Mutation of the RIIβ Subunit of Protein Kinase A Prevents Diet-Induced Insulin Resistance and Dyslipidemia in Mice

Sandra A. Schreyer,1 David E. Cummings,2 G. Stanley McKnight,3 and Renée C. LeBoeuf4

The mechanisms by which obesity contributes to diabetic phenotypes remain unclear. We evaluated the role of protein kinase A (PKA) signaling events in mediating diabetes associated with obesity. PKA comprises two regulatory subunits and two catalytic subunits and is activated by cAMP. The RIIβ regulatory subunit is abundantly expressed in adipose tissue and brain. Knockout mice lacking this subunit are lean and display remarkable resistance to diet-induced obesity. We investigated whether these mice were also resistant to diet-induced diabetes and whether this effect was dependent on reduced adiposity. Mice were fed a high-fat, high-carbohydrate diet and weight gain and diabetes phenotypes were examined. RIIβ-/- mice displayed decreased body weights, reduced insulin levels, improved insulin sensitivity, and improved total-body glucose disposal as compared with wild-type controls. Plasma levels of VLDL and LDL cholesterol were also reduced in high fat-fed RIIβ-/- mice compared with wild-type mice. Taken together, these data demonstrate that loss of RIIβ protects mice from diet-induced obesity, insulin resistance, and dyslipidemia. Diabetes 50:2555–2562, 2001

Recent reports indicate that obesity is rapidly increasing in industrialized nations. More than 35% of American adults are now considered overweight or obese (1), and obesity is an important contributing factor to the development of type 2 diabetes (2–4). Dyslipidemia is often observed in association with obesity and diabetes and is a significant contributor to the increased mortality observed in diabetic patients (5,6). Characteristics of type 2 diabetes include impaired glucose disposal, diminished insulin production, and increased hepatic glucose output. The mechanisms by which obesity contributes to these phenotypes remain unclear (7).

Signaling via protein kinase A (PKA) plays an important role in regulating metabolism and body weight (8). PKA is activated by cAMP and comprises two regulatory and two catalytic subunits (8). Four regulatory isoforms (RIα, RIIα, RIIβ, and RIIγ) and two catalytic isoforms (Cα and Cβ) are expressed in the mouse, and each is encoded by a separate gene. The RIIβ subunit is expressed principally in three tissues known to regulate energy homeostasis: brown adipose tissue, white adipose tissue, and brain (8,9). Recent studies suggest that the induction of PKA in certain tissues may decrease obesity. For example, activation of the adipose-specific β-adrenergic receptor (10), which signals via PKA, decreases obesity in both genetically obese (ob/ob) (11,12) and diet-induced obese mice (13), suggesting that signaling mechanisms through this pathway are important in preventing obesity.

Studies in mice lacking a specific PKA subunit, RIIβ, have revealed an unexpected role for this protein in regulating energy balance (9). RIIβ knockout mice (RIIβ-/-) remain remarkably lean even when challenged with a high-fat diet (9). These animals have increased metabolic activity, manifested by increases in body temperature, uncoupling protein 1 concentration, and lipid hydrolysis. Biochemical studies have shown that loss of RIIβ was compensated by increased RIα regulatory subunit, which is more sensitive to cAMP activation and results in a net increase in basal PKA activity (14). These studies suggest that increasing basal PKA activity in adipose tissue and brain ameliorates obesity.

In this study, we sought to determine whether loss of RIIβ influences the development of diabetes and dyslipidemia associated with obesity. Wild-type and RIIβ-/- mice, maintained on the C57BL/6 genetic background strain, were fed a high-fat, high-carbohydrate diet. This diet is known to induce obesity and diabetes in C57BL/6 mice (15,16). RIIβ-/- mice were resistant to weight gain and hyperinsulinemia. In vivo insulin sensitivity and glucose disposal were dramatically improved in the RIIβ-/- mice, as were plasma lipid profiles. When mice were corrected for differences in body weight, improved insulin-mediated glucose disposal was still observed in the RIIβ-/- mice, suggesting an obesity-independent effect of RIIβ on promoting insulin resistance. We suggest that PKA activity in both adipose tissue and brain is important for determining body composition, food intake, and diabetogenic parameters. Thus, PKA is an attractive therapeutic target for preventing and treating obesity and the coinciding disorders of insulin resistance and dyslipidemia.
Immunoblot procedures. Apolipoproteins were quantified using immunoblotting procedures essentially as described (19, 20). Plasma samples (2 μl) from male mice fed the diabetogenic diet for 15 weeks were applied to SDS-PAGE, electrophoresed, and transferred to nitrocellulose membranes. Specific protein bands were detected after incubation of filters with monoclonal antibodies specific for apoA-I, apoA-II, apoB, apoE, and apoCII (Cat. #325000, #110000, and #110010, respectively) followed by incubation with horseradish peroxidase–conjugated secondary antibodies (Sigma, St. Louis, MO). Band intensities were measured using an autoradiographic imaging system (Kodak, Rochester, NY). Immunoblot analysis was performed using a FluorChem 8900 and KODAK Image Station 4400 (Amerham Biosciences, Buckinghamshire, UK).

RESULTS

Longitudinal analysis of RIIβ−/− mice. Body weights increased steadily for male mice of both genotypes fed the diabetogenic diet (Study 1) (Fig. 1A). RIIβ−/− mice were 20% lighter than wild-type mice at initial (0 weeks) and final (15 weeks) time points (Table 1). Although mice of both genotypes showed comparable changes in percent body weight by the end of the study (e.g., 60% at 15 weeks), RIIβ−/− mice gained weight more slowly than did wild-type mice.

Leptin levels reflected differences in body weights for RIIβ−/− and wild-type mice. Among mice fed rodent chow (0 weeks), leptin levels were threefold lower for RIIβ−/− mice than for wild-type mice (Table 1 and Fig. 1B). Leptin levels were significantly lower for male RIIβ−/− than for wild-type mice at 15 weeks of dietary treatment (12 and 14 weeks of dietary treatment, mice were introduced to the diet at 16–20 weeks of age). A: Body weights of wild-type mice (○) were significantly higher (P < 0.001) than those of mutant mice (▲) at all time points. B: Leptin levels were significantly higher for wild-type mice than for RIIβ−/− mice between 0 and 10 weeks of diet feeding (P < 0.05). C: Cholesterol levels were significantly different between genotypes at 4 weeks (P < 0.05). D: Plasma insulin was significantly higher in wild-type mice than in mutant mice at 4 and 8 weeks (P < 0.05). Values are presented as means ± SE for n = 10–12 mice per set.

Levels of the PKA subunits, RIIβ, RIIα, and RIIα, were evaluated using procedures as described (9). Briefly, brown adipose tissue, testis, or islet protein homogenates (40 μg per lane) were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes. Uniformity of protein loading was confirmed by Coomassie Blue staining (not shown). Polyclonal antibodies raised to recombinant murine PKA subunits were provided by J. Scott and distributed as fol. RIIβ: 1:100,000; RIIα: 1:1,000; and RIIα: 1:200. Primary antibodies were visualized using horseradish peroxidase–conjugated goat antibody–antibody (1:40,000) and enhanced chemiluminescence (Amersham). Hepatic lipid measurements. Lipids were extracted from mouse livers as described (19). Triglyceride and cholesterol content were then measured on extracted samples using the colorimetric kits. Statistics. Data are presented as means ± SE. Differences between genotypes were determined using the Student’s t test. Pearson’s correlation coefficients were used to assess relations between adiposity and glucose disposal. P < 0.05 was accepted as statistically significant.

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Research design and methods. RIIβ-deficient (RIIβ−/−) mice. Mice were originally generated on a 50:50 (129X5C57BL/6J) genetic background (9). Mice have been backcrossed to C57BL/6J (Jackson Laboratory, Bar Harbor, ME) for five generations and then interbred to produce the RIIβ−/− and wild-type littermates used here.

Experimental design. The two diets used in our studies were rodent chow (Wayne Rodent BLOX 8004; Teklad, Madison, WI) and a high-fat, high-sucrose “diabetogenic” diet (No. F1850; Bioserve, Frenchtown, NJ) containing 35% (wt/wt) fat (primarily lard) and 37% carbohydrate (primarily sucrose). For all experiments, mice were maintained in a 25°C facility with a strict 12-h light/dark cycle (06:00 A.M./06:00 P.M.) and were given free access to food and water. Unless otherwise noted, food was removed from mice 4 h before the collection of blood from the retro-orbital sinus into tubes containing anti-coagulant (1 mg/ml/EDTA). Plasma was used immediately or stored at −70°C until analysis. Mice were killed by cervical dislocation. This project was approved by the Animal Care and Use Committee of the University of Washington.

Two separate experiments were performed. In the first study (Study 1), male RIIβ−/− and wild-type mice were maintained on a rodent chow until they were 16–20 weeks old and then were fed the diabetogenic diet for 15 weeks. Body weights, food intake, plasma glucose, insulin, and leptin levels were quantified. Food intake was estimated from the difference in food remaining in the food trough between the afternoon, when food was given, and the next day, when food troughs were replaced. The total amount of food eaten by one to four mice per cage, in each of four cages per genotype, was averaged over 3-day periods during weeks 2, 4, 6, 8, and 10.

In the second experiment (Study 2), comparisons between sexes were made. Male and female RIIβ−/− and wild-type mice, 9–10 weeks old, were fed the diabetogenic diet for 15 weeks. Body weights were monitored throughout the feeding study. At 12 and 14 weeks of dietary treatment, mice were subjected to an insulin sensitivity assay and an intraperitoneal glucose tolerance test (IPGTT), respectively. At 15 weeks, mice were bled and killed, and the individual fat pads were weighed (reproductive and pairs of inguinal, retroperitoneal/renal, and brown adipose tissue).

Analytical procedures. Plasma insulin and leptin were measured using radioimmunoassay kits (No. R1-13K and No. ML-82K; Linco, St. Louis, MO) with rat insulin and mouse leptin as the respective standards. Plasma glucose levels were determined colorimetrically (Cat. #915-100, Sigma, St. Louis, MO). Plasma triglyceride concentrations were assessed after the removal of free cholesterol (Diagnostic Kit #450632, Boehringer Mannheim, Indianapolis, IN) (18). Plasma cholesterol levels were determined using a colorimetric kit (Diagnostic Chemicals Ltd., Oxford, CT). Plasma lipoproteins were separated by fast-performance liquid chromatography gel filtration using a Superose 6 column (Pharmacia LKB Biotechnology, Uppsala, Sweden). A 200-μl aliquot of plasma from each mouse per diet group was analyzed at a flow rate of 0.2 ml/min using phosphate-buffered saline (PBS). Next, 100-μl aliquots from each 0.5-ml fraction were used for total cholesterol determinations.

IPGTT. IPGTTs were performed essentially as described (18). Mice were fasted overnight (18 h) and injected intraperitoneally with 10% glucose in PBS at a dose of 2 g glucose/kg body wt. Plasma glucose was monitored before glucose injection and at 30, 60, 120, and 240 min after injection. Insulin sensitivity assay. Mice were fasted overnight and injected intraperitoneally with pork insulin (Eli Lilly, Indianapolis, IN) at a dose of 2 U/kg body wt. Glucose injection was followed by injection of 1 ml/kg body wt of 20% glucose. Plasma glucose was monitored before injection and at 30, 60, and 120, and 180 min after injection.

RESULTS

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levels increased when mice were fed the high-fat diet, but the pattern of response differed between genotypes. Whereas wild-type mice showed an early and robust increase in leptin, leptin elevations were delayed in RIIβ−/− mice. By 3 weeks, leptin levels in wild-type mice increased fourfold (6.2 ± 1.1 to 27.6 ± 2.0 ng/ml; \( P < 0.001 \)), whereas those in RIIβ−/− mice showed only a twofold increase (2.1 ± 0.1 to 5.2 ± 1.0 ng/ml; \( P < 0.005 \)). These changes are consistent with the delayed weight gain of RIIβ−/− mice compared with controls. By 12–15 weeks, leptin levels were comparable between strains.

Body weights correlated significantly with leptin levels for both wild-type (\( r = 0.88, P < 0.01 \)) and RIIβ−/− (\( r = 0.95, P < 0.008 \)) mice over the 15 weeks of study. Food intake was measured to determine whether differences in weight and leptin levels were reflected as differences in caloric consumption. Average food intake over the 15-week period was higher for wild-type mice than for RIIβ−/− mice (15.4 ± 0.6 vs. 13.8 ± 0.6 cal/mouse/day, \( P = 0.06 \)). However, when food consumption was corrected to differences in body weight, the RIIβ−/− mice actually consumed more calories. Wild-type mice consumed 0.37 ± 0.02 cal/g mouse/day, whereas RIIβ−/− mice consumed 0.44 ± 0.02 cal/g mouse/day (\( P = 0.005 \)). Since the leptin levels were significantly different between the genotypes (Fig. 1B), leptin levels did not reflect food consumption in these mice.

Mice fed rodent chow (0 weeks) had plasma glucose levels that were comparable between genotypes (Table 1 and Fig. 1C). When the mice were fed the high-fat diet, glucose levels increased in both strains. Glucose levels for wild-type mice increased from 131 ± 14 mg/dl, with final values after 15 weeks of 184 ± 8 mg/dl (\( P < 0.05 \) vs. 0 weeks). RIIβ−/− mice exhibited a steady but gradual increase in glucose with time on the diet to a final value of 205 ± 18 mg/dl (\( P < 0.001 \) vs. week 0). Except for the time point at 3 weeks (\( P < 0.005 \)), plasma glucose levels did not differ significantly between genotypes.

In contrast to glucose, plasma insulin levels were consistently higher in wild-type than in RIIβ−/− mice (Fig. 1D). Initial insulin values were 26% higher for wild-type mice (Table 1) and were approximately two- to fivefold higher at all other time points. Final insulin levels were significantly higher than initial levels for both wild-type (\( P < 0.001 \)) and RIIβ−/− (\( P < 0.001 \)) mice. Overall, both genotypes were able to adjust insulin levels to compensate for increases in glucose, but insulin requirements were higher for wild-type mice than for RIIβ−/− mice.

The observation that RIIβ−/− mice were able to achieve glucose compensation with less insulin suggests improved insulin sensitivity in this strain. This is better reflected by examining the ratio of insulin to glucose, which would be expected to be higher for insulin-resistant mice. Indeed, over the course of the study, the average ratio of insulin to glucose for wild-type mice was 40% higher than that for RIIβ−/− mice (Fig. 1C inset). However, another interpretation of the reduced insulin levels in RIIβ−/− mice may be that loss of RIIβ causes defects in insulin secretion. Immunoblotting of islet tissue taken from wild-type mice showed that RIIβ protein is absent from this tissue (Fig. 2).

Thus, it is unlikely that RIIβ contributes directly to signaling pathways in β-cells involved with insulin secretion. Taken together, these data are consistent with increased insulin sensitivity in the RIIβ−/− strain. **Diabetogenic phenotypes of RIIβ−/− mice.** An additional study (Study 2) was performed to confirm whether RIIβ−/− mice showed improved insulin sensitivity and glucose responsiveness as compared with wild-type mice. In addition, because of the sex dimorphism in obesity and diabetes often seen among mice (21,22), we examined males and females separately. Body weights and adiposity were recorded for diabetogenic diet–fed mice, and IPGTT and insulin sensitivity tests were performed. Finally, the consequences of RIIβ ablation on plasma lipoprotein profiles were determined.

**Body weight and adiposity.** At week 0 of the study (8 weeks of age), body weights for RIIβ−/− mice were 15% lower than those for wild-type mice within each sex (Fig. 3), consistent with data obtained for the older males (Fig. 1A). Absolute body weights were somewhat lower than those seen in Study 1 because the mice in Study 2 were younger. For instance, males weighed 20.3 ± 0.4 vs. 24.2 ±

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**TABLE 1**

Plasma glucose, insulin, and leptin levels for male mice before and after feeding them the diabetogenic diet.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Body weight (g)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>WT</td>
<td>30 ± 1</td>
<td>131 ± 14</td>
<td>0.72 ± 0.07</td>
</tr>
<tr>
<td>Final</td>
<td>WT</td>
<td>48 ± 1§</td>
<td>184 ± 8§</td>
<td>2.78 ± 0.77§</td>
</tr>
<tr>
<td>Initial</td>
<td>RIIβ−/−</td>
<td>24 ± 1§</td>
<td>123 ± 8</td>
<td>0.53 ± 0.02†</td>
</tr>
<tr>
<td>Final</td>
<td>RIIβ−/−</td>
<td>38 ± 2§</td>
<td>205 ± 18§</td>
<td>1.26 ± 0.20†</td>
</tr>
</tbody>
</table>

Data are means ± SE for \( n = 8–12 \) mice per set. Wild-type (WT) and RIIβ−/− mice were fed rodent chow until 16–20 weeks of age (Initial) and then fed the diabetogenic diet for 15 weeks (Final). Mice were fasted for 4 h before plasma isolation. Statistical differences at \( *P < 0.05 \) and \( †P < 0.001 \) between genotypes, and \( §P < 0.001 \) within dietary treatments.
0.8 g (P < 0.001) and females weighed 15.6 ± 0.7 vs. 19.7 ± 0.5 g (P < 0.0006) for RIIβ−/− and wild-type mice, respectively. When they were fed the diabetogenic diet, mice of both sexes experienced steady increases in body weight. Final body weights were 27 ± 1 vs. 43 ± 2 g for male RIIβ−/− versus wild-type mice, respectively (P < 0.0002), and 23 ± 1 vs. 31 ± 4 g for female RIIβ−/− versus wild-type mice, respectively (P < 0.03). RIIβ−/− mice of both sexes maintained lighter phenotypes than wild-type mice when fed either the rodent chow diet or the diabetogenic diet.

In this study, a sex difference was observed. The percent body weight gain between genotypes fed the diabetogenic diet for 15 weeks was greater in male mice (41%) than in female mice (14%). Thus, in our study, sex, age, and genotype influenced the extent of body weight gain in response to the diabetogenic diet.

Based on initial characterizations of RIIβ−/− mice (9), differences in actual body weights between genotypes were probably due to differences in adiposity. To test this, the investigators killed male and female mice (Study 2) after 15 weeks of consuming the diabetogenic diet, and four fat pad pairs (reproductive, inguinal, retroperitoneal plus perirenal, and the sum of these tissue weights [WAT]. Intrascapular brown adipose tissue [BAT]) were collected and weighed (Fig. 4). Loss of RIIβ resulted in reduced adiposities involving all fat pads with the exception of brown adipose tissue in females.

**Glucose disposal.** The effect of RIIβ deficiency on total-body glucose disposal in an IPGTT was examined in chow-fed male mice and diabetogenic diet–fed mice of both sexes (Fig. 5). Results show that loss of RIIβ improves glucose disposal, regardless of dietary treatment. Compared with wild-type mice, RIIβ−/− mice displayed lower glucose levels at all time points after glucose injection. Differences between genotypes matched for age and fed the chow diet (Fig. 5A) were not statistically significant, but a trend for improved glucose disposal by RIIβ−/− mice was seen. Glucose excursion was significantly reduced in female RIIβ−/− mice compared with wild-type mice fed the diabetogenic diet (Fig. 5B). Differences were most dramatic for diabetogenic diet–fed male
mice (Fig. 5C). In RIIβ−/− mice, glucose levels returned to baseline values 120 min after glucose injection; in wild-type mice, this did not occur until 240 min after injection. The profile of glucose disposal remained the same between diabetogenic diet– and chow diet–fed RIIβ−/− mice (compare Fig. 5A with 5C), indicating that loss of RIIβ completely prevented the induction of diet-induced glucose intolerance.

**Insulin sensitivity.** To assess insulin-stimulated glucose clearance directly, in vivo insulin-mediated glucose disposal assays were performed (Fig. 6). For chow-fed, age-matched (24 weeks old) male mice, no significant difference was observed between genotypes. Feeding wild-
type mice the diabetogenic diet resulted in a marked reduction in the percent of glucose cleared after insulin injection. Importantly, this diet-induced reduction was not observed in RIIβ−/− mice (Fig. 6A). Among diabetogenic diet–fed RIIβ−/− male and female mice, the clearance of glucose after insulin injection was the same as for animals fed the chow diet, demonstrating that these mice are resistant to the development of diet-induced insulin resistance.

To determine whether differences in adiposity between genotypes could solely account for differences in insulin sensitivity, insulin-stimulated glucose clearance was assessed as a function of total white adipose tissue weight for both genotypes fed the diabetogenic diet (Fig. 6B). Results demonstrate that the percent glucose clearance was negatively correlated with adiposity only for wild-type mice (r = −0.70, P < 0.0002). This relationship was modified by the RIIβ gene deletion, because no correlation was seen between these parameters for RIIβ−/− mice. The inability to obtain RIIβ−/− mice with adiposity comparable to wild-type mice prevents us from confirming whether the improved insulin sensitivity is solely due to reduced adiposity or whether there are independent effects of RIIβ deficiency on improving insulin-mediated glucose disposal. In an attempt to address this, we evaluated insulin-mediated glucose disposal per gram mouse weight for chow versus diabetogenic diet–fed mice. Results demonstrate that in diabetogenic diet–fed wild-type mice, decreased glucose disposal per gram body weight is observed (P < 0.02) (Fig. 6C). This supports the concept that the diet itself increases insulin resistance: i.e., if insulin resistance was proportional only to increased body weight, similar values between chow diet– and diabetogenic diet–fed mice would be expected. RIIβ−/− mice showed no weight-adjusted reduction in insulin-mediated glucose disposal in response to the diabetogenic diet. Therefore, we conclude that the diabetogenic diet increases resistance to insulin-mediated glucose disposal and that loss of RIIβ ameliorates this effect.

**Plasma lipids.** Dyslipidemia is often observed in conjunction with obesity and diabetes. Plasma total cholesterol levels were significantly higher in wild-type mice than in RIIβ−/− male mice fed the diabetogenic diet for 15 weeks (250 ± 37 and 159 ± 12 mg/dl for wild-type and RIIβ−/− mice, respectively; P = 0.02). Female mice showed significantly lower levels of plasma total cholesterol than males (P < 0.05), and there was a trend toward lower levels in RIIβ−/− mice than in wild-type mice (132 ± 17 and 117 ± 10 mg/dl). No significant differences in plasma triglyceride or free fatty acid levels were observed between genotypes.

Plasma lipoprotein profiles were determined for diabetogenic diet–fed wild-type and RIIβ−/− mice (Fig. 7), and the results were consistent with total cholesterol levels. Although all mice showed an HDL fraction of comparable size and abundance, male RIIβ−/− mice had a dramatic decrease in the VLDL and LDL fraction as compared with wild-type mice. Female RIIβ−/− mice also showed reduced VLDL/LDL cholesterol compared with wild-type mice, although the difference was not as striking.

Finally, quantification of plasma apolipoproteins for male mice fed the diabetogenic diet was performed to assess how RIIβ deficiency influences lipoprotein composition. Consistent with our lipoprotein profile analysis, no differences in apoA-I, the principal protein associated with HDL, was observed. However, we also did not observe any differences in either apoB48 or apoB100, which are proteins associated with VLDL/LDL particles. In contrast, plasma levels of apoE, a protein associated with both VLDL and HDL in rodents (23), was reduced twofold in RIIβ−/− mice (P < 0.006). The higher apoE level in wild-type males is consistent with the presence of β-VLDL as the major lipoprotein within the VLDL/LDL peak (Fig. 7B). β-VLDL particles contain abundant amounts of apoE and cholesterol, with a relatively little triglyceride (24). Further studies are needed to improve resolution between lipoprotein fractions and to determine actual chemical compositions. Overall, RIIβ knockout mice were protected from the diet-induced dyslipidemia that developed in wild-type males.

**Hepatic lipid content.** Hepatic cholesterol and triglyceride levels were quantified for male mice fed the diabetogenic diet for 15 weeks. Liver cholesterol levels were similar between genotypes (~3.1 mg cholesterol/g liver), whereas the RIIβ−/− mice showed a 2.5-fold reduction in triglyceride levels (wild-type: 22.3 ± 2.2 mg/g; RIIβ−/−: 8.5 ± 1.2 mg/g; P < 0.0003). Thus, RIIβ−/− mice were
relatively resistant to diet-induced fatty livers, as reported earlier on a different diet (9).

**DISCUSSION**

We show that a major consequence of PKA RIIβ deficiency is to protect mice from obesity, insulin resistance, and dyslipidemia induced by a high-fat, high-carbohydrate diet. RIIβ−/− mice displayed reduced weight gain, lower insulin levels, and improved total-body glucose disposal as compared with wild-type mice fed a high-fat, high-sucrose diet. The magnitude of effect was dependent on both the sex and age of the mice studied. These findings demonstrate that targeting RIIβ activity would provide an excellent therapy to reduce both obesity and metabolic disorders associated with obesity.

RIIβ expression is limited to a few tissues, which include white and brown adipose tissue and brain (8,25). In murine brain, targeted disruption of the RIIβ gene results in marked reduction of total PKA activity in the striatum, cortex, and hypothalamus (26,27). In adipose tissue, RIIα substitutes for RIIβ and causes a four- to fivefold increase in basal PKA activity, resulting in chronic stimulation of thermogenesis and basal lipolysis. Since RIIβ is absent from pancreatic islets, effects on insulin secretion in response to circulating glucose would be indirect. Taken together, these findings imply that PKA activity in both brain and adipose tissue is involved in regulating both susceptibility to adiposity and insulin-mediated glucose clearance.

There are several mechanisms that are likely contributing to the lean phenotype observed in the RIIβ−/− mice. Possible contributing factors include increased thermogenesis, increased lipolysis, and decreased food intake. RIIβ−/− mice fed normal chow diets have elevations in thermogenesis (9) and basal lipolysis (28), both of which may promote leanness. These attributes may contribute significantly to the leanness seen in diabeticogen diet–fed mice, since food intake was similar based on total body weight and significantly higher based on gram mouse weight for the RIIβ−/− mice. Therefore, differences in caloric consumption cannot fully account for the unusual leanness in the RIIβ−/− mice. Leptin was markedly reduced in RIIβ mutants compared with wild types at the onset of this study and during most of the time on the diabeticogen diet. Leptin is a long-term satiety hormone secreted by adipocytes in proportion to adiposity (29–31). Since the RIIβ−/− mice ate comparably to the wild-type mice despite having lower leptin levels, this suggests that RIIβ deficiency dysregulates leptin-induced satiety responses. It is likely that RIIβ−/− mice are protected against developing insulin resistance when fed the diabeticogen diet at least in part because of their relative resistance to diet-induced obesity. We were unable to obtain a sufficient number of RIIβ−/− mice that had white adipose tissue weights similar to those of wild-type mice. The availability of this population would allow us to directly test whether RIIβ has direct effects on influencing insulin resistance. However, when we corrected insulin-mediated glucose disposal to differences in body weights, we observed that diabeticogen diet–fed RIIβ−/− mice retained a similar amount of insulin-mediated glucose disposed per gram mouse weight as compared with chow-fed RIIβ−/− mice, whereas wild-type mice showed decreased glucose disposal per gram mouse weight. This finding suggests that elimination of RIIβ improves insulin-mediated glucose disposal through a mechanism independent of alterations in body composition. PKA is known to antagonize insulin’s activation of the mitogen-activated protein kinase cascade, probably by blocking events at the levels of ras or raf (32). It is possible that this action requires an RIIβ-containing PKA holoenzyme and cannot be subserved by the compensating RIIα isoform present in RIIβ knockout mice. Insulin action in adipose may thus be enhanced in RIIβ−/− mice due to the absence of PKA-mediated counterregulation of insulin signaling.

Elevations in LDL and VLDL occur commonly among mice fed high-fat diets (33–36). In fact, the major lipoprotein increase is seen in β-VLDL (36), cholesterol-rich particles containing both apoB and apoE (24). Unique to this report was the marked loss of lipoproteins from the VLDL/LDL fraction in the RIIβ−/− mice. Concomitant with this loss was a twofold reduction in plasma levels of apoE, supporting the concept that β-VLDL is nearly absent in RIIβ−/− animals fed a high-fat diet. Remaining apoE is presumed to reside in the HDL fraction (35). It remains to be determined whether the difference in β-VLDL between RIIβ−/− and wild-type mice results from direct effects of RIIβ on lipoprotein production or clearance, as opposed to secondary effects arising from differences in diet-induced insulin resistance.

A consistent feature of obesity and insulin resistance in rodents is sex dimorphism. Male mice are more susceptible to body weight gain and diabetes due to eating high-fat diets (22) or to genetic mutations (21) as compared with female mice. Molecular mechanisms for this difference are not clear but may involve the expression of hepatic sex steroid sulfotransferases (37). Here, female RIIβ−/− mice gained proportionally more body weight than male mice, although they retained insulin sensitivity. This is a somewhat unusual finding among diabetic rodent models and may reflect a role for PKA in modulating hormonal control of body weight.

In early studies of RIIβ−/− mice from the mixed 129 and C57BL/6 genetic background, we found greater disparities in diet-induced weight gain between genotypes than were seen for the >97% C57BL/6 background mice presented in this report. This may be due to allelic contributions from 129, known to present a lean phenotype in other studies. Thus, the influence of the RIIβ mutation is magnified in mice containing 129 genes and demonstrates that the influence of RIIβ function is dependent on unknown loci present within the 129 genome. Importantly, however, the effects of the RIIβ mutation on body weight are significant regardless of genetic background, which suggests that targeting this gene in human therapies will benefit a range of human populations.

Overall, our studies support independent roles for PKA RIIβ in the modulation of weight gain and insulin resistance associated with obesity. It remains to be determined which of these effects are modulated by PKA activity within adipose versus central nervous system–mediated responses. Our results suggest that alleles at loci coding for PKA regulatory subunits should be included in the list.
of candidate genes determining susceptibility to obesity and diabetes associated with obesity.

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