Effects of Aging and a High Fat Diet on Body Weight and Glucose Tolerance in Glucagon-Like Peptide-1 Receptor\(^{-/-}\) Mice*

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ABSTRACT

Disruption of glucagon-like peptide-1 (GLP-1) receptor signaling in mice results in mild glucose intolerance, principally due to elimination of the incretin effect of GLP-1. Despite the inhibitory effects of GLP-1 on food intake, 6- to 8-week-old GLP-1 receptor\(^{-/-}\) (GLP-1R\(^{-/-}\)) mice were not obese and did not exhibit disturbances of feeding behavior. As both diabetes and obesity frequently become more phenotype evident in older rodents, we studied the consequences of aging and a high fat diet on glucose control and body weight in GLP-1R\(^{-/-}\) mice. No evidence of obesity or deterioration in glucose control was detected in 11- and 18-month-old GLP-1R\(^{-/-}\) mice (mean weight, 34.7 ± 2.0, 30.5 ± 1.5, and 34.6 ± 2.8 g in male and 25.3 ± 1.6, 28.4 ± 1.2, and 31.9 ± 2.9 g in female GLP-1R\(^{-/-}\), GLP-1R\(^{-/-}\), and GLP-1R\(^{-/-}\) mice, respectively; \(P = \text{NS}\)). After 18 weeks of high fat feeding, GLP-1R\(^{-/-}\) mice gained similar (males) or less (females) weight than age- and sex-matched CD1 controls. No significant deterioration in glucose tolerance was observed after high fat feeding in GLP-1R\(^{-/-}\) mice. These observations demonstrate that long term disruption of GLP-1 signaling in the central nervous system and peripheral tissues of older mice is not associated with the development of obesity or deterioration in glucose homeostasis. (Endocrinology 139: 3127–3132, 1998)

GLUCAGON-LIKE peptide-1 (GLP-1) produced in the enteroendocrine cells of the small and large intestine is a potent incretin and stimulates both glucose-dependent insulin secretion (1–5) and proinsulin gene expression (6, 7). GLP-1 appears to induce glucose competence in islet \(\beta\)-cells by recruiting glucose-resistant \(\beta\)-cells, actions that further enhance the capacity for insulin-stimulated insulin secretion (8). The glucose-lowering properties of GLP-1 are not restricted to the islet \(\beta\)-cell, as GLP-1 also inhibits gastric emptying and glucagon secretion in normal subjects and patients with diabetes (5, 9–12). Antagonism of GLP-1 receptor (GLP-1R) function, as carried out with the GLP-1R antagonist exendin-(9–39), results in increased blood glucose and diminished glucose-stimulated insulin secretion (13–15), providing key evidence in support of the biological importance of GLP-1 in the control of postabsorptive glucose disposal. These glucose-lowering properties of GLP-1 have raised the possibility that GLP-1 or its analogs may be a useful adjunct for treatment of patients with diabetes mellitus.

GLP-1 is also synthesized in the central nervous system (CNS), predominantly in the brain stem and hypothalamus (16), and brain stem projections have been identified that appear to transport GLP-1 to diverse regions of the CNS (17, 18). The initial observation that GLP-1 activated adenylate cyclase activity in hypothalamic and pituitary membrane preparations was followed by studies demonstrating GLP-1-binding sites and GLP-1 expression in different brain regions (19, 20); however, the precise physiological role(s) for GLP-1 in the CNS remained unclear. The demonstration that GLP-1 was a potent inhibitor of food intake coupled with the high density of GLP-1-binding sites in hypothalamic nuclei important for control of satiety strongly suggested that GLP-1 was an important peptide mediator of feeding behavior and weight control (21). Furthermore, GLP-1 inhibited the neuropeptide Y (NPY)-induced stimulation of food intake and blockade of CNS GLP-1Rs with the antagonist exendin-(9–39) potentiated the NPY-induced stimulation of feeding (21). These experiments provided important new evidence implicating the GLP-1R as a key mediator of peptide regulation of feeding in vivo.

Molecular cloning of GLP-1Rs from pancreatic islets, heart, lung, and brain (22–25) has provided direct evidence that the amino acid sequence of the GLP-1R is identical in all of these tissues. Accordingly, derivation of mice with a complete disruption of GLP-1 signaling provides a useful opportunity to assess the phenotypic consequences of GLP-1R deficiency in both the CNS and peripheral tissues in vivo. GLP-1R\(^{-/-}\) mice exhibit fasting hyperglycemia and abnormal glycemic excursions after both oral and ip glucose challenge (26). These abnormalities together with reduced levels of glucose-stimulated insulin (26) define an essential role for GLP-1 in the control of blood glucose. Surprisingly, despite complete elimination of GLP-1-binding sites in the hypothalamus, GLP-1R\(^{-/-}\) mice are not obese and do not exhibit abnormalities in feeding behavior (26). Nevertheless, the initial characterization of glucose tolerance and feeding behavior in GLP-1R\(^{-/-}\) mice was carried out on young mice, 8–10 weeks of age. Accordingly, it remained possible that analysis of older animals might reveal additional defects in glucose metabolism.

Received December 17, 1997.

* This work was supported in part by an operating grant from the Juvenile Diabetes Foundation International, a Juvenile Diabetes Foundation International Fellowship (to L.A.S.), and a Scientist Award from the Medical Research Council of Canada (to D.J.D.).

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homeostasis and possibly abnormalities in satiety control or weight regulation. We now report the results of studies examining the response to high-fat feeding and the effects of aging in GLP-1R−/− mice.

Materials and Methods

Animals

Both wild-type CD1 and GLP-1R−/− mice were used and sex-matched and housed at the Toronto Hospital Animal Facility (Toronto, Canada) for up to 16 months (aging study) or for 20 weeks (high-fat feeding study) under conditions of constant temperature and humidity with a 12-h light, 12-h dark cycle as previously described (26). GLP-1R−/− mice were generated using targeted embryonic stem (ES) cells from a 129/SV mouse ES RI cell line (27). Targeted ES cells were aggregated with CD1 morulae to generate chimera CD1 mice as previously described (28). These chimera founder mice were mated with wild-type CD1 mice to generate the GLP-1R−/− line. Control mice were wild-type CD1 mice obtained from Charles River Canada (Toronto, Ontario, Canada).

Aging studies. Male and female wild-type (+/−), heterozygous (+/−), and homozygous (−/−) littermates derived from matings of mice heterozygous for the GLP-1R (genotype confirmed by Southern blotting) were placed into separate cages at weaning (n = 5/group) under normal housing conditions with a 12-h light, 12-h dark cycle and ad libitum access to water and rodent chow. All mice were weighed at 11 months of age and again at the end of the experiment at 16 months of age.

High fat studies. For high-fat feeding studies, 12-week-old wild-type CD1 and GLP-1R−/− mice (n = 30/group) were provided ad libitum access for 18 weeks to a defined rodent chow (D12451) containing 45% of the total calories from fat (Research Diets, New Brunswick, NJ). At all other times mice received Lab Diet 5001, containing 4.5% of the total calories from fat (PMI Feeds, St. Louis, MO). Once each week the mice were weighed, and the amount of chow consumed was recorded. After 18 weeks of high-fat diet, oral glucose tolerance tests (OGTTs) were performed on (now 30-week-old) wild-type and GLP-1R−/− mice (n = 5/group) as described above.

Glucose and insulin determinations

For assessment of glucose tolerance by OGTT, mice were fasted overnight for 18 h. OGTT was carried out by administering 1.5 mg glucose/g BW orally through a gavage tube. Blood was withdrawn from a tail vein at 0, 10, 20, 30, 60, 90, and 120 min, and blood glucose levels were measured with a ONE TOUCH BASIC glucose monitor (LifeScan Canada, Delta, Canada). Plasma insulin levels were determined in duplicate using an insulin RIA kit (Linco, St. Charles, MO) with rat insulin as a standard. Values are the mean ± SEM. Blood for insulin determinations was collected by tail bleeds induced by a 1- to 2-mm tail nick from minimally restrained, hand-held mice. The blood was collected 20–30 min after glucose administration into 5000 kallikrein inhibitory units/ml Trasylol-32 mM EDTA-0.1 mM dipotassium A; plasma was separated by centrifugation and stored at −80°C until assayed. Statistical analysis was performed using the Instat program (Graph Pad Software, San Diego, CA) for the Macintosh. All animal studies were carried out in accordance with guidelines and protocols approved by the Toronto Hospital animal care committee.

Results

Analysis of mice maintained on normal laboratory chow for 11 months did not reveal any significant differences in body weight among GLP-1R−/−, GLP-1R+/−, and age- and sex-matched wild-type control mice (Fig. 1A). The identical groups of mice were subsequently maintained on normal rodent chow for a further 5 months. Although a few 16-month-old GLP-1R−/− females were heavier than wild-type controls, this difference was not statistically significant (Fig. 1A). Furthermore, no difference in weights between 16-month-old control and GLP-1R−/− male mice was detected. These observations demonstrate that unlike other rodent models of obesity (29), GLP-1R−/− mice do not develop increased weight gain with advancing age.

As previous studies of aging rodents have also demonstrated a deterioration in glucose tolerance in older animals, we carried out OGTTs on the 16-month-old mice (Fig. 1B). Glucose tolerance was impaired in 16-month-old female GLP-1R−/− mice, and the difference in blood sugars (compared with age-matched wild-type controls) was statistically significant (P < 0.05–0.01). In contrast to the abnormal glucose tolerance previously demonstrated in 6- to 8-week-old male GLP-1R−/− mice (26), glucose tolerance was not significantly different (from controls) in 16-month-old male GLP-1R−/− mice (Fig. 1B). Furthermore, we did not detect a significant difference in the levels of glucose-stimulated insulin in 16-month-old GLP-1R−/− mice (Fig. 1C). Taken together, these experiments clearly demonstrate that the phenotype of glucose intolerance does not markedly deteriorate and may even improve (relative to that in age-matched wild-type controls) in older mice with disruption of GLP-1 signaling.

To determine the effects of high-fat feeding on body weight and glucose control in the absence of central or peripheral GLP-1 action, control and GLP-1R−/− mice were maintained for 18 weeks on a high-fat rodent diet (45% of the total calories from fat). As shown in Fig. 2A, although both wild-type and GLP-1R−/− female mice gained weight on the high-fat diet, wild-type mice gained significantly more weight (31% increase from 35.5 ± 0.5 to 46.4 ± 1.3 g) than did female GLP-1R−/− mice (18% increase from 29.3 ± 0.9 to 34.4 ± 1.8 g; P < 0.001, control vs. GLP-1R−/− mice) from 10–18 weeks. In contrast, the weights of male wild-type control CD1 mice increased by 28%, whereas the weights of GLP-1R−/− mice consuming the identical high-fat diet increased by 25% (Fig. 2A; P = NS).

As glucose intolerance was previously noted to be comparably more perturbed in younger male GLP-1R−/− mice (26), we performed OGTTs on age-matched wild-type mice and GLP-1R−/− mice (Fig. 2B) maintained on either regular rodent or high-fat chow for 18 weeks. Although blood glucose excursion was slightly greater in GLP-1R−/− mice maintained on a high-fat diet, this difference was not statistically significant (Fig. 2B). In contrast, after high-fat feeding, the levels of circulating insulin after glucose challenge were markedly elevated in GLP-1R−/− mice (Fig. 2C). These observations raise the possibility of a differential induction of insulin resistance and/or β-cell sensitivity in response to the high-fat feeding, possibly to fatty acids, in GLP-1R−/− mice that merits further investigation.

Discussion

The observation that intracerebroventricular administration of GLP-1 in rats potently inhibited short-term feeding has stimulated considerable interest in the possible role of GLP-1 as a satiety factor (21). Subsequent studies demonstrated that intracerebroventricular (icv), but not ip, injection of GLP-1 inhibits food consumption in food-restricted rats, and both icv and ip GLP-1 inhibited angiotension II-induced drinking behavior and water intake in rats (30). The mechanisms un-
derlying the effects of GLP-1 on food intake remain controversial. The demonstration that central infusion of GLP-1 may be associated with the development of conditioned taste aversion in rats (31) raised the possibility that the reduced food intake observed after icv GLP-1 administration reflects an aversive, and not necessarily a direct, anorectic effect of GLP-1 action. In contrast, similar experiments by other investigators have failed to demonstrate either locomotor abnormalities or conditioned taste aversion associated with icv GLP-1 injection in rats (30, 32), raising the possibility that the actions of GLP-1 may indeed reflect direct anorectic effects on hypothalamic feeding centers in vivo. The short term inhibitory effects of GLP-1 on food intake have recently been extended to human subjects, as normal volunteers receiving GLP-1 by iv infusion experienced enhanced sensations of increased satiety in association with decreased food intake (33). The results of the experiments presented here extend our initial finding that 6- to 8-week-old GLP-1R+/− mice are lean by demonstrating that obesity fails to develop in GLP-1R+/− mice with aging or after 18 weeks of high fat feeding.

Although both leptin and GLP-1 inhibit food intake, comparison of the inhibitory effects of leptin vs. GLP-1 revealed several important differences. First, analysis of c-Fos-like immunoreactivity in the brains of peptide-injected rats demonstrated differential stimulation of fos activation in different brain nuclei by these two peptides, implying unique pathways for the actions of leptin and GLP-1 (34). Furthermore, whereas both leptin and GLP-1 inhibit feeding in short-term studies, analysis of 16-h food consumption and 24-h changes in body weight after icv peptide administration demonstrated that only the inhibitory effects of leptin, but not GLP-1, were significant at these longer time points (31). These observations together with experimental data demonstrating that GLP-1 inhibits short term, but not long term, food intake in lean and obese rats (35) suggest that the inhibitory effects of GLP-1 on food intake may be relatively transient. These temporal differences between GLP-1 and leptin on suppression of food intake may explain why disruption of leptin signaling is associated with marked changes in food intake and body weight (36), whereas disruption of GLP-1 signaling, as shown here, produces no long term detectable phenotypic changes in food intake or body weight (26).

As obesity commonly arises secondary to a mismatch between caloric intake and expenditure, we hypothesized that exposure of GLP-1R−/− mice to a high fat diet might pre-dispose the mice to the development of obesity. No difference in weight gain was observed in male wild-type vs. GLP-1R−/− mice, and remarkably, female GLP-1R−/− mice

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Fig. 1. A, Body weight, glucose tolerance, and glucose-stimulated insulin levels in 11- and 16-month-old wild-type (+/+, n = 5), GLP-1R heterozygote (+/−; n = 5), and homozygote (−/−; n = 5 for male and

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9 for female) mice. Body weight measurements of wild-type (solid black bars), heterozygote (hatched bars), and homozygous GLP-1R−/− mice (open bars) are shown as the mean ± SEM. B, OGTT results for 16-month-old female (top panel) and male (lower panel) GLP-1R+/+ control, GLP-1R+/−, and GLP-1R−/− mice. Statistical significance between groups (control vs. GLP-1R) was determined by ANOVA: *, P < 0.05; **, P < 0.01. C, Plasma insulin levels after oral glucose challenge in wild-type (solid bars), GLP-1R−/− heterozygote (hatched bars), and GLP-1R−/− mice (open bars). Values are expressed as the mean ± SEM. Statistical significance between groups (control vs. GLP-1R) was determined by ANOVA: *, P < 0.05; **, P < 0.01.
Fig. 2. Body weights, glucose tolerance, and glucose-stimulated insulin levels in wild-type (+/+) and GLP-1R (−/−) male (n = 20/group) and female (n = 30/group) mice maintained on a high fat (containing 45% fat) rodent chow diet. A. Comparison of relative change in body weights between female (upper panel) and male (middle panel) wild-type (open circles) or GLP-1R (−/−) mice (closed circles) maintained on a high fat diet for a period of 18 weeks. Values are expressed as a percentage of the initial body weight (mean ± SEM) at the start of the high fat diet. The lower panel shows actual body weights of wild-type (+/+) and GLP-1R (−/−) mice before (open bars) and after (solid black bars) 18 weeks of the high fat diet. Statistical significance between groups in the top and middle panels was determined by ANOVA. *** P < 0.001. B. OGTT results for wild-type (top panel) or GLP-1R (−/−) (lower panel) male mice maintained on standard rodent chow (open circles or squares) or high fat rodent chow (closed circles or squares). Values are expressed as the mean ± SEM. C. Plasma insulin levels after oral glucose challenge in wild-type (solid bar) and GLP-1R (−/−) (open bar) male mice. Values are expressed as the mean ± SEM. Statistical significance between groups (control vs. GLP-1R (−/−)) was determined by ANOVA. *** P < 0.001.

Gained significantly less weight, compared with wild-type controls, after 18 weeks of high fat feeding. Despite exposure to a high fat diet for 18 weeks, control CD-1 mice did not develop obesity or glucose intolerance. The murine response to high fat feeding and susceptibility to the development of obesity appear to be highly strain specific (37). The failure of GLP-1R (−/−) mice to develop obesity may therefore be related in part to the CD-1 genetic background that harbors the
GLP-1R mutation. Alternatively, multiple compensatory changes in the factors that regulate appetite control and body weight might mitigate against increased body weight in the absence of hypothalamic GLP-1 signaling.

Evidence of the existence of compensatory mechanisms in the control of food intake derives from studies of mice with NPY deficiency. Despite the central role of neuropeptide Y in the stimulatory control of food intake, mice with disruption of the NPY gene do not exhibit disturbances of feeding or body weight (38); however, mice with mutations at both the ob and NPY loci eat less, have increased energy expenditure, and are significantly less obese than ob/ob mice with normal NPY function (39). These observations together with the demonstration that NPY-deficient mice are leptin sensitive (38) demonstrate important functional interactions among peptideergic networks that control body weight and raise the possibility that redundancy at the level of CNS GLP-1 signaling might compensate for the lack of inhibitory GLP-1 action on feeding behavior in vivo.

Intriguingly, we observed a gender-specific difference in the response to high fat feeding, with GLP-1R−/− female mice gaining comparatively less weight than their male counterparts. Studies of heterozygous mice with a mutation in one glucokinase allele also demonstrated that female mice gained less weight on a high fat diet (40). We previously observed that younger male GLP-1R−/− mice exhibited a greater degree of glucose intolerance than age-matched female littermates (26). The mechanism(s) responsible for these gender-specific differences remains unknown; however, gender-specific differences in murine diabetes are not uncommon. For example, the cumulative incidence of diabetes in the NZY mouse is 98% in males and 31% in females at 48 weeks of age (41). Similarly, a greater degree of glucose intolerance has been observed in male transgenic mice of different genetic backgrounds in several different studies (42, 43).

Surprisingly, despite previous observations that glucose intolerance in rodents often deteriorates with increasing age (41), we did not detect worsening of oral glucose tolerance in older GLP-1R−/− mice. The glycemic excursion after oral glucose challenge was actually similar in 16-month-old male wild-type CD1 and GLP-1R−/− mice. These results may be explained in part by a mild deterioration in glucose excursion with aging in older CD1 control mice. Furthermore, despite the well known deleterious effects of increased fatty acids on insulin resistance and insulin secretion (44), glucose tolerance did not deteriorate in GLP-1R−/− mice consuming a high fat diet. The reason for the markedly increased glucosestimulated insulin levels in GLP-1R−/− mice after high fat feeding remains unknown, but may reflect the need of GLP-1 R−/−-s inlets to overcome peripheral insulin resistance through increased insulin secretion. Comparison of the glucose-stimulated insulin levels in 16-month-old mice reported here vs. levels in 8-week-old GLP-1R−/− characterized previously (26) demonstrates that glucose-stimulated insulin levels are actually higher in the older GLP-1R−/− mice, consistent with the relatively normal glycemic excursion detected in older GLP-1R−/− mice. These observations highlight the importance of longitudinal studies in the phenotypic assessment of mouse models of diabetes, as metabolic abnormalities detected in younger mice may be subject to modification as the animal ages. Taken together, the demonstration that aging and high fat feeding do not induce obesity in GLP-1R−/− mice suggests that either GLP-1 is not essential for regulation of body weight or multiple compensatory mechanisms exist for the control of feeding and body weight in vivo.

Acknowledgment

We thank Lorraine DeForest for technical assistance with the feeding studies.

References

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