0013-7227/98/$03.00/0
Endocrinology

Vol. 139, No. 7
Printed in U.S.A.

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

LOUISE A. SCROCCHI AND DANIEL J. DRUCKER

Department of Medicine, Banting and Best Diabetes Center, Toronto Hospital, University of Toronto, Toronto, Ontario, Canada M5G 2C4

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 phenotypically 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 16-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 = 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 β-cells by recruiting glucose-resistant β-cells, actions that further enhance the capacity for glucose-stimulated insulin secretion (8). The glucose-lowering properties of GLP-1 are not restricted to the islet β-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-1R 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 peptidergic 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-1R 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

Received December 17, 1997.
Address all correspondence and requests for reprints to: Dr. D. Drucker, Toronto Hospital, 200 Elizabeth Street, CCRW3–838, Toronto, Ontario, Canada M5G 2C4. E-mail: d.drucker@utoronto.ca.
* 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.).

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 age 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 R1 cell line (27). Targeted ES cells were aggregated with CD-1 morulae to generate chimeric CD-1 mice as previously described (28). These chimeric founder mice were mated with wild-type CD-1 mice to generate the GLP-1R$^{-/-}$ line. Control mice were wild-type CD-1 mice obtained from Charles River Canada (Toronto, Ontario, Canada).

Aging studies. Male and female wild-type (+/+), heterozygote (+/−), and homozygote (−/−) littermates derived from matings of mice heterozygous for the GLP-IR (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-IR $^{-/-}$ 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-IR $^{-/-}$ 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 nM diprotin 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-IR$^{-/-}$ 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-IR $^{-/-}$ 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 comparatively 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 angiotensin II-induced drinking behavior and water intake in rats (30). The mechanisms un-

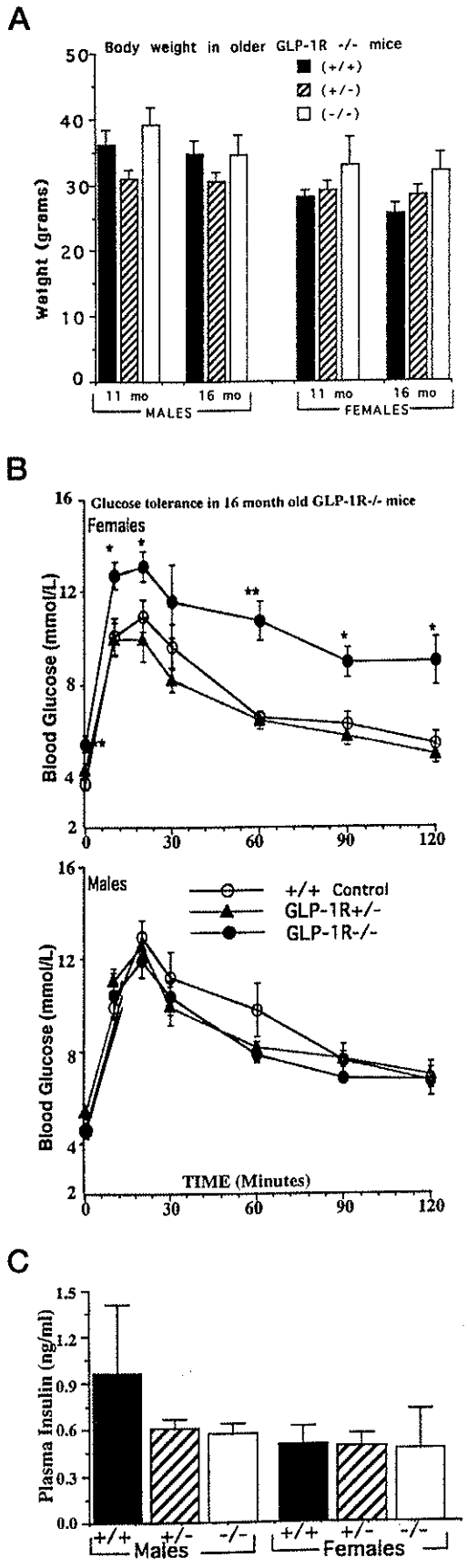

FIG. 1. A, Body weight, glucose tolerance, and glucose-stimulated insulin levels in 11- and 16-month-old wild-type (+/+; n = 5), GLP-1R heterozygote (+/−; n = 5), and homozygote ($^{-/-}$; n = 5 for male and 9 for female) mice. Body weight measurements of wild-type (*solid black bars*), heterozygote (*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: *, $P < 0.05$; **, $P < 0.01$.

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 locomoter 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 predispose 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

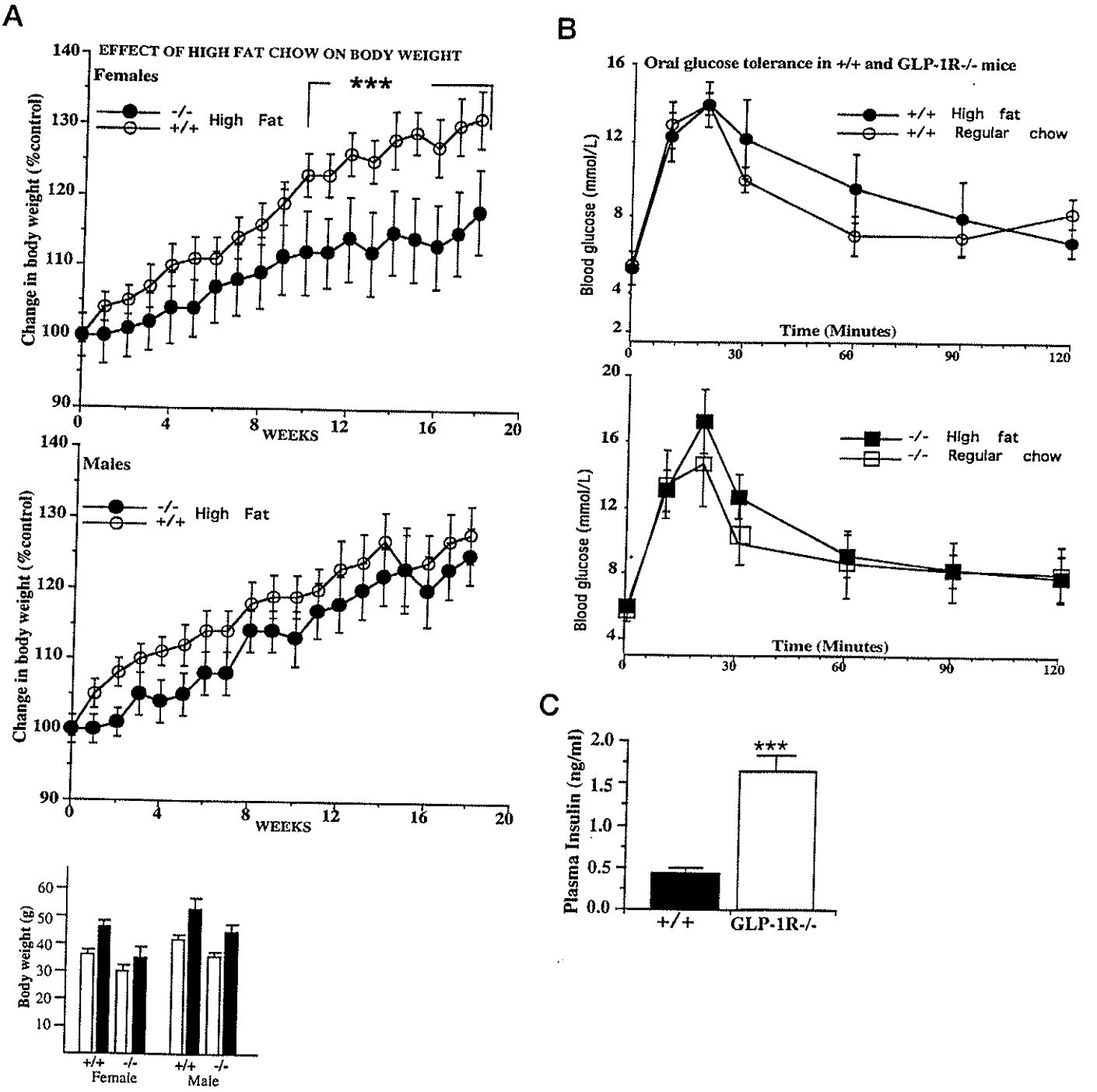

FIG. 2. Body weights, glucose tolerance, and glucose-stimulated insulin levels in wild-type (+/+) and GLP-IR (−/−) male (n = 20/group) and female (n = 30/group) mice maintained on a high fat (containing 45% fat) rodent chow diet. A, Comparison of relative change in body weights between female (*upper panel*) and male (*middle panel*) wild-type (*open circles*) or GLP-1R$^{-/-}$ mice (*closed circles*) maintained on a high 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 peptidergic 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 NSY 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 glucose-stimulated insulin levels in GLP-1R$^{-/-}$ mice after high fat feeding remains unknown, but may reflect the need of GLP-1 R$^{-/-}$ islets 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

1. **Mojsov S, Weir GC, Habener JF** 1987 Insulinotropin: glucagon-like peptide I (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79:616–619
2. **Kreymann B, Ghatei MA, Williams G, Bloom SR** 1987 Glucagon-like peptide-1 7–36: a physiological incretin in man. Lancet 2:1300–1304
3. **Holst JJ, Orskov C, Nielsen OV, Schwartz TW** 1987 Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett 211:169–174
4. **Gutniak M, Orskov C, Holst JJ, Ahrén B, Efendic S** 1992 Antidiabetogenic effect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes mellitus. N Engl J Med 326:1316–1322
5. **Ahren B, Larsson H, Holst JJ** 1997 Effects of glucagon-like peptide-1 on islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 82:473–478
6. **Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF** 1987 Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proc Natl Acad Sci USA 84:3434–3438
7. **Fehmann H-C, Habener JF** 1992 Insulinotropic hormone glucagon-like peptide-I(7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma βTC-1 cells. Endocrinology 130:159–166
8. **Holz GG, Kühtreiber WM, Habener JF** 1993 Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7–37). Nature 361:362–365
9. **Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA** 1996 Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7–36)amide in type 2 (non-insulin dependent) diabetic patients. J Clin Endocrinol Metab 81:327–332
10. **Nauck MA, Wollschlager D, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Willms B** 1996 Effects of subcutaneous glucagon-like peptide 1 (GLP-1[7–36 amide]) in patients with NIDDM. Diabetologia 39:1546–1553
11. **Toft-Nielsen M, Madsbad S Holst JJ** 1996 The effect of glucagon-like peptide 1 (GLP-1) on glucose elimination in healthy subjects depends on the pancreatic glucoregulatory hormones. Diabetes 45:552–556
12. **Rachman J, Gribble FM, Barrow BA, Levy JC, Buchanan KD, Turner RC** 1996 Normalization of insulin responses to glucose by overnight infusion of glucagon-like peptide 1(7–36)amide in patients with NIDDM. Diabetes 45:1524–1530
13. **D'Alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker D, Eng J, Ensinck JW** 1996 Elimination of the action of glucagon-like peptide 1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. J Clin Invest 97:133–138
14. **Kolligs F, Fehmann H-C, Göke R, Göke B** 1995 Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9–39) amide. Diabetes 44:16–19
15. **Wang Z, Wang RM, Owji AA, Smith DM, Ghatei MA, Bloom SR** 1995 Glucagon-like peptide 1 is a physiological incretin in rat. J Clin Invest 95:417–421
16. **Drucker DJ, Asa S** 1988 Glucagon gene expression in vertebrate brain. J Biol Chem 263:13475–13478
17. **Han VKM, Hynes MA, Jin C, Towle AC, Lauder JM, Lund PK** 1986 Cellular localization of proglucagon/glucagon-like peptide I messenger RNAs in rat brain. J Neurosci Res 16:97–107
18. **Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C** 1997 Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience 77:257–270
19. **Comb M, Mermod N, Hyman SE, Pearlberg, Ross ME, Goodman HM** 1989 Proteins bound at adjacent DNA elements act synergistically to regulate human proenkephalin cAMP inducible transcription. EMBO J 7:3793–3805
20. **Uttenthal LO, Toledano A, Blazquez E** 1992 Autoradiographic localization of receptors for glucagon-like peptide-1(7–36)amide in rat brain. Neuropeptides 21:143–146
21. **Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi**

SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72

22. **Thorens B** 1992 Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci USA 89:8641–8645
23. **Wei Y, Mojsov S** 1995 Tissue-specific expression of the human receptor for glucagon-like peptide 1: brain, heart and pancreatic forms have the same deduced amino acid sequences. FEBS Lett 358:219–224
24. **Dillon JS, Tanizawa Y, Wheeler MB, Leng X-H, Ligon BB, Rabin DU, Yoo-Warren H, Permutt MA, Boyd III AE** 1993 Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. Endocrinology 133:1907–1910
25. **Lankat-Buttgereit B, Goke R, Fehmann H-C, Richter G, Goke B** 1994 Molecular cloning of a cDNA encoding for the GLP-1 receptor expressed in rat lung. Exp Clin Endocrinol 102:341–347
26. **Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ** 1996 Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide-receptor gene. Nat Med 2:1254–1258
27. **Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC** 1993 Derivation of completely cell culture-derived mice from early passage embryonic stem cells. Proc Natl Acad Sci USA 90:8424–8428
28. **Nagy A, Rossant J** 1993 In: Joyner AL (ed) Production of completely ES cell-derived fetuses. Gene Targeting: A Practical Approach. Oxford University Press, Oxford, pp 147–180
29. **Coleman DL, Eicher EM** 1990 Fat (fat) and tubby (tub): two autosomal recessive mutations causing obesity syndromes in the mouse. J Hered 81:424–427
30. **Tang-Christensen M, Larsen PJ, Goke R, Fink-Jensen A, Jessop DS, Moller M, Sheikh SP** 1996 Central administration of GLP-1(7–36) amide inhibits food and water intake in rats. Am J Physiol 271:R848–R856
31. **Thiele TE, Van Dijk G, Campfield LA, Smith FJ, Burn P, Woods SC, Bernstein H, Seeley RJ** 1997 Central infusion of GLP-1, but not leptin, produces conditioned taste aversion in rats. Am J Physiol 272:R726–R730
32. **McMahon LR, Wellman PJ** 1998 PVN infusion of GLP-1-(7–36) amide suppresses feeding but does not induce aversion or alter locomotion in rats. Am J Physiol 274:R23–R29
33. **Flint A, Raben A, Astrup A, Holst JJ** 1998 Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 101:515–520
34. **Van Dijk G, Thiele TE, Donahey JCK, Campfield LA, Smith FJ, Burn P, Bernstein IL, Woods SC, Seeley RJ** 1996 Central infusions of leptin and GLP-1-(7–36) amide differentially stimulate c-FLI in the rat brain. Am J Physiol 271:R1096–R1100
35. **Donahey JCK, Van Dijk G, Woods SC, Seeley RJ** 1998 Intraventricular GLP-1 reduces short- but not long-term food intake or body weight in lean and obese rats. Brain Res 779:75–83
36. **Chua SC, Chung WK, Wu-Peng XS, Zhang Y, Lui SM, Tartaglia L, Leibel RL** 1996 Phenotypes of mouse diabetes and rat fatty due to mutation in the Ob (leptin) receptor. Science 271:994–996
37. **Surwit RS, Petro AE, Parekh P, Collins S** 1997 Low plasma leptin in response to dietary fat in diabetes-and obesity-prone mice. Diabetes 46:1516–1520
38. **Erickson JC, Clegg KE, Palmiter RD** 1996 Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 381:415–418
39. **Erickson JC, Hollopeter G, Palmiter RD** 1996 Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science 274:1704–1707
40. **Grupe A, Hultgren B, Ryan A, Ma YH, Bauer M, Stewart TA** 1995 Transgenic knockouts reveal a critical requirement for pancreatic β cell glucokinase in maintaining glucose homeostasis. Cell 83:69–78
41. **Ueda H, Ikegami H, Yamato E, Fu J, Fukuda M, Shen G, Kawaguchi Y, Takekawa K, Fujioka Y, Fujisawa T, Nakagawa Y, Hamada Y, Shibata M, Ogihara T** 1995 The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. Diabetologia 38:503–508
42. **Klebig ML, Wilkinson JE, Geisler JG, Woychik RP** 1995 Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes and yellow fur. Proc Natl Acad Sci USA 92:4728–4732
43. **Stenbit AE, Tsao T-S, Burcelin R, Geenen DL, Factor SM, Houseknecht K, Katz EB, Charron MJ** 1997 GLUT4 heterozygous knockout mice develop muscle insulin resistance and diabetes. Nat Med 10:1096–1101
44. **Unger RH** 1994 Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. Biochemistry 33:7460–7469