

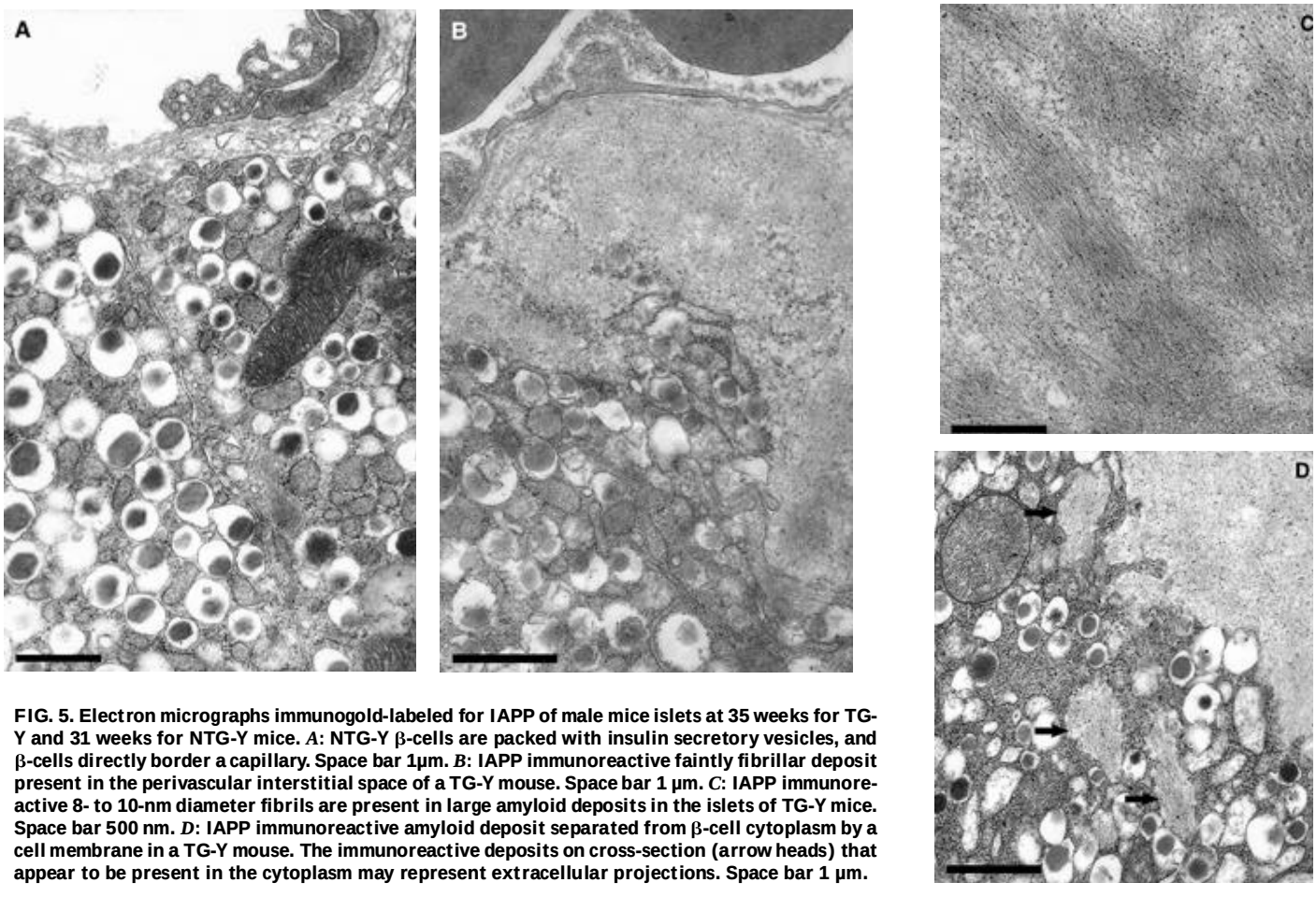

FIG. 5. Electron micrographs immunogold-labeled for IAPP of male mice islets at 35 weeks for TG-Y and 31 weeks for NTG-Y mice. *A*: NTG-Y β-cells are packed with insulin secretory vesicles, and β-cells directly border a capillary. Space bar 1μm. *B*: IAPP immunoreactive faintly fibrillar deposit present in the perivascular interstitial space of a TG-Y mouse. Space bar 1 μm. *C*: IAPP immunoreactive 8- to 10-nm diameter fibrils are present in large amyloid deposits in the islets of TG-Y mice. Space bar 500 nm. *D*: IAPP immunoreactive amyloid deposit separated from β-cell cytoplasm by a cell membrane in a TG-Y mouse. The immunoreactive deposits on cross-section (arrow heads) that appear to be present in the cytoplasm may represent extracellular projections. Space bar 1 μm.

cause β-cell death. Also, in the dexamethasone- and growth hormone–treated mice, marked cytotoxicity was present after 4 weeks of therapy when extensive intracellular small IAPP aggregates were present, but (by light microscopy) only small extracellular islet amyloid deposits were detected (11).

Another novel aspect of the present studies is our demonstration that the deposition of amyloid is clearly influenced by the genetic background of the mice. Thus the hIAPP hemizygous transgenics, on an inbred FVB/N background, display no phenotype up to 2 years of age (11). This study has shown that the same transgenic line on a hybrid FVB/N-C57BL/6 genetic background (TG-A) accumulates detectable amyloid deposits by 10 months. This finding is not surprising considering that expression of type 2 diabetes in humans is known to be highly dependent on the genetic background of individuals (26). The present model in which the development of type 2 diabetes is dependent on the genetic background of the mice provides an exciting tool for a genetic approach to identify susceptibility genes (12,24,27,28).

We have recently observed increased obesity in another transgenic model with high rates of expression of mouse IAPP (W.C.S., J.J., J.C.P., M.D.C., R. Nelson, J. Stock, P.C.B., R.W.S., unpublished observations). The present studies (Fig. 3) appear consistent with that observation. The mechanism of the apparent obesity-stimulating effect of IAPP remains unknown but may be secondary to induction of insulin resistance in early life with increased plasma insulin concentrations (22). This effect is in all likelihood mediated by soluble, circulating IAPP. Reports by other laboratories indicating that IAPP can inhibit insulin secretion in a paracrine fashion (29,30), coupled with our own findings that murine IAPP can affect glucose homeostasis in young transgenic mice (31), suggest that circulating IAPP can contribute in some part to the disease process we have observed in the TG-Y mice. Distinguishing between the pathogenic effects of circulating and aggregated IAPP requires further investigation.

In conclusion, in the present study, we report a novel mouse model that was developed by cross-breeding a homozygous transgenic mouse for hIAPP with a well-established insulin-resistant obese mouse model. The gradual mid-life development of obesity, insulin resistance, and ultimately diabetes is a characteristic shared by type 2 diabetes in humans. This mouse model, therefore, appears to be particularly well suited for further investigations of the pathogenesis and genetic basis of type 2 diabetes in humans and for the development of therapeutic strategies for its prevention and treatment.

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