Long-Term Treatment With Rosiglitazone and Metformin Reduces the Extent of, but Does Not Prevent, Islet Amyloid Deposition in Mice Expressing the Gene for Human Islet Amyloid Polypeptide

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Islet amyloid deposition in type 2 diabetes is associated with reduced β-cell mass. Therefore, interventions aimed at reducing islet amyloid formation may help preserve β-cell mass in type 2 diabetes. Rosiglitazone and metformin act by different mechanisms to improve insulin sensitivity and thereby reduce β-cell secretory demand, resulting in decreased release of insulin and islet amyloid polypeptide (IAPP), the unique constituent of islet amyloid deposits. We hypothesized that this reduced β-cell secretory demand would lead to reduced islet amyloid formation. Human IAPP (hIAPP) transgenic mice, a model of islet amyloid, were treated for 12 months with rosiglitazone (1.5 mg·kg⁻¹·day⁻¹, n = 19), metformin (1 g·kg⁻¹·day⁻¹, n = 18), or control (n = 17). At the end of the study, islet amyloid prevalence (percent islets containing amyloid) and severity (percent islet area occupied by amyloid), islet mass, β-cell mass, and insulin release were determined. Islet amyloid prevalence (44 ± 8, 13 ± 4, and 11 ± 3% for control, metformin-, and rosiglitazone-treated mice, respectively) and severity (9.2 ± 3.0, 0.22 ± 0.11, and 0.10 ± 0.05% for control, metformin-, and rosiglitazone-treated mice, respectively) were markedly reduced with both rosiglitazone (P < 0.001 for both measures) and metformin treatment (P < 0.001 for both measures). Both treatments were associated with reduced insulin release assessed as the acute insulin response to intravenous glucose (2,189 ± 857, 621 ± 256, and 14 ± 158 pmol/l for control, metformin-, and rosiglitazone-treated mice, respectively; P < 0.05 for metformin vs. control and P < 0.005 for rosiglitazone vs. control), consistent with reduced secretory demand. Similarly, islet mass (33.4 ± 7.0, 16.6 ± 3.6, and 12.2 ± 2.1 mg for control, metformin-, and rosiglitazone-treated mice, respectively) was not different with metformin treatment (P = 0.06 vs. control) but was significantly lower with rosiglitazone treatment (P < 0.05 vs. control). When the decreased islet mass was accounted for, the islet amyloid–related decrease in β-cell mass (percent β-cell mass/islet mass) was ameliorated in both rosiglitazone- and metformin-treated animals (57.9 ± 3.1, 64.7 ± 1.4, and 66.1 ± 1.6% for control, metformin-, and rosiglitazone-treated mice, respectively; P < 0.05 for metformin or rosiglitazone vs. control). In summary, rosiglitazone and metformin protect β-cells from the deleterious effects of islet amyloid, and this effect may contribute to the ability of these treatments to alleviate the progressive loss of β-cell mass and function in type 2 diabetes.

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Type 2 diabetes is characterized by insulin resistance and islet β-cell secretory dysfunction. In addition, several studies (1–3) have shown that reduced β-cell mass is a feature of the disease. Whether this β-cell mass loss is progressive in nature is not clear from available data, but a recent study (3) has shown β-cell mass to be already reduced in individuals with impaired fasting glucose, suggesting that decreased β-cell mass precedes the clinical diagnosis of type 2 diabetes.

One contributor to decreased β-cell mass is islet amyloid. Islet amyloid deposits occur in the majority of subjects with type 2 diabetes (4) and contain as their unique component the β-cell peptide islet amyloid polypeptide (IAPP) (5,6). In vitro data demonstrate that small aggregates or fibrils formed from amyloidogenic human IAPP (hIAPP) are toxic to β-cells in culture (7). This effect is specific for the amyloidogenic form of IAPP and is not observed with rodent IAPP, which is nonamyloidogenic (8). Moreover, in vivo data from several groups that have generated hIAPP transgenic mice as models of islet amyloid formation show that hIAPP expression and/or amyloid fibril formation is associated with increased β-cell apoptosis (9) and that nature, light microscopy–visible amyloid is associated with reduced β-cell mass (10–12). Longitudinal data from Macaca nigra monkeys, which spontaneously develop islet amyloid deposits and diabetes, showed that continued islet amyloid deposition over time paralleled the development of hyperglyce-
mia (13). In humans, extensive islet amyloid deposition has been documented in type 2 diabetic individuals with decreased β-cell mass (2,3), suggesting that islet amyloid may play a role in the decrease in β-cell mass that characterizes type 2 diabetes. Thus, interventions that reduce islet amyloid deposition may be beneficial in maintaining β-cell mass before and during the progression of type 2 diabetes.

The thiazolidinediones, a family of peroxisome proliferator–activated receptor γ ligands, are antidiabetic agents that primarily act by improving peripheral insulin sensitivity. Since insulin sensitivity is an important modulator of insulin release from the β-cell (14), thiazolidinediones also result in reduced secretory demand on the β-cell and have been shown to improve β-cell function in individuals with type 2 diabetes (15) and individuals at increased risk for developing type 2 diabetes (16). Rosiglitazone and pioglitazone, both thiazolidinediones, have also been shown to improve islet architecture in animal models of diabetes (17,18). Whether this improvement results from a direct effect of these agents on the islet, from improved insulin sensitivity and reduced secretory demand on the β-cell, or a combination of both remains unclear. Peroxisome proliferator–activated receptor γ expression has been shown in human and rodent islets (19,20). However, studies (21,22) examining direct effects of thiazolidinediones on the β-cell have yielded mixed results.

Metformin is another glucose-lowering agent that acts predominantly at the level of the liver to suppress hepatic glucose output (23) and is an effective treatment in individuals with type 2 diabetes. It is also effective in individuals at increased risk of developing type 2 diabetes, having been shown in the Diabetes Prevention Program to reduce the development of diabetes by 31% (24). In addition to its effect to improve insulin action, metformin may also have a direct effect at the level of the β-cell, where it may counteract the effects of elevated glucose and free fatty acids to decrease β-cell secretory function and viability (25–28).

Thus, both rosiglitazone and metformin have been shown to have direct and/or indirect beneficial effects on the β-cell. In the present study, we sought to determine whether prolonged treatment with rosiglitazone or metformin could reduce islet amyloid formation in hIAPP transgenic mice and thereby alleviate the deleterious effects of islet amyloid on the β-cell.

RESEARCH DESIGN AND METHODS

Transgenic mice. Hemizygous transgenic mice expressing hIAPP in their pancreatic islet β-cells (29) were generated by breeding hIAPP transgenic C57BL/6 female mice with DBA/2 wild-type male mice. Transgenic status was determined by PCR of genomic DNA using primers directed against the hIAPP transgene, as previously described (30). Only F1 male hIAPP transgenic mice were studied, as we have previously observed islet amyloid in 81% of male hIAPP transgenic mice but only in 11% of female transgenic littermates (31). The study was approved by the Institutional Animal Care and Use Committee at the Seattle Veterans Affairs Puget Sound Health Care System.

Groups and medications. At 6–8 weeks of age, hIAPP transgenic mice were randomly assigned to one of three medication groups: control (n = 17), metformin (1 g · kg⁻¹ · day⁻¹; n = 19), or rosiglitazone (1.5 mg · kg⁻¹ · day⁻¹; n = 10) and followed for 1 year. Medications were provided in the drinking water; control mice received plain drinking water only. Water intake was randomly monitored throughout the study, was found to increase in proportion to body weight (r = 0.69, P < 0.001), and was not different among treatment groups (0.003 ± 0.004, 0.005 ± 0.011, and 0.005 ± 0.004 mg · g⁻¹ · day⁻¹ for control, metformin-, and rosiglitazone-treated mice, respectively). Thus, based on the water intake measurements, the mean achieved dose for metformin was 0.98 ± 0.11 g · kg⁻¹ · day⁻¹ and for rosiglitazone was 1.43 ± 0.06 mg · kg⁻¹ · day⁻¹. Mice were fed a diet containing 18% kcal from fat (9% fat by weight; Purina autoclavable mouse diet no. 5021), which is a moderate-fat diet and has been previously shown to induce islet amyloid deposition (31). Mice were given free access to food and water, unless otherwise stated.

Body weight and body composition. Body weight was assessed after commencing the medications and then following 1, 3, 6, 9, and 12 months of treatment. Before sacrifice, fat mass was determined by proton magnetic resonance spectroscopy in pentobarbital-anesthetized mice (100 mg/kg i.p.) (32). This measurement was performed in 9 rosiglitazone-treated, 14 metformin-treated, and 11 control mice. When the mice were killed, body fat distribution was assessed in all mice by weighing visceral (omental plus mesenteric) as well as subcutaneous (inguinal) fat pads.

**Glucose-stimulated insulin secretion.** After 12 months, glucose-stimulated insulin secretion was assessed in mice (rosiglitazone n = 13, metformin n = 9, and control n = 11) in response to an intravenous glucose load (1 g/kg dextrose) administered into the jugular vein under pentobarbital anesthesia (100 mg/kg i.p.). Blood samples were drawn before and 2, 5, and 10 min following glucose injection. Plasma was separated by centrifugation and stored at −20°C before assay for glucose and immunoreactive insulin (IRI). Plasma glucose was determined by glucose assay and pancreatic IRI was measured in all mice by radioimmunoassay (33). Serum insulin was measured by radioimmunoassay (30). Plasma total cholesterol and triglyceride levels were obtained from overnight-fasted animals under pentobarbital anesthesia (100 mg/kg i.p.) before they were killed. When they were killed, a small portion of the pancreas was snap frozen for homogenization in 50% (vol/vol) isopropanol/1% (vol/vol) trifluoroacetic acid and subsequent measurement of pancreatic IRI, hIAPP-LI, mouse IAPP-LI, and protein content.

**Assays.** Plasma glucose was determined using a glucose oxidase method. Plasma levels and pancreatic content of IRI were measured by radioimmunoassay (30). Plasma and pancreatic hIAPP-LI were quantified by enzyme immunoabsorbance assay, with F024 and F002 as the capture and detection antibodies (a kind gift from Amylin Pharmaceuticals, San Diego, CA). Pancreatic mouse IAPP-LI was determined using a previously described radioimmunoassay for rodent IAPP (30). Total protein for determinations of pancreatic IRI, hIAPP-LI, and mouse IAPP-LI content was assessed using a bicinchoninic acid kit (Pierce Biotechnology, Rockford, IL). Serum free fatty acids were measured using the nonesterified fatty acid (NEFA)-C kit (Wako Chemicals, Richmond, VA). Plasma triglycerides were determined by enzymatic hydrolysis using a triglyceride GB kit (Roche Diagnostics, Indianapolis, IN). Total plasma cholesterol was measured using a total serum cholesterol kit (Diagnostica Stago, Parsippany, NJ).

**Histological assessment of islet amyloid, islet area, and β-cell area.** When the mice were killed, pancreata were excised, weighed, and fixed in 4% (wt/vol) phosphate-buffered paraffin/paraffin embedded in paraffin. Five-micrometer sections were cut and stained with thioflavin S to visualize amyloid deposits and anti-insulin antibody (Sigma; 1:2,000) followed by cy3-conjugated anti-mouse immunoglobulins to visualize β-cells. Histological assessments were made on an average of 34 islets per mouse on three sections representing the head, body, and tail of the pancreas. We have previously shown this sampling technique to be sufficiently representative of a whole mouse pancreas (11).

**Assessment of β-cell replication and apoptosis.** Serial sections were costained with insulin together with anti-Ki-67 antibody to visualize β-cells. Histochemical assessments were made on 25 sections representing the head, body, and tail of the pancreas. The pancreas was excised. Islets were separated on a histopaque gradient and hand picked. A sample of islets from each isolation procedure was taken for determination of islet amyloid formation. Following isolation, islets were cultured overnight in RPMI-1640 containing 10% fetal bovine serum, 5 μg/ml gentamicin, and 11.1 mmol/l glucose and then cultured for 7 days in RPMI-1640, 10% fetal bovine serum, 5 μg/ml gentamicin, and 16.7 mmol/l glucose alone or in the presence of islet amyloid (1.5 μg/ml) or metformin (2.4 μg/ml). Each experiment (n = 3) comprised 100 islets in duplicate per culture condition.
After 7 days of culture, islets (n = 50 in duplicate) were fixed in 4% (wt/vol) phosphate-buffered paraformaldehyde. Islets were embedded in agar, refixed in 4% paraformaldehyde, embedded in paraffin, and processed for histology. Five-micrometer sections were cut throughout the islet pellet, and sections at 50-μm intervals were stained for amyloid with thioflavin S as described above.

Static secretion studies were performed on parallel sets of islets (n = 10 or 20 islets in duplicate; n = 3 independent experiments). Insulin release in response to basal (1.67 mmol/l) and elevated (16.7 mmol/l) glucose concentrations was assessed. Insulin content per islet was determined for each culture condition.

Calculations and data analysis. For in vivo studies, incremental body weight was calculated as body weight at the time mice were killed minus body weight at baseline (6–8 weeks of age). Body fat distribution was quantified in each mouse as the ratio of visceral (omental plus mesenteric)-to-subcutaneous (inguinal) fat pad mass. Insulin release in vivo was determined as the acute insulin response following intravenous glucose and was calculated as the mean IRI level from 2 to 10 min following glucose administration, with basal IRI (time 0) subtracted from this value.

Subsets of mice from each treatment group were randomly selected for determination of body fat mass and insulin release. A proportion of these mice underwent both body fat and insulin secretion measurements (n = 5, n = 5, and n = 4 for control, metformin, and rosiglitazone-treated mice, respectively). Measurements of amyloid deposition, β-cell mass, fat mass, fat distribution, and insulin release in this subset of mice were not different from the whole cohort.

Islet amyloid, and β-cell areas (insulin-positive area) were computed for pancreas sections and isolated islet samples, as we have previously done (11). Pancreatic section area was also determined for the in vivo study. From these measures, islet amyloid prevalence (percentage of islets containing amyloid) and severity (Σ amyloid area/Σ islet area × 100%) were derived. Islet mass and β-cell mass were calculated as follows: Σ islet area/pancreatic section area × pancreatic weight and Σ insulin-positive area/pancreatic section area × pancreatic weight, respectively, and the proportion of β-cell mass to islet mass was calculated as (β-cell mass/islet mass) × 100%. The proportion of β-cells undergoing replication and apoptosis were quantified as the number of Ki-67 or activated caspase 3–positive islet β-cells, respectively/total number of β-cells assessed × 100%. Insulin release in vitro to response to 1.67 or 16.7 mmol/l glucose was assessed as fractional release [medium IRI/(medium IRI + islet IRI content) × 100%].

Data are expressed as means ± SE. Body weight data were analyzed using a repeated measures ANOVA, with time on study and treatment as independent variables. Comparisons between the three groups were performed using one-way ANOVA, using the LSD post hoc test to determine differences between two groups. Correlation analyses were performed using simple linear regression. Multiple linear regression models were constructed to determine whether the effects of metformin and rosiglitazone to change islet amyloid formation were independent of their effects on fat mass and fat distribution, respectively. Log transformations were performed upon dependent variables when required to satisfy the assumptions of linear regression. P ≤ 0.05 was considered significant.

RESULTS

Body weight and body fat distribution. No difference in body weight was observed among groups at the beginning of the study (25.4 ± 1.2, 24.4 ± 0.7, and 26.8 ± 0.9 g for control, metformin, and rosiglitazone-treated mice, respectively; Fig. 1A). Body weight increased throughout the study in all three groups (P < 0.001), with final (55.8 ± 2.5, 49.8 ± 1.6, and 59.7 ± 1.4 g, respectively) and incremental (30.4 ± 2.1, 25.3 ± 1.5, and 32.9 ± 1.2 g; Fig. 1B) body weights being lower in the mice that received metformin (P < 0.05 compared with controls for both measures). While rosiglitazone-treated mice were heavier than controls at the end of the study, this difference did not reach statistical significance.

Fat mass was significantly different among the three groups at the end of the study (20.1 ± 1.0, 15.1 ± 1.2, and 23.5 ± 0.9 g for control, metformin, and rosiglitazone, P < 0.001; Fig. 1C), with metformin-treated mice having significantly less (P < 0.005) and rosiglitazone-treated mice having significantly more (P < 0.05) body fat than controls. Body fat distribution, assessed as the ratio between visceral and subcutaneous fat (Fig. 1D), was not different between metformin-treated mice and controls (0.37 ± 0.02 and 0.43 ± 0.02, respectively). However, rosiglitazone treatment was associated with altered body fat distribution, resulting in a lower visceral–to–subcutaneous fat ratio (0.25 ± 0.02, P < 0.001 compared with control mice).

Glucose-stimulated insulin release. To ensure that the administration of metformin and rosiglitazone led to the expected reduction in β-cell secretory demand, insulin secretion in response to intravenous glucose was assessed at the end of the study. Insulin release was significantly lower in metformin- and rosiglitazone-treated mice, reflected by marked reductions in acute insulin response to glucose (2.189 ± 850, 621 ± 256, and 14 ± 156 pmol/l, P = 0.01; Fig. 2). In three of the rosiglitazone-treated mice, this response was paradoxically negative.

Plasma measurements and pancreatic peptide content. At the time the mice were killed, fasting plasma glucose and cholesterol levels were not different among the three treatment groups, although cholesterol levels tended to be lower in the metformin-treated mice (Table 1). Fasting free fatty acids and triglycerides were significantly lower with rosiglitazone treatment (P < 0.01 and P < 0.05, respectively), while fasting plasma insulin did not differ among groups (P = 0.06 and P = 0.09, respectively, for metformin- and rosiglitazone-treated mice compared with controls). Fasting plasma hIAPP was lower in metformin-treated (P < 0.01) but not in rosiglitazone-treated mice (P = 0.1) compared with controls. Pancreatic insulin content was lower in metformin-treated mice than in controls (P < 0.05), while mIAPP and hIAPP contents were not different (P = 0.07 and P = 0.2, respectively). In rosiglitazone-treated mice, pancreatic insulin, mIAPP, and hIAPP contents were significantly lower than in control mice (P < 0.01, P < 0.01, and P < 0.05, respectively). These changes in pancreatic peptide contents occurred in proportion to one another, so that no differences were observed in the ratios of hIAPP to mIAPP, mIAPP to IRI, or hIAPP to IRI between any groups (Table 1).

Islet amyloid formation in vivo. The proportion of animals that developed islet amyloid after 12 months of treatment did not differ among the three groups (77, 72, and 84% for control, metformin, and rosiglitazone, respectively). In contrast, the prevalence of islet amyloid (percent islets with amyloid) was markedly reduced by a similar amount in both metformin- and rosiglitazone-treated mice (44 ± 8, 13 ± 4, and 11 ± 3% for control, metformin, and rosiglitazone, respectively, P < 0.001; Fig. 3A). Similarly, the severity of islet amyloid (percent islet area occupied by amyloid) was also vastly reduced in mice treated with both metformin and rosiglitazone (9.23 ± 2.95, 0.22 ± 0.11, and 0.10 ± 0.05%, P < 0.001; Fig. 3B).

Islet morphology. Mean islet mass was not different from controls in metformin-treated mice but was significantly lower in rosiglitazone-treated mice (33.4 ± 7.0, 16.6 ± 3.6, and 12.2 ± 2.1 mg, P = 0.06 for control vs. metformin and P < 0.05 for control vs. rosiglitazone; Fig. 4A). Similarly, β-cell mass was not different in metformin-treated mice and was significantly lower in rosiglitazone-treated mice compared with controls (16.9 ± 2.9, 19.9 ± 2.5, and 8.4 ± 1.7 mg, P = 0.09 for control vs. metformin and P < 0.05 for
control vs. rosiglitazone; Fig. 4B). When the difference in islet mass among groups was accounted for, the proportion of β-cell mass to islet mass was significantly different among groups, being lower in control mice than in metformin- and rosiglitazone-treated mice (57.9 ± 3.1, 64.7 ± 1.5, and 66.1 ± 1.6%, P < 0.05 for control vs. metformin or rosiglitazone; Fig. 4C). This measure was also strongly negatively correlated with amyloid severity in the control mice (r = −0.88, P < 0.001; Fig. 4D) but not in the metformin-treated (r = −0.20) or rosiglitazone-treated (r = −0.17) mice. Islet β-cell replication, assessed as the percentage of Ki-67–positive β-cells, did not differ among groups (0.02 ± 0.01, 0.04 ± 0.01, and 0.03 ± 0.01%). Similarly, β-cell apoptosis assessed as the percentage of activated caspase 3–positive β-cells did not differ among groups (2.0 ± 0.3, 1.1 ± 0.2, and 1.8 ± 0.4%).

Determinants of decreased islet amyloid formation with metformin and rosiglitazone treatment. To determine whether the effects of metformin and rosiglitazone to reduce body fat mass or alter fat distribution were responsible for their ability to reduce islet amyloid formation, we performed multiple linear regression analyses (Table 2). Dependent variables were amyloid prevalence and severity, islet mass, and β-cell mass, with covariates being fat mass and fat distribution (visceral-to-subcutaneous ratio).

Metformin treatment did not significantly reduce islet mass or β-cell mass, but the reduction in islet amyloid prevalence was no longer significant when changes in fat mass were adjusted for. However, the effect of metformin to decrease islet amyloid severity remained significant even after adjusting for fat mass. On the other hand, the lack of change in fat distribution with metformin meant that addition of this to the model did not explain any of the effects of metformin on amyloid.

The effect of rosiglitazone treatment to reduce islet mass and β-cell mass was completely accounted for by the effect of the thiazolidinedione to change fat distribution and was not related to fat mass per se. In contrast to metformin, the effect of rosiglitazone to reduce both islet
amplified prevalence and severity remained highly significant even after accounting for the effect of rosiglitazone to change fat mass or to alter fat distribution.

**Islet amyloid formation and insulin release in vitro.**

To determine whether the effects of metformin and rosiglitazone to reduce islet amyloid deposition that were not accounted for by changes in fat mass or fat distribution may have included a direct effect of these compounds at the level of the islet, isolated hIAPP transgenic mouse islets were cultured for 7 days in the presence of high glucose alone or in the presence of metformin or rosiglitazone. Islet amyloid was not present in hIAPP transgenic islets immediately following isolation.

Incubation of hIAPP transgenic mouse islets in the presence of high glucose for 7 days was associated with the reproducible development of light microscopy–visible amyloid deposits (Fig. 5A, left panel). As expected, no islet amyloid deposits were present in islets isolated from nontransgenic mice (Fig. 5A, right panel). The prevalence and severity of islet amyloid in hIAPP transgenic islets cultured in high glucose alone were 42.9 ± 3.2 and 1.14 ± 0.32%, respectively (Fig. 5B and C, respectively). Treatment with metformin or rosiglitazone was associated with no alteration in islet amyloid formation in vitro, with the prevalence of islet amyloid being 34.8 ± 6.1% for metformin-treated and 39.1 ± 5.7% for rosiglitazone-treated islets (Fig. 5B) and the severity of islet amyloid being 0.82 ± 0.30% for metformin-treated and 0.81 ± 0.29% for rosiglitazone-treated islets (Fig. 5C).

Insulin content following 7 days of culture in high glucose was comparable among control, metformin-, and rosiglitazone-treated islets (1,357 ± 444, 1,346 ± 301, and 1,375 ± 264 pmol/islet, respectively). Fractional insulin release in response to 1.67 mmol/l glucose was similar among groups, being 1.54 ± 0.73% in the control, 1.27 ± 0.50% in the metformin-treated, and 1.22 ± 0.43% in the rosiglitazone-treated islets. Similarly, fractional insulin release in response to 16.7 mmol/l glucose was comparable among groups, being 6.44 ± 0.81, 6.03 ± 1.14, and 7.66 ± 0.64% for control, metformin-, and rosiglitazone-treated islets, respectively.

**DISCUSSION**

In the present study, we have demonstrated that islet amyloid formation in vivo is markedly reduced by the antihyperglycemic medications rosiglitazone and metformin in hIAPP transgenic male mice fed a moderate-fat diet for 1 year. Interestingly, however, neither medication was effective in preventing islet amyloid formation completely. Thus, the moderately increased dietary fat utilized in the present study was sufficient to induce amyloid formation, albeit to a more reduced extent, even when β-cell secretory demand was reduced by rosiglitazone or metformin treatment.

Rigoslitazone and metformin treatment resulted in reduced islet and β-cell mass compared with controls. Frequently, in conditions where β-cell mass is altered, β-cell function is also changed. A very important example of this is type 2 diabetes, where β-cell mass is increased and β-cell function is decreased (1–3). This reduction in β-cell mass occurs despite the fact that individuals with type 2 diabetes are frequently obese and insulin resistant, conditions where β-cell mass has been shown to be increased in humans (1,3). In the present study, rosiglitazone and metformin treatment resulted in reduced β-cell secretory function.

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**TABLE 1**

Plasma and pancreatic peptide measurements in overnight-fasted hIAPP transgenic mice following 12 months of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Metformin</th>
<th>Rosiglitazone</th>
<th>P (control vs. metformin)</th>
<th>P (control vs. rosiglitazone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>8.9 ± 0.8</td>
<td>7.8 ± 1.1</td>
<td>9.0 ± 0.7</td>
<td>0.4</td>
<td>0.9</td>
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<tr>
<td>Plasma insulin (pmol/l)</td>
<td>955 ± 195</td>
<td>483 ± 119</td>
<td>549 ± 171</td>
<td>0.06</td>
<td>0.08</td>
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<tr>
<td>Plasma hIAPP (pmol/l)</td>
<td>49 ± 13</td>
<td>14 ± 3</td>
<td>30 ± 7</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>Serum free fatty acids (mmol/l)</td>
<td>0.75 ± 0.09</td>
<td>0.61 ± 0.07</td>
<td>0.49 ± 0.06</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>76.9 ± 6.5</td>
<td>75.3 ± 4.2</td>
<td>53.6 ± 8.4</td>
<td>0.7</td>
<td>0.01</td>
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<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>168 ± 15</td>
<td>137 ± 9</td>
<td>198 ± 11</td>
<td>0.052</td>
<td>0.005</td>
</tr>
<tr>
<td>Pancreatic IRI (pmol/mg protein)</td>
<td>1,470 ± 270</td>
<td>868 ± 153</td>
<td>702 ± 70</td>
<td>0.02</td>
<td>0.005</td>
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<td>Pancreatic mIAPP (pmol/mg protein)</td>
<td>37 ± 7</td>
<td>24 ± 5</td>
<td>12 ± 2</td>
<td>0.07</td>
<td>0.006</td>
</tr>
<tr>
<td>Pancreatic hIAPP (pmol/mg protein)</td>
<td>21 ± 3</td>
<td>15 ± 3</td>
<td>12 ± 2</td>
<td>0.2</td>
<td>0.05</td>
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<tr>
<td>Pancreatic hIAPP-to-mIAPP ratio (%)</td>
<td>6.25 ± 5</td>
<td>58 ± 5</td>
<td>65 ± 3</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Pancreatic hIAPP-to-IRI ratio (%)</td>
<td>2.5 ± 0.2</td>
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<td>Pancreatic hIAPP-to-IRI ratio (%)</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>0.3</td>
<td>0.4</td>
</tr>
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</table>

Data are means ± SE.
three mice negative values for the acute insulin response to glucose in treated mice was unexpectedly low, due to paradoxical lycemia. The mean insulin response in the rosiglitazone-response to intravenous glucose, with maintenance of euglycemia. The peripheral actions of metformin and rosiglitazone after long-term treatment of nondiabetic animals in the present study are consistent with their known effects in humans and in animal models of diabetes. The mechanism for an improvement in insulin sensitivity for both treatments may be a reduction in visceral fat, whose accumulation is closely associated with worsening insulin sensitivity in humans (35). While rosiglitazone treatment in humans is associated with increased fat mass (36) that would be anticipated to be associated with decreased insulin sensitivity, body fat distribution is also altered, resulting in a relative reduction in visceral fat and improved insulin sensitivity (36,37). In contrast, metformin treatment in humans is not associated with body fat redistribution (37) and may be associated with small amounts of weight loss, although if it occurs, it is not always maintained over time (24,38). These findings in humans differ from those of the present study, where the metformin-treated mice exhibited a significant and sustained reduction in weight gain (and thereby an absolute reduction in visceral fat). Thus, the current findings of reduced amyloid formation in the mice may not be fully applicable to the effects of metformin treatment in humans, especially as the reduction in amyloid formation with metformin was largely the result of the reduction in fat mass.

Multivariate regression analysis showed that the effect of rosiglitazone to reduce islet and β-cell mass was mediated completely through its action to reduce relative visceral adiposity. However, it is also possible that the differential effects of rosiglitazone and metformin to alter islet mass could have resulted from different effects of the medications on normal islet growth, since medication administration commenced when the animals were relatively young. In the multivariate analysis, rosiglitazone’s effect to reduce islet amyloid prevalence and severity
demand, as shown by reduced insulin secretion in response to intravenous glucose, with maintenance of euglycemia. The mean insulin response in the rosiglitazone-treated mice was unexpectedly low, due to paradoxical negative values for the acute insulin response to glucose in three mice. Thus, in this case, the reduction of islet mass with rosiglitazone and metformin appears to have been appropriate for the agents’ effects to reduce the secretory stimulus to the β-cell.

When the difference in islet mass was accounted for among treatment groups, the proportion of β-cell mass to islet mass was reduced in the control group, suggesting that a loss of β-cells had occurred. This reduction in the proportion of β-cell mass to islet mass was strongly correlated with increased islet amyloid severity, consistent with an effect of amyloid to result in β-cell loss. Thus,
treatment with both rosiglitazone and metformin, while resulting in reduced β-cell mass through reduced secretory demand, acted to preserve the remaining β-cell population through suppression of islet amyloid formation. This effect of rosiglitazone and metformin to reduce islet amyloid formation through decreased secretory demand is consistent with studies showing that increased secretory demand due to obesity and insulin resistance is associated with marked islet amyloid deposition in hIAPP transgenic mice (10–12,33), whereas decreased insulin output by disrupting β-cell glucokinase resulted in a significant reduction in islet amyloid deposition (30).

The reductions in β-cell mass seen with rosiglitazone or metformin treatment were not accompanied by detectable differences in β-cell replication or apoptosis among treatment groups. This observation is probably due to the fact that at the end of the study, when changes in body weight and thus insulin sensitivity and β-cell secretory demand were relatively small, β-cell mass was at a steady state. The proportion of β-cells undergoing apoptosis was higher than the proportion undergoing replication in all three treatment groups. While this may seem surprising, the same phenomenon has been observed by others (3,34) and may well reflect a difference in the rate of turnover of the markers of apoptosis and replication (activated caspase 3 and Ki67, respectively).

The peripheral actions of metformin and rosiglitazone after long-term treatment of nondiabetic animals in the present study are consistent with their known effects in humans and in animal models of diabetes. The mechanism for an improvement in insulin sensitivity for both treatments may be a reduction in visceral fat, whose accumulation is closely associated with worsening insulin sensitivity in humans (35). While rosiglitazone treatment in humans is associated with increased fat mass (36) that would be anticipated to be associated with decreased insulin sensitivity, body fat distribution is also altered, resulting in a relative reduction in visceral fat and improved insulin sensitivity (36,37). In contrast, metformin treatment in humans is not associated with body fat redistribution (37) and may be associated with small amounts of weight loss, although if it occurs, it is not always maintained over time (24,38). These findings in humans differ from those of the present study, where the metformin-treated mice exhibited a significant and sustained reduction in weight gain (and thereby an absolute reduction in visceral fat). Thus, the current findings of reduced amyloid formation in the mice may not be fully applicable to the effects of metformin treatment in humans, especially as the reduction in amyloid formation with metformin was largely the result of the reduction in fat mass.

Multivariate regression analysis showed that the effect of rosiglitazone to reduce islet and β-cell mass was mediated completely through its action to reduce relative visceral adiposity. However, it is also possible that the differential effects of rosiglitazone and metformin to alter islet mass could have resulted from different effects of the medications on normal islet growth, since medication administration commenced when the animals were relatively young. In the multivariate analysis, rosiglitazone’s effect to reduce islet amyloid prevalence and severity

FIG. 3. A: Islet amyloid prevalence (percent islets containing amyloid) quantified in all mice after 12 months on study was markedly lower in metformin-treated (□; P < 0.001) and rosiglitazone-treated (●; P < 0.001) mice compared with controls (■). B: Islet amyloid severity (percent islet area occupied by amyloid) was reduced in metformin-treated (□; P < 0.001) and rosiglitazone-treated (●; P < 0.001) mice compared with controls (■) after 12 months of treatment.
remained highly significant after adjustment for altered fat distribution, suggesting that an additional independent effect contributed to reduced amyloid deposition. The effect of metformin to decrease islet amyloid prevalence was largely explained by its effect to reduce total body fat, while the reduction in amyloid severity remained even after accounting for metformin’s effect to reduce body fat. The effects of metformin and rosiglitazone to reduce islet amyloid deposition in the present study appear to be independent of the well-known effects of both metformin and thiazolidinediones to lower plasma glucose in individuals with type 2 diabetes (15,23) and in those at increased

![Image](https://via.placeholder.com/150)

**FIG. 4.** A: Islet mass at the end of the study was not different in mice receiving metformin (p > 0.05) and was significantly lower in mice treated with rosiglitazone (p < 0.05) for 1 year compared with controls. B: β-Cell mass was not different between controls and metformin-treated mice but was lower in rosiglitazone-treated mice. C: The proportion of β-cell mass to islet mass was significantly higher in metformin and rosiglitazone mice compared with controls. D: Increased islet amyloid severity was strongly associated with a decrease in the proportion of β-cell mass to islet mass in control mice but not in metformin-treated or rosiglitazone-treated mice.

### TABLE 2

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Covariate</th>
<th>P (control vs. metformin)</th>
<th>P (control vs. rosiglitazone)</th>
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<tr>
<td>Amyloid prevalence</td>
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<td>&lt;0.001</td>
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<td></td>
<td>Fat mass</td>
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<td></td>
<td>Visceral/subcutaneous fat</td>
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<tr>
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<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fat mass</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Visceral/subcutaneous fat</td>
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risk of developing the disease (16,24,39), as no difference in plasma glucose levels were observed in either group of treated mice. The fasting plasma glucose levels seen in the present study are similar to our previous observations in hIAPP transgenic mice fed a 45% kcal diet for 1 year (12). We have previously reported islet amyloid deposition to be associated with hyperglycemia in our hIAPP transgenic mice (31). However, these elevated plasma glucose levels were detected after 4–6 h of food withdrawal and thus would be predicted to be higher than glucose levels measured after an overnight fast as in the present study.

The actions of metformin or rosiglitazone to reduce islet amyloid deposition appear not to have included a direct effect at the level of the islet. Culture of hIAPP transgenic, but not nontransgenic, mouse islets for 7 days in 16.7 mmol/l glucose was associated with the development of islet amyloid. The formation of hIAPP amyloid deposition visible by electron microscopy has previously been demonstrated in cultured islets from hIAPP transgenic mice (40–43); but, to our knowledge, this is the first demonstration of in vitro deposition of light microscopy–visible amyloid. Our finding that rosiglitazone and metformin did not reduce in vitro islet amyloid formation and did not alter insulin secretion in islets cultured in high glucose is in keeping with previous observations of no effect of the same doses of these medications on insulin release in isolated human islets cultured for 24 h in elevated glucose (22,27).

The actions of rosiglitazone and metformin in vivo to protect the islet from the adverse effects of free fatty acids have been shown to lead to β-cell dysfunction in vivo and in vitro, manifest as impaired glucose–stimulated insulin biosynthesis (45) and secretion (46), and lead to β-cell apoptosis (28,47). Direct interaction of free fatty acids with hIAPP also increases amyloid fibril formation (48). A recent study (22) in cultured human islets showed that rosiglitazone treatment was capable of protecting β-cells from the deleterious effects of free fatty acids, resulting in improved glucose-stimulated insulin secretion and improved proinsulin biosynthesis. In addition, rosiglitazone has been suggested to have antipoptotic effects (17). Similarly, metformin has been shown to normalize glucose and lipid oxidation in isolated islets exposed to elevated glucose or free fatty acids (26,27) and to protect against fatty acid–induced β-cell apoptosis (28). Taken together, these data suggest that the actions of rosiglitazone and metformin to reduce diet-associated increases in plasma free fatty acids and/or protect against deleterious effects of residual free fatty acids contributed to the reduced islet amyloid formation observed with these agents.

While rosiglitazone and metformin are typically used in the treatment of type 2 diabetes, recent studies have demonstrated that both metformin (24) and the thiazolidinediones troglitazone (49) and rosiglitazone (50) are capable of reducing the rate of conversion from impaired glucose tolerance to diabetes. Whether reduced islet amyloid formation is a basis for these effects is an interesting and important question.

In summary, we have shown that rosiglitazone or metformin treatment significantly reduce both the prevalence and severity of islet amyloid in hIAPP transgenic mice. These effects appear to be mediated in part through the
ability of these agents to alter visceral fat deposition, thus reducing secretory demand on the compromised β-cell that is capable of forming islet amyloid. Further, it would appear that both agents might have an effect beyond simply changing visceral fat, with this effect being greater with rosiglitazone. However, our data do not support a direct effect on the islet by either compound to modulate islet amyloid formation. The findings in the current study could explain, in part, the observations made in human prevention studies, which suggest that treatments such as rosiglitazone and metformin that reduce β-cell secretory demand may reduce the risk of developing diabetes. These interventions may ultimately reduce the progressive loss of β-cell mass and function observed before and during the development of diabetes.

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