Estrogen Can Prevent or Reverse Obesity and Diabetes in Mice Expressing Human Islet Amyloid Polypeptide

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Type 2 diabetes is characterized by loss of β-cell mass and concomitant deposition of amyloid derived from islet amyloid polypeptide (IAPP). Previously we have shown that expression of human IAPP (huIAPP) in islets of transgenic mice results in either a rapid onset of hyperglycemia in mice homozygous for the huIAPP transgene on a lean background (FVB/N) or a gradual hyperglycemia in mice hemizygous for the huIAPP transgene on an obese background (Aβ/A). In both strains, only the males routinely develop diabetes. To investigate this sexual dimorphism, we treated prediabetic Aβ/A mice transgenic for huIAPP (huIAPP-Aβ) with 17β-estradiol (E2). The treatment completely blocked the progression to hyperglycemia but also prevented the associated weight gain in these mice. Immunohistochemistry of pancreatic sections demonstrated normal islet morphology with no apparent deposition of islet amyloid. E2 treatment of 1-year-old huIAPP-Aβ diabetic males rapidly reverses obesity and hyperglycemia. To determine the effects of E2 in a nonobese model, we also treated prediabetic, ad libitum–fed and pair-fed Lean-huIAPP transgenic males. E2 completely blocked the progression to hyperglycemia with no significant effect on body weight. Pancreatic insulin content and plasma insulin concentration of Lean-huIAPP transgenic mice increased in a dose-dependent manner. We demonstrated the presence of estrogen receptor (ER)-α mRNA in mouse and human islets. By also confirming the presence of ER-α protein in islets, we discovered a novel 58-kDa ER-α isoform in mice and a 52-kDa isoform in humans, in the absence of the classic 67-kDa protein found in most tissues of both species. The demonstrated presence of ER-α in mouse and human islets is consistent with a direct effect on islet function. We conclude that exogenous E2 administered to male mice may block human IAPP-mediated β-cell loss both by direct action on β-cells and by decreasing insulin demand through inhibition of weight gain or increasing insulin action. Diabetes 51:2158–2169, 2002

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AR, androgen receptor; E2, 17β-estradiol; ER, estrogen receptor; EST, estrogen sulfotransferase; FBG, fasting blood glucose; huIAPP, human IAPP; IAPP, islet amyloid polypeptide; PPAR, peroxisome proliferator–activated receptor; TBST, Tris-buffered saline plus Tween.

Type 2 diabetes is characterized by both impaired insulin action (1) and failure of β-cells to compensate with increased insulin secretion (2). Some studies have found an association of this β-cell failure with the deposition of islet amyloid in the pancreas (3). We and others have previously reported the generation of transgenic animal models that exhibit human islet amyloid polypeptide (huIAPP)-dependent hyperglycemia associated with loss of β-cell mass and/or amyloid deposition (3–5). One model is described herein as the Lean-huIAPP transgenic line [FVB/N-Tg(IAPP)6Jdm/Tg(IAPP)6Jdm] (6). It was generated with a transgene that directs expression of huIAPP to pancreatic β-cells under the regulation of rat insulin II promoter fragment, in mice bred to homozygosity for the transgene. This model exhibits a rapid onset of severe hyperglycemia associated with the progressive loss of islet area and insulin immunoreactivity in the pancreas. This β-cell loss is specific for huIAPP since the expression of murine IAPP to comparable levels in transgenic mice has the normal complement of β-cells (7). With the aim of obtaining a phenotype more akin to human type 2 diabetes, we crossed Lean-huIAPP transgenics to the Agouti viable yellow (Aβ/a) mouse on the C57BL/6 background to generate an obese hemizygous transgenic mouse [FVB8F1-Tg(IAPP)6Jdm/-Aβ/a] (8). These mice, herein described as huIAPP-Aβ, develop a much more gradual onset of hyperglycemia that is associated with the appearance of copious deposits of islet amyloid in the pancreas. Interestingly, in both these models, either the females are much less severely affected (huIAPP-Aβ) (8) or diabetes appears at a much lower frequency (~5% of Lean-huIAPP males) (6).

Such sexual dimorphism has been described in a number of rodent models of diabetes or insulin resistance: Cpefat (9), Leptdb/Leptdb mice on a C3HeB/FeJ genetic background (10), obese Agouti viable yellow (Aβ/a) mice overexpressing Agouti (11,12). Sexual dimorphism may be caused by several factors stemming from the different levels of circulating estrogen between sexes. First, a cohort of genes may be transcriptionally modulated by estrogen if they possess an estrogen response element within their promoters (13). Second, estrogen sulfotransferases (ESTs) inactivate estrogen and may play a role in diminishing available reservoirs, which thereby androgenizes or estrogenizes the liver (10,14). High levels of EST
mRNA were detected in male rat livers, whereas no EST mRNA was found in the livers of females (15), which would maintain higher levels of 17β-estradiol (E2) for females within the local hepatic environment. A null mutation of the mouse EST did not alter circulating levels of estrogen or testosterone, and mice were phenotypically normal for both sexes (16). Finally, one report has shown that estrogen can increase the number of insulin receptors in the liver (17). The sexual dimorphic phenotype suggests either that E2 is having some protective effect against hyperglycemia or that androgens have a detrimental effect. A role for E2 in the regulation of lipid and glucose metabolism has recently emerged in the characterization of aromatase-deficient mice (ArKO) (18,19) and estrogen receptor (ER)-α/−deficient mice (20). ArKO mice have increased adiposity, hepatic steatosis, and hyperinsulinemia. ER-α null mutant mice display increased adiposity, insulin resistance, and glucose intolerance. In humans, the E2 antagonist tamoxifen can induce hepatic steatosis (21).

RESEARCH DESIGN AND METHODS

Animal maintenance and techniques. All animal maintenance protocols and procedures on animals performed in these studies were carried out in accordance with the NIH guidelines. E2 was administered as subcutaneously implanted pellets containing 16 mg/ml sodium pentobarbital as a general anesthetic. The pancreas was excised under general anaesthesia from transgenic animals.

Statistical analysis. A standard two-tailed Student’s t test was used with a 5% significance level or a P value <0.05.

Western blot. FVB/N male mouse brain, liver, and islet protein or human islets, liver, brain, skeletal muscle, and pancreas were extracted from isolated

Plasma leptin and insulin levels. Plasma samples obtained from retro-orbital sinus bleeds were stored at −20°C until analysis. Plasma E2 levels were measured using Estradiol ELISA (American Laboratory Products Co., Windham, NH), following the manufacturer’s instructions.

Inulin infusion. Inulin (Pharmacia, Uppsala, Sweden) was infused into the abdominal cavity provided access to the pancreas. The ampulla of Vater was clamped off. A small nick in the common bile duct was made from the origin (near the liver). A 22-gauge needle was inserted into the bile duct, and 5 ml cold Hanks was infused to distend the pancreas. Pancreas was removed while the mice were anesthetized with a 1:40 or 1:100 dilution of this in Hanks provided a working solution for the assay. The assay for insulin has previously been described (23).

RT-PCR. Hand-picked islets of Langerhans, liver tissue, and brain tissue from each E2 treated mouse were digested and used in a total of 60 PCR reactions (Gibco BRL). Total RNA was extracted according to the manufacturer’s recommendations. RNA was resuspended in 200 μl DEPC-treated water and treated with 10 μl DNase (Promega) for 1 h at 37°C. DNase treatment was stopped by incubating reaction for 10 min at 70°C, followed by ETOH precipitation. RNA was resuspended and quantified, and 5 μg was used for RT using SuperScript II (Gibco) according to the manufacturer’s recommendation. For each PCR reaction, 1 μg of reverse-transcribed total RNA was used. As a control for DNA contamination, 1 μg of non-RT (−) total RNA was also assayed for each oligonucleotide set. Mouse ER-α oligonucleotide set was 5′-ACCATGG TACACGTGACGAGACTC-3′ and 5′-TATGCTGAGGTTGGTCAATAAGC-3′, which generates a 381-bp fragment. ER-β oligonucleotide set was 5′-TCTATGCAGAACCCTAAAGACGC-3′ and 5′-TCTCTCCGTGATCACCCTGTTG-3′, which generates a 382-bp fragment. Human ER-α primers were 5′-ATTGGACCTCTCACAGAACCCTAAAGACGC-3′, which generates a 665-bp fragment. Human ER-β primers were 5′-ATTGACCTCTCACAGAACCCTAAAGACGC-3′ and 5′-ACGTGACGAGACTC-3′, which generates a 679-bp fragment. Expand High Fidelity PCR System (Roche Diagnostics, Indianapolis, IN) was used under the following conditions: denature at 95°C for 30 s, anneal at 68°C for 30 s, and extend at 72°C for 1 min, 10–40 cycles.

Islet isolation. One-year-old male huIAPP-AV transgenic mice or AV/A mice were anesthetized using −0.3 cm3 16 mg/ml sodium pentobarbital. An incision into the abdominal cavity provided access to the pancreas. The ampulla of Vater was clamped off. A small nick in the common bile duct was made from the origin (near the liver). A 22-gauge needle was inserted into the bile duct, and 5 ml cold Hanks was infused to distend the pancreas. Pancreas was excised away from other tissues and diced into fine pieces using a scissors. Tissue was transferred to a 15-ml test tube and washed several times with cold Hanks, and light centrifugation was performed under a light microscope and transferred into fresh Krebs-Ringer bicarbonate buffer with 1% BSA. Islets were collected and transferred to a 1.5-ml Eppendorf tube and centrifuged at low speed, the supernatant was discarded, and 1 ml Trizol (Gibco BRL) was used to directly dissolve islet tissue with vortexing. Human islets were provided as a gift from the JDF Human Islet Distribution Program at the University of Alberta. Islets provided in tissue culture media were centrifuged to collect islets, washed in PBS, and centrifuged again, and the resulting pellet was resuspended in 1 ml Trizol (Gibco BRL).

Plasma samples obtained from retro-orbital sinus bleeds of E2- or vehicle-treated mice and stored at −20°C. Leptin concentrations were determined using a Mouse Leptin-RIA kit (Linco, St. Charles, MO), and insulin concentrations were determined using a Mouse Ultrasensitive Insulin ELISA (Alpco Diagnostics, Windham, NH) according to the manufacturer’s instructions.
tissue using 500 μl M-PER Mammalian Protein Extraction Reagent (Pierce, Rockford, IL) with 10 μl of 0.1 mol/l dithiothreitol (DTT), 2.5 μl of 200 mmol/l phenylmethylsulfonyl fluoride (PMSF), and a 1/1,000 Protease Inhibitor Cocktail Tablet (Roche Diagnostics, Mannheim, Germany). Tissues were briefly sonicated on ice, followed by 20 min of incubation on ice. Protein concentrations were determined using Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer’s recommendation. Protein samples (10, 20, 40, or 80 μg) were heated to 100°C in 1× NuPAGE LDS Sample Buffer (Invitrogen, Carlsbad, CA) 5 min or 2 μl/lane of Cruz Markers (Santa Cruz Biotechnology, Santa Cruz, CA) without heat and run on a NuPAGE 10% Bis-Tris Gel in 1× NuPAGE MOPS SDS Running Buffer (Invitrogen) 100 V for ~2 h, and protein gel transfer was conducted using a PVDF Membrane Filter and 1× NuPAGE Transfer Buffer (Invitrogen) 30 V for 1 h at room temperature. Blots were preincubated in 10 ml of 1× TBST (Tris-buffered saline, pH 8.0, with 0.5% Tween-20) with 5% Carnation Non-Fat Dry Milk (Nestle, Solon, OH) (1 h at room temperature), followed by replacing the solution with 10 ml 1× TBST with 5% nonfat dried milk and 1 μl monoclonal anti-ER-α antibody MAB463 (Chemicon, Temecula, CA) for 1 h at room temperature with gentle shaking. Nylon filter was washed several times with 10 ml 1× TBST 5 min with shaking, followed by adding 10 ml 1× TBST, 5% nonfat dried milk, and 12.5 μl anti-mouse IgG-HRP conjugate (Santa Cruz Biotechnology) for 1 h at room temperature. Western blot was washed several times for 5 min with 1× TBST, then developed using ECL Western Blotting Detection Reagents (Amersham Pharmacia Biotech, Arlington Heights, IL) solutions for 5 min and analyzed on a Kodak Image Station 440 CF (Eastman Kodak Company, Rochester, NY) for a 3-min exposure. Band size was determined by Kodak DS 1D version 3.0.2 software (Kodak Imaging Systems, New Haven, CT).

RESULTS

Our initial intent was to determine if estrogen could prevent the onset of hyperglycemia in the huIAPP-A^γ gradual-onset obese model. HuIAPP-A^γ males that were still euglycemic at 16 weeks of age were subjected to vehicle or E2 slow-release pellet (0.72 mg/90-day release) implantation. Fasting blood glucose concentrations and body weight were monitored for the next 22 weeks (Fig. 1A). Implanted pellets were replenished at week 32. The fasting blood glucose concentrations of the vehicle-treated males steadily climbed to a plateau of 7.3 mmol/l by week 37. In contrast, the E2-treated mice remained euglycemic (4.4 mmol/l) throughout this time period. The mean body weight of the vehicle group climbed from 25 g at the time

FIG. 1. Prediabetic huIAPP transgenic males treated with E2. E2 (0.72 mg/90-day release) or vehicle pellets were implanted in seven huIAPP-A^γ males beginning at 16 weeks of age (4 months). E2 and vehicle pellets were replenished at 32 weeks of age in each of the corresponding groups. A: Fasting blood glucose concentrations (mmol/l glucose) monitored over the 21-week study period. B: Fasted body weight (g) versus time of mice monitored in A. C: Double staining for amyloid (thioflavine S) and insulin (anti-insulin conjugated with Alexa 594) in the E2- and vehicle-treated mice shows that islets are devoid of amyloid and have intact β-cells. Vehicle-treated animals develop large amyloid plaques, rendering the greater plaque area of the islet absent of insulin or β-cells. *P < 0.05.
From an average of 16.3 to 5.9 mmol/l in 19 days.

In E2-treated animals, blood glucose levels rapidly dropped.

Glucose concentrations (mmol/l glucose) monitored over a 12-week period. In E2- and vehicle-treated mice in A, the mean pancreatic insulin content of the E2-treated males was 2.4-fold lower than the vehicle-treated males (6.22 ± 0.92 ng/ml, n = 8) and E2-treated males (6.04 ± 1.86 ng/ml, n = 4); however, both groups were clearly elevated relative to the younger cohort in Fig. 1 (P < 0.01).

To determine if such effects would also occur in a euglycemic mouse prone to insulin resistance and obesity but not diabetes, we treated 16-week-old nontransgenic A<sup>Vy</sup>/A males with two successive implants of E2 (0.72 mg/90-day release) (Fig. 3A and B).

No significant differences in fasting blood glucose concentrations were observed. However, the body weight steadily increased in the vehicle population from 30.7 to 49.0 g by week 37, whereas the E2-treated animals maintained the same weight of 31.0 g. Thus, the resistance to body weight gain was just as dramatic as that seen in the huIAPP-A<sup>Vy</sup> males (Fig. 1B). The mean pancreatic insulin content of the E2-treated males was 2.4-fold lower than the vehicle-treated group (221 vs. 520 μg), consistent with an E2-mediated increase in insulin sensitivity due to lower concentrations and body weight from 7 to 13 weeks of age.

We next shifted our attention to the Lean-huIAPP transgenic mouse. This strain also exhibits a strong sexual dimorphism with respect to the diabetic phenotype. However, unlike the huIAPP-A<sup>Vy</sup> strain, this animal is a model of insulin deficiency that lacks the obese and insulin resistance phenotype of the A<sup>Vy</sup>/A mouse. Vehicle- and E2-treated males were monitored for blood glucose concentrations and body weight from 7 to 13 weeks of age. Vehicle-treated mice exhibited a very rapid onset of fasting hyperglycemia (18.8 mmol/l blood glucose by 13 weeks of age) (Fig. 4A) as reported previously (6). E2-treated mice remained euglycemic (6.2 mmol/l). A slight body weight lowering was noted between 10 and 11 weeks of age (P > 0.2) (Fig. 4B).

To determine if this slight drop in body weight could be due to an E2-mediated effect on food intake, we measured the 5-day average ad libitum food consumption of a new...
cohort of the Lean-huIAPP transgenic mice. These mice had been implanted with vehicle, 0.18 mg/90-day E2 slow-release pellets, or 0.72 mg/90-day E2 slow-release pellets (Fig. 5A). E2 at either dose suppressed food consumption by 33%, consistent with a recent report (24). At the end of the 5-day food intake study, the vehicle-treated mice were food restricted to match the average intake of the E2-treated mice, and all three groups were monitored for fasting blood glucose concentrations and body weight for the next 5 weeks (Fig. 5B and C). Again, the vehicle-treated Lean-huIAPP transgenic males became extremely hyperglycemic (>20 mmol/l) by 8 weeks of age, while both the low- and high-dose E2-treated mice remained euglycemic. The mean body weight of the vehicle-treated mice did not differ significantly from either estradiol-treated group ($P > 0.2$ for all postpellet implantation time points). Plasma insulin concentrations were determined for these three groups and compared with euglycemic, vehicle-treated Lean-huIAPP females and both sexes of nontransgenic FVB/N mice (Fig. 5D). Plasma insulin concentration rose in a dose-dependent manner greater than twofold relative to the vehicle-treated Lean-huIAPP males ($P < 0.01$). Both sexes of the FVB/N mice and the female Lean-huIAPP mice had plasma insulin levels threefold greater than vehicle-treated Lean-huIAPP males ($P < 0.001$). Only the low-dose E2-treated cohort was significantly lower ($P < 0.05$) than male and female wild-type mice, but not Lean-huIAPP females ($P > 0.1$).

Plasma E2 concentrations were measured for Lean-huIAPP transgenic males treated with 0.18 mg/90-day pellets, 0.72 mg/90-day pellets, or vehicle after 4 weeks of treatment (Fig. 6A). The mean E2 concentration in plasma of vehicle-treated mice was 13.8 pg/ml. The low- and high-dose group E2 concentrations were 323 and 838 pg/ml, respectively. The low-dose value represents an ~10-fold increase of what is typically observed in a
nonpregnant female mouse (~30 pg/ml) (25) or an ~5-fold increase if pregnant (~60 pg/ml) (26). The pancreas was removed and weighed from each of these animals, and the insulin content was determined (Fig. 6B). Pancreatic insulin content increased dramatically and dose-dependently with E2 treatment (Fig. 6C). To determine the morphology of the Lean-huIAPP transgenic mice with or without E2 treatment, pancreatic sections from the first Lean-huIAPP transgenic cohort (Fig. 4) were treated with anti-insulin antisera to visualize insulin and thioflavin S to detect amyloid fibrils (Fig. 7A). A stained section from a huIAPP-A<sup>−/−</sup> male is shown for comparison. No thioflavin S staining was detected for either vehicle-treated or E2-treated mice. This confirms previous reports that failed to observe islet amyloid deposits under light microscopy but noted the detection of amyloid fibrils in these mice only by electron microscopy (6, 27). Insulin staining in the 13-week-old vehicle-treated pancreata was weak. In contrast, E2-treated mice stained intensely for insulin in a level comparable to that seen in wild-type nontransgenic FVB/N (data not shown). The average islet size of vehicle-treated mice was dramatically smaller than that seen for E2-treated mice. The tissue sections from two mice of each group were then subjected to morphometric analysis. Islet area was measured for every islet detected on three whole pancreatic cross-sections stained for insulin. The mean islet areas for two of the vehicle-treated mice (17 and 22 mmol/l fasting blood glucose) were 150 and 650 μm<sup>2</sup>, respectively, while the values for two of the E2-treated mice (5 and 6 mmol/l fasting blood glucose) were 2,650 and 1,600 μm<sup>2</sup>, respectively (Fig. 7B). To visualize the distribution of islet sizes within each group, the islet number was plotted against the islet area in 1,000 μm<sup>2</sup> increments. Islet size was very heterogeneous in every section. However, it is clear that the vast majority of vehicle-treated islets were smaller than 500 μm<sup>2</sup> in size, and none were larger than 5,000 μm<sup>2</sup>. Islets in the E2-treated mice are skewed to the larger increments, with some areas as large as 45,000 μm<sup>2</sup> (data not shown).

To determine if the effects of estrogen could be due to the estrogen receptor inducing transcription of leptin, we analyzed plasma samples of both E2- and vehicle-treated mice. The plasma leptin concentration of the ER-treated Lean-huIAPP transgenic males (3.00 ± 0.33 ng/ml, n = 10 mice) was not significantly different from either the vehicle-treated Lean-huIAPP transgenic males (2.60 ± 0.32 ng/ml, n = 10 mice) (P = 0.43) or the vehicle-treated Lean-huIAPP transgenic females (2.50 ± 0.35 ng/ml, n = 10 mice) (P = 0.29). The glucose-lowering effects of E2 observed in a model of insulin deficiency (Lean-huIAPP transgenic) suggests that some of its effects could be mediated directly on the islet itself. We took the first step in exploring this possibility by determining if the two ER isoforms and AR are expressed in mouse islets. RNA was extracted from purified mouse islets and subjected to RT-PCR utilizing primers specific for murine ER-α, ER-β, and AR. Mouse liver and brain RNAs, as well as RNA in the absence of reverse transcriptase [(−)RT], were run as controls. The results are shown in Fig. 8A. ER-α primers generated a 381-bp PCR product from brain and liver RNA, as expected. These primers also demonstrated the presence of ER-α RNA in islets. ER-β-specific primers generated a 372-bp product in brain but not liver, as expected. No ER-β-specific product was detected in islet RNA. AR (665-bp product) was detected in all three tissues. No PCR products were formed in the absence of reverse transcriptase [(−)RT], and to PCR a human ER-α gene fragment (Fig. 8B). The expected 679-bp product was detected, whereas no product was found in the (−)RT. Thus murine and human islets specifically express ER-α, consistent with a direct role for E2 in maintaining islet function and/or viability. To confirm translation of ER-α message in islets, we analyzed mouse islet protein for ER-α product by Western blot (Fig. 8C). Surprisingly, Western blot analysis revealed an ER-α isofrom in an islet tissue of novel size (i.e., 58 kDa, ~9 kDa smaller than the known 67-kDa product in brain or liver). The abundance of this novel isofrom in islets is about half of that seen of the 67-kDa product in liver or brain, and the classic 67-kDa ER-α is not detected at all. To verify if humans also have a truncated isofrom specifically expressed in islets, Western blot analysis was conducted using human islets, whole pancreas, liver, brain, and skeletal muscle (Fig. 8D). Again, a novel 52-kDa ER-α isofrom was detected in human islets.
in the absence of the classic 67-kDa protein detected in most tissues. Since whole pancreas detects the 67-kDa protein and not the 52-kDa isoform and islets detect only the 52-kDa isoform, these Western blot results suggest that the exocrine portion of the pancreas expresses the classic ER-α and the endocrine portion of the pancreas expresses the novel isoform.

DISCUSSION
In one mouse model of islet amyloid, Kahn et al. (28) reported that ovariectomy promoted islet amyloid formation in females. However, no effects on blood glucose concentration were observed. Sexual dimorphism in the diabetic phenotype of rodents has been extensively documented in the literature (9–12). Our primary aim in the present study was to determine if E2 can stop the progression to hyperglycemia in two distinct huIAPP-dependent murine models of diabetes. In the huIAPP-AV transgenic mouse, E2 inhibited the weight gain and the associated onset of hyperglycemia normally observed in this mouse model. Examination of the islet morphology determined that E2 also prevented the degeneration of β-cells and
deposition of islet amyloid. The effect of E2 on weight gain was also manifested in nontransgenic A\textsuperscript{Avy}/A mice but with no significant effect on fasting blood glucose concentrations. More surprisingly, E2 was able to restore euglycemia in older huIAPP-A\textsuperscript{Avy} males with established frank diabetes. This restoration was also associated with a dramatic and rapid weight loss (23 g in 2.5 weeks) primarily due to reduction of adipose tissue mass (data not shown). Because weight gain primarily in the form of ammassed adipose tissue is well known to induce insulin resistance, a likely explanation for the prevention and reversal of hyperglycemia in the huIAPP-A\textsuperscript{Avy} model would be a restoration of insulin sensitivity mediated by the reduction in adipose mass. Unlike the significant E2-mediated lowering of plasma insulin concentrations seen in the younger huIAPP-A\textsuperscript{Avy} and nontransgenic A\textsuperscript{Avy}/A mice, the hyperinsulinemia in 1-year-old huIAPP-A\textsuperscript{Avy} males was not reduced despite the restoration of euglycemia. This suggests that some insulin resistance independent of adipose mass persists in these E2-treated mice.

That E2 plays a role in body fat deposition has long been known from studies of ovariectomized rats (29). Several recent studies of null mutation animal models have further elucidated a role for E2 in energy homeostasis and regu-
lation of adipose mass in rodents. Jones et al. (18) examined the adipose depots of male and female mice null for aromatase and thus incapable of synthesizing endogenous estrogen. These mice were found to possess fatty livers and accumulate more adipose tissue, especially in the abdominal area, relative to their wild-type counterparts. Surprisingly, the increase in weight gain was not due to increased food intake or decreased resting energy expenditure. Rather, these mice displayed reduced physical activity and reduced glucose oxidation. Such null muta-
tions were also independently generated by Nemoto et al. (19). Although this group did not comment on adipose stores they did observe impaired hepatic expression of enzymes involved in fatty acid oxidation in their model. This resulted in hepatic steatosis that could be reversed by estradiol treatment. These findings are consistent with reports of hepatic steatosis in humans induced by the E2 antagonist tamoxifen (21,30). Reports of sex-related defects in lipid and glucose metabolism in peroxisome proliferator–activated receptor (PPAR)-α null mice point to cross-talk between signaling pathways involving ERs and PPARs in the maintenance of energy metabolism (31).

Another key finding supporting a role for E2 in energy homeostasis is increased white adipose tissue in ER-α null mice of both sexes (20). These mice exhibit not only adipocyte hypertrrophy and hyperplasia but also insulin resistance and glucose intolerance. This occurs in the absence of any change in food intake. These findings indicate that E2/ER-α signaling in both sexes plays an important role in development and function of white adipose tissue, which in turn affects overall energy metabolism. It also has recently been reported that ER-α null mice have lower metabolic rates (32).

In searching for possible mechanisms whereby E2 could be mediating this effect, leptin is one obvious target, given its crucial role in regulating whole-body energy homeostasis. Plasma leptin concentrations are higher in females independent of body composition (33) and ovariectomy in rats leads to decreases in adipose leptin mRNA levels and serum leptin concentrations that can be reversed by E2 replacement (34). Furthermore, E2 can regulate leptin synthesis and secretion directly through ER-mediated processes (35). It also has recently been discovered that the leptin promoter contains an ER response element (ERE) and in vitro functionality studies demonstrate that a combination of E2 and ER-α can induce expression from the leptin promoter (36). In light of this data, we measured plasma leptin levels in E2- and vehicle-treated Lean-huIAPP and huIAPP-Avy transgenic mice, recapitulating the phenotype of female littermates and resolving the diabetic sexual dimorphism. Such protection may stem from a lower demand by peripheral tissues for insulin and thereby feedback to decrease expression of the huIAPP transgene under the Rip2 promoter or from a local effect within the environment of the β-cell. Although E2 has a pleiotropic effect in lowering food intake, decreasing body weight in the obese animals and lowering blood glucose, the effect of preventing amyloid formation may be a result of the novel ER-α isoform specifically expressed in islets. The mechanism for E2 effect in peripheral tissues or within the islets is unknown. It should be noted that even with the lower dose formulation, the plasma concentrations of E2 achieved in these mice are 11-fold elevated compared to the endogenous concentrations found in nonpregnant female mice. Although the ER-α null and aromatase null mice argue for physiological rather than pharmacological effects of E2 on glucose metabolism, the studies described here should be extended to male mice subjected to physiological doses of E2.

Relevance of these findings to obesity and diabetes in humans is suggested by the phenotype of people possessing point mutations or variations in ER-α or aromatase. The patient with an ER-α deficiency was glucose intolerant and hyperinsulinemic (22). Hyperinsulinemia was also reported in male individuals harboring single amino acid
substitutions in the aromatase gene (44). Although treat-
ment of disorders of glucose and lipid metabolism with E2 itself would not be suitable, elucidation of the signal-
ning pathway in the various target organs/tissues would be useful in identifying other targets for therapeutic
intervention.

Interestingly, humans also appear to display sexual
dimorphism related to glucose metabolism and diabetes.
Normal women in the general population have twice the
adipose mass with only two-thirds the skeletal muscle/
bone mass (45), yet the incidence of type 2 diabetes
remains roughly equal between sexes up until the time of
menopause. It was shown in Western society in the early
20th century (46) and confirmed in the later half of the
time (47) that there is a significant spike in the incid-
ence of diabetes for women aged 55–60 years. The time
frame and data suggest that metabolic protection is attenu-
ated when E2 levels fall during menopause, perhaps
through decline in lipid partitioning (48).

Nearly 100% of type 2 diabetic subjects who have been
tested for islet amyloid deposition have some deposits in
contrast to nondiabetic subjects (49). The relationship
between islet amyloid and hyperglycemia has been known
since 1901 (50); however, the mechanism or factor which
causes the monomeric IAPP to form to fibrils in persons
with diabetes is still unknown (51). There is a strong
association between obesity and diabetes (52), and it is
known that fat metabolites have a pleiotropic effect
(53–55). Although a relationship between lipids and amy-
loid formation is still unclear, it is possible that fat
metabolites may also play a role in shifting the balance
from IAPP to amyloid, perhaps by acting as a locus for
seeding or altering expression of chaperone proteins.

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