Extended life span is associated with insulin resistance in a transgenic mouse model of insulinoma secreting human islet amyloid polypeptide

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Andrikopoulos, Sofianos, Rebecca L. Hull, C. Bruce Verchere, Feng Wang, Shani M. Wilbur, Thomas N. Wight, Lucy Marzban, and Steven E. Kahn. Extended life span is associated with insulin resistance in a transgenic mouse model of insulinoma secreting human islet amyloid polypeptide. Am J Physiol Endocrinol Metab 286: E418–E424, 2004. First published November 12, 2003; 10.1152/ajpendo.00137.2003.—Pancreatic amyloid is found in patients with insulinomas and type 2 diabetes. To study mechanisms of islet amyloidogenesis, we produced transgenic mice expressing the unique component of human islet amyloid, human islet amyloid polypeptide (hIAPP). These mice develop islet amyloid after 12 mo of high-fat feeding. To determine whether we could accelerate the rate of islet amyloid formation, we crossbred our hIAPP transgenic animals with RIP-Tag mice that develop islet tumors and die at 12 wk of age from hypoglycemia. At 12 wk of age, this new line of hIAPP×RIP-Tag mice was heavier (29.7 ± 1.0 vs. 25.0 ± 1.3 g, P < 0.05) and had increased plasma glucose levels (4.6 ± 0.4 vs. 2.9 ± 0.6 mmol/l, P < 0.05) compared with littermate RIP-Tag mice. However, the hIAPP×RIP-Tag mice did not display islet amyloid or amyloid fibrils despite high circulating hIAPP levels (24.6 ± 7.0 pmol/l). Interestingly, hIAPP×RIP-Tag mice had a longer life span than RIP-Tag mice (121 ± 8 vs. 102 ± 5 days, P < 0.05). This increase in life span in hIAPP×RIP-Tag was positively correlated with body weight (r = 0.48, P < 0.05) and was associated with decreased insulin sensitivity compared with RIP-Tag mice. hIAPP×RIP-Tag mice did not develop amyloid during their 4-mo life span, suggesting that increased hIAPP secretion is insufficient for islet amyloid formation within such a short time. However, hIAPP×RIP-Tag mice did have an increase in life span that was associated with insulin resistance, suggesting that hIAPP has extrapancreatic effects, possibly on peripheral glucose metabolism.

IAPP is a 37-amino acid peptide localized to secretory granules and cosecreted with insulin in a molar ratio of ~1:100 (25). The mechanism of IAPP deposition as islet amyloid is not known, although an amyloidogenic sequence between amino acids 20–29 such as that present in the primate and cat peptides seems to be essential (32). This region has the propensity to form β-pleated sheets, a secondary structure necessary for amyloid formation (45). The lack of the amyloidogenic sequence in rodent IAPP is thought to be the reason that rats and mice do not develop islet amyloid. However, the sequence alone is not sufficient for amyloidogenesis, since humans without type 2 diabetes or insulinomas rarely develop islet amyloid (8).

To study mechanisms responsible for islet amyloid formation, we generated transgenic mice that express human IAPP (hIAPP) in the β-cells of their pancreas (13) and develop islet amyloid deposits and hyperglycemia when fed a high-fat diet for 12–16 mo (39). The length of time (12–16 mo) required for islet amyloid to be deposited in most of these transgenic mice is compatible with the middle-age onset of the syndrome in humans. However, such an approach is time consuming and expensive. Therefore, mechanisms to accelerate this process were deemed to be potentially useful so that intervention studies could be pursued in a shorter time frame. We hypothesized that, because insulinomas, like type 2 diabetes, are associated with islet amyloid deposition (33), hIAPP overproduction by such islet endocrine tumors may lead to more rapid islet amyloid deposition.

The RIP-Tag-transgenic mouse is an animal model of insulinomas. These mice express the large T-antigen of the SV-40 virus driven by the rat insulin promoter, so that transgene expression is targeted to the β-cells of the pancreas (18). These mice develop islet hyperplasia and hyperinsulinemia, with 1–2% of these islets becoming solid, vascularized tumors (7). As a result, these mice die at ~12 wk of age from hypoglycemia. Tumors from these mice have been excised and cultured in vitro to develop immortal cell lines (BTC-3, BTC-6), which have been extensively used to study synthesis and secretion of β-cell hormones (2, 15, 27).

To study islet amyloid formation in insulinomas, we therefore crossbred our hIAPP transgenic mice with RIP-Tag trans-
genic mice to produce a new, double-transgenic line (hIAPP×RIP-Tag) and characterized these new transgenic mice, which develop islet tumors and secrete hIAPP.

MATERIALS AND METHODS

Transgenic mice. RIP-Tag mice (C57BL/6) were kindly provided by Dr. Douglas Hanahan (University of California, San Francisco, CA) (18) and maintained at the Animal Research Facility of the Seattle Division, Veterans Affairs (VA) Puget Sound Health Care System. hIAPP transgenic mice (C57BL/6 × DBA/2) were bred from a previously described line that had used a 676-bp fragment of cDNA encoding the entire human preproIAPP sequence (13). These mice produce hIAPP in their islets and secrete the peptide with insulin in the typical biphasic pattern when challenged with glucose (40).

To produce the new line of transgenic mice, hemizygous RIP-Tag transgenic male mice (C57BL/6) were mated with hemizygous hIAPP transgenic females (C57BL/6 × DBA/2), and the resulting offspring (75% C57BL/6 × 25% DBA/2) were genotyped using the polymerase chain reaction of tail DNA (14). hIAPP and RIP-Tag were detected using specific primers, with the internal control being the β2-microglobulin gene. All of the studies were approved by the Animal Care Committee at the VA Puget Sound Health Care System.

Breeding of hIAPP and RIP-Tag hemizygous transgenic mice resulted in offspring containing no, one, or both transgenes. Only mice containing no (wild type), the RIP-Tag, or both hIAPP and RIP-Tag transgenes were studied. Thus all mice used in this study were littermates. Both hIAPP×RIP-Tag and RIP-Tag mice developed islet tumors. All animals were kept in a specific pathogen-free environment with a 12:12-h light-dark cycle and had free access to water and a high-fat diet containing 24.4% (wt/wt) fat (Research Diets, Bruns-wick, NJ). Both male and female mice were used in this study. All the mice were weighed and bled at 6, 9, and 12 wk of age for determination of body weight, plasma glucose, and immunoreactive insulin (IRI). They were then followed until natural death. The dates of birth and death were recorded so that life span (in days) could be calculated.

Plasma measurements and assays. Mice were fasted for 4 h and anesthetized with an intraperitoneal injection of pentobarbital sodium (100 mg/kg) before blood sampling. Blood was obtained from the retroorbital sinus by use of heparinized hematocrit capillary tubes, and the animals were allowed to recover. Plasma samples were stored at −20°C until assayed. Mice from which pancreas samples were obtained were killed at 12 wk of age after the final blood sample was collected. After cervical dislocation, a laparotomy was performed and the pancreas excised for histological analysis.

Plasma glucose was measured by an automated glucose oxidase method (Beckman Glucose Analyzer II). Plasma IRI was measured by a modification of the double-antibody radioimmunoassay method of Morgan and Lazarow (31), using rat insulin as the standard. Plasma human IAPP-like immunoreactivity (hIAPP-LI) was measured by an enzyme immunoabsorbance assay by using F024 as the capture and F002 as the detection antibody [kind gift of Amylin Pharmaceuticals, La Jolla, CA (34)]. Plasma mouse IAPP-like immunoreactivity (mIAPP-LI) was measured as previously described (3).

Euglycemic hyperinsulinemic clamp procedure. Twelve-week-old mice were anesthetized as described above. Catheters were inserted into the left jugular vein for the infusion of insulin and glucose and the right carotid artery for blood sampling. Insulin was infused at a rate of 25 mU·kg−1·min−1, and plasma glucose concentrations were kept at euglycemia with the infusion of a 5% glucose solution. Stable plasma glucose concentrations were achieved after 60–90 min of insulin infusion, at which time the glucose infusion rate was determined and used as a measure of insulin sensitivity. To account for differences in the steady-state plasma glucose and IRI levels, insulin sensitivity was calculated as glucose infusion rate/(clamped insulin level × clamped glucose level) (6).

Immunocytochemical analysis. For light microscopy, pancreata were fixed in 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde, rinsed in 0.1 M phosphate buffer (pH 7.4), and embedded in paraffin. Sections (5 μm) were deparaffinized and rehydrated before staining.

Immunodetection of pancreatic hormones was performed with the following antibodies diluted in 0.1 M phosphate buffer (pH 7.4)/0.5% BSA: insulin antibody (Sigma, St. Louis, MO) at a dilution of 1:2,000; rodent IAPP antibody (8342, which recognizes both mouse and rat but not human IAPP) at a dilution of 1:500; somatostatin antibody (AS-10, kind gift from Dr. John Ensinck) at a dilution of 1:2,000; and glucagon antibody [14C, gift from Dr. Robert McEvoy (5)] at a dilution of 1:1,000. Slides were rinsed and treated with the Vector ABC kit (Vectastain ABC Kit Elite PK-6100 Standard) and then treated with the Vector DAB Peroxidase Substrate Kit. Slides were counterstained with hematoxylin and dehydrated, and coverslips were mounted with permount.

Western blotting analysis. Tissue was powdered in liquid nitrogen and lysed in lysis buffer containing 50 mM Tris-HCl (pH 8), 150 mM NaCl, 0.02% sodium azide, 0.1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 20 μg/ml leupeptin, 1 μM pepstatin A, 1% Nonidet P-40, and 0.5% sodium deoxycholate for 25 min on ice. Samples were centrifuged at 15,000 g, and the supernatant was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Ten milligrams of protein were electrophoresed on a Tris-tricine gel, transferred to a polyvinylidene difluoride membrane, and blocked with 5% skim milk for 2 h at room temperature. The membrane was incubated with 1:1,000 rabbit anti-rat IAPP antibody (cat. no. RIN7323; Peninsula Laboratories, San Carlos, CA) for 1 h followed by 1:5,000 hors eradish peroxidase-conjugated antirabbit antibody for 1 h at room temperature. Detection was achieved using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ).

Statistical analysis. The Mann-Whitney U-test was used to compare data from the two groups of mice. The Wilcoxon signed rank test was used when data were paired. Analysis of life span was performed using Kaplan-Meier survival statistics. A P value of ≤0.05 was considered statistically significant.

RESULTS

Characterization of offspring from hIAPP and RIP-Tag transgenic mice. Results of weight, plasma glucose, and IRI determinations for wild-type, RIP-Tag, and hIAPP×RIP-Tag littermate mice are presented in Table 1. Both wild-type and hIAPP×RIP-Tag mice showed an increase in body weight over the 6-wk period of observation between 6 and 12 wk of age. RIP-Tag mice did not gain weight from 9 wk of age onward. Body weight was higher in hIAPP×RIP-Tag mice at both 9 and 12 wk of age compared with RIP-Tag mice. Plasma glucose levels decreased in both hIAPP×RIP-Tag and RIP-Tag mice from 6 to 12 wk of age. hIAPP×RIP-Tag mice had higher plasma glucose levels compared with RIP-Tag mice only at 12 wk of age. Wild-type mice had higher plasma glucose levels at all ages studied compared with both hIAPP×RIP-Tag and RIP-Tag mice. Plasma IRI levels increased with age in both hIAPP×RIP-Tag and RIP-Tag mice, although no difference was observed between these genotypes. Wild-type mice had significantly lower plasma IRI levels at all ages tested compared with both hIAPP×RIP-Tag and RIP-Tag mice.

Plasma hIAPP-LI was measured with a two-site immunoab sorption assay in all mice at 12 wk of age. The capture antibody binds to the amidated COOH terminus of IAPP, and the detection antibody binds to the 20- to 29-amino acid region
Table 1. Characteristics of offspring from an intercross of hIAPP and RIP-Tag transgenic mice between 6 and 12 wk of age

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>RIP-Tag</th>
<th>hIAPP×RIP-Tag</th>
</tr>
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<tbody>
<tr>
<td><strong>6 wk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>31</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>Weight, g</td>
<td>21.3±0.8</td>
<td>22.0±0.9</td>
<td>23.2±0.7</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>12.0±0.5</td>
<td>7.2±0.3</td>
<td>6.7±0.3‡</td>
</tr>
<tr>
<td>IRI, pmol/l</td>
<td>148.9±17.5</td>
<td>671.4±112.9‡</td>
<td>503.3±127.4‡</td>
</tr>
<tr>
<td><strong>9 wk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>30</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Weight, g</td>
<td>25.2±1.0</td>
<td>24.9±1.1</td>
<td>27.3±0.9*</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>12.5±0.7</td>
<td>4.9±0.3</td>
<td>5.9±0.3‡</td>
</tr>
<tr>
<td>IRI, pmol/l</td>
<td>177.3±26.2</td>
<td>834.5±227.2‡</td>
<td>699.4±92.9‡</td>
</tr>
<tr>
<td><strong>12 wk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>30</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Weight, g</td>
<td>27.2±1.1</td>
<td>24.5±1.2</td>
<td>29.7±1.1†</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>12.0±0.5</td>
<td>3.0±0.5</td>
<td>4.3±0.3‡</td>
</tr>
<tr>
<td>IRI, pmol/l</td>
<td>206.2±23.8</td>
<td>1345.2±212.5‡</td>
<td>1265.0±187.8‡</td>
</tr>
</tbody>
</table>

Results are presented as means ± SE. RIP-Tag, C57BL/6 transgenic mice; hIAPP×RIP-Tag, human islet amyloid polypeptide transgenic mice crossed with RIP-Tag mice; IRI, immunoreactive insulin. *P < 0.05 compared with RIP-Tag transgenic mice. †P < 0.01 compared with RIP-Tag transgenic mice. ‡P < 0.01 compared with wild-type mice.

of human IAPP, so that the assay measures only hIAPP-LI. Thus, in mice that did not express the hIAPP gene (i.e., wild-type and RIP-Tag), plasma hIAPP-LI was not detected. In hIAPP×RIP-Tag mice, the mean plasma hIAPP-LI concentration was 24.6 ± 7.0 pmol/l, which is ~2.5 times higher than in hIAPP transgenic littermates (9.3 ± 2.6 pmol/l, P < 0.05) that had been fed the same diet and had blood sampled at 12 wk of age.

Because of the limited blood volume of mice, mIAPP-LI in plasma was measured by pooling plasma samples from 3–4 animals obtained at death (12 wk of age). The results suggest an increased amount of mIAPP-LI in hIAPP×RIP-Tag (47.1 ± 11.8 pmol/l, n = 2) and RIP-Tag mice (28.6 ± 4.1 pmol/l, n = 2) compared with wild-type mice (5.7 ± 0.3, n = 4).

Pancreatic histology. Results of islet morphology and immunostaining of serial sections of pancreas for insulin, IAPP, glucagon, and somatostatin are shown in Fig. 1. Sections through tumorous and normal-sized islets from hIAPP×RIP-Tag mouse pancreata revealed immunolabeling for insulin and IAPP throughout. Glucagon and somatostatin immunoreactivity in tumors was rare, confirming previous reports that the islet tumors in RIP-Tag mice were composed almost exclusively of β-cells (18, 35). In normal-sized islets from hIAPP×RIP-Tag mice, glucagon and somatostatin immunoreactivity was located in the periphery, where α- and δ-cells were expected to reside. The pattern of immunostaining in RIP-Tag mouse pancreata was essentially the same as in hIAPP×RIP-Tag pancreata (data not shown).

Wild-type mice displayed insulin and IAPP immunoreactivity throughout regularly shaped islets, whereas glucagon and somatostatin labeling was primarily located in the periphery of the islets (Fig. 1).

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Fig. 1. Immunostaining for islet hormones in pancreatic sections from a wild-type mouse (left) and tumor and islets from a transgenic human islet amyloid polypeptide (hIAPP)×RIP-Tag (C57BL/6) mouse (middle and right, respectively). Immunostaining for insulin (A–C) and rodent IAPP (D–F) was present in islets and tumor tissue from both wild-type and hIAPP×RIP-Tag mice. Immunostaining for glucagon (G–I) and somatostatin (J–L) was present in the periphery of wild-type islets (G and J) and normal hIAPP×RIP-Tag islets (I and L) but was rarely observed in hIAPP×RIP-Tag tumors (J and K). Scale bars, 100 μm.
Western blotting. Impaired proteolytic processing of proinsulin to mature insulin is a feature of insulinomas. Western blotting was performed to determine whether processing of IAPP from its precursor proIAPP was impaired in tumor-bearing mice and to ascertain whether differences existed in IAPP processing between RIP-Tag and hIAPP×RIP-Tag tumors. Figure 2 shows that, although the major band corresponded to mature IAPP (4 kDa), both RIP-Tag and hIAPP×RIP-Tag tumors had more higher molecular mass bands corresponding to unprocessed proIAPP (~8 kDa) than wild-type islets. There was no large difference in the intensity of these higher molecular mass bands between RIP-Tag and hIAPP×RIP-Tag tumors (Fig. 2).

Microscopy for identification of amyloid and amyloid fibrils. Light microscopy of thioflavin S-stained tumors and islets from hIAPP×RIP-Tag and RIP-Tag transgenic mice showed no evidence of amyloid at 12 wk of age.

Because the presence of amyloid deposits visible by light microscopy is preceded by the development of fibrils visible only by electron microscopy, we examined four hIAPP×RIP-Tag mice at 12 wk of age and two hIAPP×RIP-Tag mice at 22 wk of age for the presence of fibrils. Despite extensive examination, amyloid fibrils were not found in hIAPP×RIP-Tag mice. As expected, assessment of tumors from RIP-Tag mice (n = 6) did not display any fibrils, as they produce and secrete rodent IAPP, which is nonamyloidogenic.

Life span assessment of RIP-Tag vs. hIAPP×RIP-Tag transgenic mice. During follow-up of these transgenic mice, we observed that hIAPP×RIP-Tag had higher body weights and plasma glucose concentrations at 12 wk of age compared with RIP-Tag mice. We also observed that, in our initial cohort, the decrement in plasma glucose between 6 and 12 wk of age was smaller in hIAPP×RIP-Tag compared with RIP-Tag mice (−2.6 ± 0.5 vs. −4.2 ± 0.6 mmol/l, respectively, P = 0.05).

We thus hypothesized that hIAPP×RIP-Tag mice may live longer than RIP-Tag mice due to a slower decrease in plasma glucose levels. A group of hIAPP×RIP-Tag and RIP-Tag mice were thus bled at 6 and 12 wk of age and then followed until natural death. In this group, weight, plasma glucose, and IRI were not different between hIAPP×RIP-Tag and RIP-Tag mice at 6 wk of age (Table 2). However, as we observed in the original cohort, body weight and plasma glucose levels were higher in hIAPP×RIP-Tag mice compared with RIP-Tag mice at 12 wk of age (P < 0.05), whereas plasma IRI concentrations were similar in both groups of mice.

The life span of hIAPP×RIP-Tag mice (120.9 ± 7.5 days, n = 13) was significantly longer compared with their RIP-Tag littermates (101.5 ± 5.3 days, n = 10, P < 0.05). This mean difference of 19.4 days represents a 19% increase in life span for the hIAPP×RIP-Tag mice. Analysis of the data with Kaplan-Meier survival statistics confirmed this and showed marked divergence of the two survival curves with hIAPP×RIP-Tag mice living longer than RIP-Tag mice (P < 0.05; Fig. 3). Life span correlated significantly with body weight at 12 wk of age (r = 0.48, P < 0.05; Fig. 4A) but did not correlate with plasma glucose (r = 0.33, P = 0.13) or plasma IRI (r = 0.17, P = 0.42) concentrations.

Insulin sensitivity of RIP-Tag vs. hIAPP×RIP-Tag transgenic mice. Euglycemic hyperinsulinemic clamps were performed to determine whether the higher body weight and plasma glucose levels and consequent increased life span in hIAPP×RIP-Tag mice were due to reduced insulin sensitivity. Plasma glucose levels were significantly higher and plasma IRI concentrations tended to be higher in hIAPP×RIP-Tag compared with RIP-Tag transgenic mice (Table 3). Although the glucose infusion rate was not different, when corrected for differences in the plasma glucose and IRI levels at steady state, this parameter was significantly lower in hIAPP×RIP-Tag compared with RIP-Tag transgenic mice (Table 3). Furthermore, there was a significant inverse correlation between body weight at 12 wk of age and insulin sensitivity (r = 0.70, P < 0.05; Fig. 4B). This finding is compatible with the heavier hIAPP×RIP-Tag mice being more insulin resistant compared with RIP-Tag transgenic mice.

![Fig. 2. Western blot analysis for IAPP-like immunoreactive peptides in a wildtype (Wt) islet and RIP-Tag and hIAPP×RIP-Tag islet tumors. The intense band at 4 kDa represents mature IAPP, whereas the other bands of higher molecular mass represent incompletely processed (pro)IAPP peptides.](image1)

![Fig. 3. Kaplan-Meier survival curve analysis of 13 hIAPP×RIP-Tag (C) and 10 RIP-Tag mice (●), demonstrating that hIAPP×RIP-Tag mice lived longer than RIP-Tag mice.](image2)

**Table 2. Characteristics at 6 and 12 wk of age of hIAPP×RIP-Tag and RIP-Tag transgenic mice used in the life span assessment study**

<table>
<thead>
<tr>
<th></th>
<th>RIP-Tag</th>
<th>hIAPP×RIP-Tag</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Weight, g</td>
<td>21.5±1.2</td>
<td>23.3±0.9</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>6.9±0.5</td>
<td>6.8±0.5</td>
</tr>
<tr>
<td>IRI, pmol/l</td>
<td>675.3±117.8</td>
<td>579.6±234.5</td>
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</tbody>
</table>

Results are presented as means ± SE. *P < 0.05 compared with RIP-Tag transgenic mice.
DISCUSSION

Amyloid deposits are characteristically found in insulinomas and in the islets from patients with type 2 diabetes. The mechanism(s) responsible for amyloid formation has not been identified (24). To investigate islet amyloidogenesis, we generated transgenic mice expressing hIAPP in pancreatic β-cells and found that, when these mice were fed a high-fat diet for 12–16 mo, they developed islet amyloid (39). The length of time necessary for islet amyloid deposition in these hIAPP transgenic mice, although consistent with the middle-age onset of type 2 diabetes in humans, is somewhat disadvantageous, as it is time consuming and expensive. We therefore hypothesized that we could increase deposition of islet amyloid by increasing hIAPP secretion and thereby shorten the time required for amyloid formation. We thus generated an animal model of increased hIAPP secretion by creating a new transgenic mouse line that develops insulinomas expressing and secreting hIAPP.

Close examination of islet tumors from hIAPP×RIP-Tag mice at both the light and electron microscopic level did not reveal any evidence of amyloid deposits or fibrils. This is despite increased plasma hIAPP-LI levels in hIAPP×RIP-Tag mice compared with age-matched hIAPP transgenic littermates. The most probable reason for the absence of amyloid is the fact that hIAPP×RIP-Tag mice have a considerably shortened life span compared with normal hIAPP transgenic or wild-type littermates. Although we examined islet tumors from 12- and 22-wk-old (longest surviving) high-fat-fed hIAPP×RIP-Tag mice, this may not be sufficient time for amyloid deposits or fibrils to form. We (39) have previously shown that islet amyloid forms after hIAPP transgenic mice are fed a high-fat diet for at least 12 mo. Furthermore, islet amyloid deposition was reduced in 12-mo-old mice that were hyperglycemic and had impaired hIAPP release due to disruption of the β-cell glucokinase gene (3). These studies and the present one suggest that, although a certain level of secretion needs to be maintained, increased hIAPP production and secretion alone are not associated with an acceleration of islet amyloid deposition.

Another possible reason for the lack of amyloid formation in the islet tumors may be that the increased production of insulin and its precursors may impair fibril formation in vivo, as has been demonstrated to occur in vitro (22, 28, 47). Because insulinomas secrete increased amounts of proinsulin, insulin, and C-peptide (10), it is possible that these peptides may act to impair fibril formation. Furthermore, it has been shown that insulin can form heteromolecular complexes with hIAPP in vitro that suppress amyloid fibril formation (21), and mouse IAPP can also antagonize and dose-dependently inhibit fibril formation by hIAPP (43). It is also likely that these tumors release increased amounts of prohIAPP, as this propeptide and proinsulin are proteolytically cleaved by the same processing enzymes (19, 41) and, as mentioned above, insulinomas are known to produce and release increased amounts of proinsulin (10, 36). We have now been able to demonstrate by Western blotting that the tumors do in fact contain increased amounts of a high molecular mass band that, based on its molecular mass, is consistent with it being prohIAPP. Although the tumors contained increased amounts of this larger IAPP-like peptide, the similar intensities of the bands did not allow us to determine whether there was a difference in the amount of higher molecular mass IAPP produced by the RIP-Tag and hIAPP×RIP-Tag tumors. However, although prohIAPP output may be increased, we do not believe that prohIAPP is likely to be inhibitory to the amyloidogenic process in hIAPP×RIP-Tag for the following reasons. 1) As stated earlier, pancreatic islet tumors are known to contain islet amyloid (1, 38, 48) and to be associated with increased propeptide secretion (10); 2) islet amyloid and increased proinsulin release occur in patients with type 2 diabetes (42), and disproportionate proinsulin levels predict the development of this disease (26); and 3) histological evidence has shown that the NH2-terminal portion of the prohIAPP molecule is associated with islet amyloid in humans with type 2 diabetes (44, 46).

Table 3. Metabolic characteristics of 12-wk-old hIAPP×RIP-Tag and RIP-Tag transgenic mice during euglycemic hyperinsulinemic clamps

<table>
<thead>
<tr>
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<th>RIP-Tag</th>
<th>hIAPP×RIP-Tag</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>No. of animals</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>7.2±0.1</td>
<td>7.9±0.3</td>
<td>0.021</td>
</tr>
<tr>
<td>IRI, pmol/l</td>
<td>12,653±2,288</td>
<td>43,079±13,246</td>
<td>0.070</td>
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<tr>
<td>GIR, μmol·kg⁻¹·min⁻¹</td>
<td>10.2±0.6</td>
<td>7.6±1.7</td>
<td>0.219</td>
</tr>
<tr>
<td>GIR/(plasma glucose × plasma IRI)</td>
<td>6.3±1.0×10⁻⁶</td>
<td>2.7±1.0×10⁻⁶</td>
<td>0.032</td>
</tr>
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</table>

Results are presented as means ± SE. GIR, glucose infusion rate.
An interesting consequence of hIAPP expression and production in hIAPP×RIP-Tag transgenic mice was the increased life span compared with RIP-Tag mice. This increased longevity of our new line of double-transgenic mice is of interest and implies that, besides islet amyloidogenesis, hIAPP may have a biological role in carbohydrate metabolism. It has previously been suggested that IAPP may cause insulin resistance by inhibiting glucose uptake and glycogen storage in muscle (29). Chronic systemic administration of IAPP has been shown to be associated with increased body weight gain and increased fat mass (12). We performed hyperinsulinemic clamps and observed that hIAPP×RIP-Tag mice had reduced insulin sensitivity compared with RIP-Tag mice. Thus the presence of the hIAPP transgene resulted in increased circulating hIAPP and reduced insulin sensitivity. Because the concentrations of both plasma human and mouse IAPP in hIAPP×RIP-Tag transgenic mice were in the physiological range (3, 20, 37), it is unlikely that the effects on insulin sensitivity were due to a pharmacological effect of these peptides. As indicated by the significant inverse correlation between insulin sensitivity and body weight (Fig. 4B), this reduction in insulin sensitivity may contribute to, or be the result of, the higher body weight and plasma glucose levels in the hIAPP×RIP-Tag mice, thereby increasing their life span. This reduction in insulin sensitivity in the hIAPP×RIP-Tag mice is also compatible with the trend for the steady-state plasma insulin levels during the clamp to be increased as a manifestation of a reduction in insulin clearance (16). IAPP may also affect insulin release, as suggested by a study of IAPP-null mice, which found that the absence of IAPP was associated with increased insulin release and improved glucose tolerance (17). Because hIAPP was present in the circulation of the hIAPP×RIP-Tag mice and therefore they had greater circulating total (human and mouse) IAPP levels than the RIP-Tag mice, we cannot exclude the possibility that increased release of IAPP in these double-transgenic mice may also have resulted in a reduction in insulin release and contributed to the increased life span of these mice. Because insulinomas are poorly responsive to glucose stimulation, it was not possible to perform a reliable assessment of stimulated insulin secretion. However, the possibility that insulin release was reduced in the hIAPP×RIP-Tag mice is supported indirectly by the fact that plasma insulin levels tended to be lower in these mice despite the fact that they were insulin resistant and had higher plasma glucose levels, two scenarios that would be expected to raise insulin levels.

Another possible reason for the increased life span in the double-transgenic mice is that hIAPP is killing β-cells, leading to reduced insulin release. Human IAPP has been shown to be cytotoxic to and induce apoptosis in pancreatic islet cells (4, 23, 30). To determine whether hIAPP production in hIAPP×RIP-Tag mice may be killing tumorous β-cells, we performed propidium iodide staining of pancreatic sections but did not observe amyloid fibrils in islets or tumors from hIAPP×RIP-Tag mice, and the plasma IRI levels were comparable in hIAPP×RIP-Tag and RIP-Tag mice. Thus we do not believe that islet cell cytotoxicity is a likely explanation for the increased life span of the hIAPP×RIP-Tag mice.

In conclusion, we have generated a new line of transgenic mice that develop pancreatic islet tumors that produce and secrete hIAPP, resulting in elevated plasma hIAPP levels. Despite this increase in hIAPP release, these mice did not develop islet amyloid, suggesting that overproduction of hIAPP is not likely to greatly accelerate amyloid formation in vivo. Interestingly, these mice also develop insulin resistance and live significantly longer than mice that develop islet tumors but do not produce hIAPP, suggesting a possible role of IAPP to impair glucose metabolism.

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