12. Cannon, M.P., Pisa, P., Fox, R. & Cooper, N. Epstein-Barr virus induces aggressive lymphoproliferative disorders of human B cell origin in SCID/hu chimeric mice. *J. Clin. Invest.* **85**, 1333–1337 (1990).
13. Mocarski, E., Bonyhadi, M., Salimi, S., McCune, J.M. & Kaneshima, H. Human cytomegalovirus in a SCID-hu mouse: Thymic epithelial cells are prominent targets of viral replication. *Proc. Natl. Acad. Sci. USA* **90**, 104–108 (1993).
14. Naniche, D., *et al.* Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *J. Virol.* **67**, 6025–6032 (1993).
15. Dorig, R., Marcel, A., Chopra, A. & Richardson, C.D. The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell* **75**, 295–305 (1993).
16. Manchester, M., Liszewski, M.K., Atkinson, J.P. & Oldstone, M.B.A. Multiple isoforms of CD46 (membrane cofactor protein) serve as receptors for measles virus. *Proc. Natl. Acad. Sci. USA* **91**, 2161–2165 (1994).
17. Slifka, M.K., Matloubian, M. & Ahmed, R. Bone marrow is a major site of long-term antibody production after acute viral infection. *J. Virol.* **69**, 1895–902 (1995).
18. Ahmed, R. & Gray, D. Immunologic memory and protective immunity: Understanding their relation. *Science* **272**, 54–60 (1996).
19. Ueki, Y., *et al.* Clonal analysis of a human antibody response. Quantification of precursors and antibody producing cells and generation and characterization of monoclonal IgM, IgG, and IgA to rabies virus. *J. Exp. Med.* **171**, 19–34 (1990).
20. McChesney, M.B. & Oldstone, M.B.A. Virus-induced immunosuppression: Infections with measles virus and human immunodeficiency virus. *Adv. Immunol.* **45**, 335–380 (1989).
21. Yanagi, Y., Cubitt, B. & Oldstone, M.B.A. Measles virus inhibits mitogen-induced T cell proliferation but does not directly perturb the T cell activation process inside the cell. *Virology* **187**, 280–289 (1992).
22. von Pirquet, C.E. Das Verhalten der kutanen Tuberkulin-reaktion wahrend der Masern. *Deutsch Med. Wochenschr.* **34**, 1297–1300 (1908).
23. McChesney, M.B., Fujinami, R.S., Lampert, P.W. & Oldstone, M.B.A. Viruses disrupt functions of human T lymphocytes. II. Measles virus suppresses antibody production by acting on B lymphocytes. *J. Exp. Med.* **163**, 1331–1336 (1986).
24. McChesney, M.B., Kehrl, J.H., Valsamakis, A., Fauci, A.S. & Oldstone, M.B.A. Measles virus infection of B lymphocytes permits cellular activation but blocks progression through the cell cycle. *J. Virol.* **61**, 3441–3447 (1987).
25. McChesney, M.B., Altman, A. & Oldstone, M.B.A. Suppression of T lymphocyte function by measles virus is due to cell cycle arrest in G1. *J. Immunol.* **140**, 1269–1273 (1988).
26. Casali, P., Rice, G.P.A. & Oldstone, M.B.A. Viruses disrupt functions of human lymphocytes: Effects of measles virus and influenza virus on lymphocyte-mediated killing and antibody production. *J. Exp. Med.* **159**, 1322–1337 (1984).
27. Fenner, F., McAuslan, B.R., Mims, C.A., Sambrook, J. & White, D. *The Biology of Animal Viruses* (Academic Press, New York, 1974).
28. McChesney, M.B., Fujinami, R.S., Lerche, N.W., Marx, P.A. & Oldstone, M.B.A. Virus induced immunosuppression: Infection of peripheral blood mononuclear cells and suppression of immunoglobulin synthesis during natural measles virus infection of rhesus monkeys. *J. Infect. Dis.* **159**, 757–760 (1989).
29. Rebai, N. & Malissen, B. Structural and genetic analyses of HLA class I molecules using monoclonal xenoantibodies. *Tissue Antigens* **22**, 107–117 (1983).
30. Meizlik, E.H. & Carpenter, A.M. Beneficial effect of measles on nephrosis: Report of 3 cases. *Am. J. Dis. Child.* **76**, 83–90 (1948).
31. Gellen, B. & Katz, S. Putting a stop to a serial killer: Measles. *J. Infect. Dis.* **170**, S1–S3 (1994).
32. Markowitz, L.E. & Bernier, R.H. Immunization of young infants with Edmonston-Zagreb measles vaccine. *Pediatr. Infect. Dis. J.* **6**, 809–812 (1987).
33. Sambrook, J., Fritsch, E.F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual*, edn. 2 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989).

Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene

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Glucagon-like peptide 1 (GLP1) is postulated to regulate blood glucose and satiety, but the biological importance of GLP1 as an incretin and neuropeptide remains controversial. The regulation of nutrient-induced insulin secretion is dependent on the secretion of incretins, gut-derived peptides that potentiate insulin secretion from the pancreatic islets[1]. To ascertain the relative physiological importance of GLP1 as a regulator of feeding behavior and insulin secretion, we have generated mice with a targeted disruption of the GLP1 receptor gene (*GLP1R*). These *GLP1R*$^{-/-}$ mice are viable, develop normally but exhibit increased levels of blood glucose following oral glucose challenge in association with diminished levels of circulating insulin. It is surprising that they also exhibit abnormal levels of blood glucose following intraperitoneal glucose challenge. Intracerebroventricular administration of GLP1 inhibited feeding in wild-type mice but not in *GLP1R*$^{-/-}$ mice; however, no evidence for abnormal body weight or feeding behavior was observed in *GLP1R*$^{-/-}$ mice. These observations demonstrate that GLP1 plays a central role in the regulation of glycemia; however, disruption of GLP1/GLP1R signaling in the central nervous system is not associated with perturbation of feeding behavior or obesity *in vivo*.

The incretins gastric inhibitory polypeptide (GIP) and GLP1 are released from enteroendocrine cells and stimulate insulin release following oral but not intravenous glucose administration in rodents and normal human subjects[2–5]. GLP1 also inhibits eating[6] and lowers blood glucose in patients with both non-insulin-dependent diabetes mellitus[7,8] and insulin-dependent diabetes mellitus[9,10], providing a rationale for the use of GLP1 in the treatment of diabetes mellitus. *GLP1R*$^{-/-}$ mice eat and gain weight normally, but exhibit both fasting hyperglycemia and abnormal glucose tolerance with diminished insulin secretion after glucose challenge, demonstrating a central role for GLP1 in the regulation of blood glucose *in vivo*.

The GLP1 receptor gene is expressed predominantly in islets, lung and brain[11]. A role for GLP1 in the control of satiety has recently been suggested by experiments demonstrating that intracerebroventricular (ICV) injection of GLP1 inhibits feeding in rats[6]. A GLP1 receptor antagonist, exendin (amino acids 9–39), doubled food intake following ICV injection in fasted rats and potentiated the neuropeptide Y (NPY)-mediated stimulation of food intake[6]. These observations predict that the GLP1-mediated reduction in feeding might also be beneficial for the control of blood glucose and led to the suggestion that the actions of GLP1 in the central control of satiety may be physiologically relevant *in vivo*[6].

Although the actions of GLP1 in stimulating glucose-dependent insulin secretion are well established, the relative biological potencies of GLP1 and GIP for augmenting insulin secre-

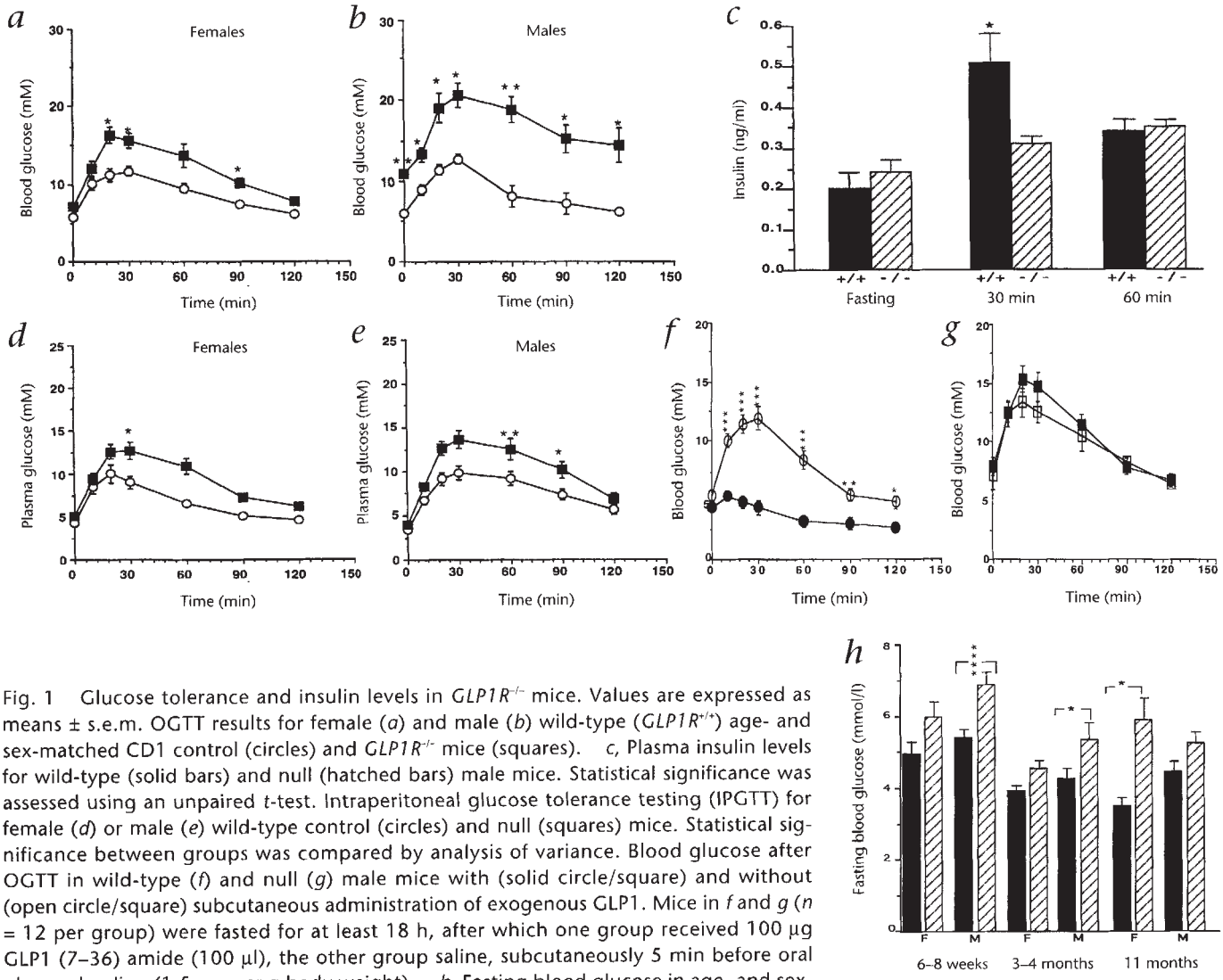

Fig. 1 Glucose tolerance and insulin levels in *GLP1R*$^{-/-}$ mice. Values are expressed as means ± s.e.m. OGTT results for female (*a*) and male (*b*) wild-type (*GLP1R*$^{+/+}$) age- and sex-matched CD1 control (circles) and *GLP1R*$^{-/-}$ mice (squares). *c*, Plasma insulin levels for wild-type (solid bars) and null (hatched bars) male mice. Statistical significance was assessed using an unpaired *t*-test. Intraperitoneal glucose tolerance testing (IPGTT) for female (*d*) or male (*e*) wild-type control (circles) and null (squares) mice. Statistical significance between groups was compared by analysis of variance. Blood glucose after OGTT in wild-type (*f*) and null (*g*) male mice with (solid circle/square) and without (open circle/square) subcutaneous administration of exogenous GLP1. Mice in *f* and *g* (n = 12 per group) were fasted for at least 18 h, after which one group received 100 µg GLP1 (7–36) amide (100 µl), the other group saline, subcutaneously 5 min before oral glucose loading (1.5 mg per g body weight). *h*, Fasting blood glucose in age- and sex-matched female (F) and male (M) wild-type (solid bars) and null (hatched bars) for mice 6–8 weeks, 3–4 months and 11 months of age. Statistical significance between groups was compared by analysis of variance. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

tion remain controversial[5]. Furthermore, GLP1 may have important extrapancreatic effects on inhibition of gastric emptying[12] and stimulation of insulin-dependent glucose uptake[13,14], although the mechanisms responsible for GLP1-mediated effects on glucose uptake have not been clearly elucidated[15]. GLP1 signaling is transduced through a single G-protein-linked receptor predominantly expressed in pancreatic islets, lung, stomach and brain[11]. Although the amino-terminally truncated lizard peptide exendin (9–39) binds to the GLP1 receptor, functions as a GLP1 receptor antagonist and inhibits GLP1-stimulated cyclic AMP formation[5,16], experiments with the cloned human islet GIP receptor demonstrated that exendin (9–39) also functions as a GIP receptor antagonist[17], hence studies with exendin (9–39) do not permit definitive conclusions about the action of GLP1 *in vivo*. To define the physiological importance of GLP1 for regulation of satiety and blood glucose, we isolated the mouse *GLP1R* gene and used homologous recombination in mouse embryonic stem (ES) cells to generate mice harboring a null mutation in both *GLP1R* alleles.

As the GLP1 receptor is a member of the G-protein-coupled receptor superfamily[11], the targeting vector was designed to inactivate the *GLP1R* gene by replacing exons encoding for membrane-spanning and both intra- and extracellular receptor domains. DNA from ES cells and *GLP1R* mutant mice made from ES cell chimeras was analyzed using probes specific to 5′- and 3′-genomic sequences flanking the targeted *GLP1R* integration site. Genomic DNA from wild-type and recombinant ES cell clones and transgenic mice was analyzed for the wild-type and correctly targeted recombinant alleles, and both PCR and Southern analyses were consistent with the correct targeting of the *GLP1R* locus (data not shown).

Both male and female *GLP1R*$^{+/-}$ and *GLP1R*$^{-/-}$ mice appeared normal, and the targeted *GLP1R* recombinant allele was transmitted through the germ line with the expected mendelian frequency. GLP1 receptor mRNA transcripts were not detected in the pancreas, lung or hypothalamus of *GLP1R*$^{-/-}$ mice by northern analysis or polymerase chain reaction coupled with reverse transcriptase (RT-PCR) (data not shown). No histological abnormality was observed following analysis of pancreas, lung or brain obtained from *GLP1R*$^{-/-}$ mice (data not shown).

To determine the importance of the GLP1/GLP1R axis for control of blood glucose, we carried out oral glucose tolerance tests

(OGTT) in both male and female mice. As shown in Fig. 1*a*, female null mice exhibited clearly abnormal levels of blood glucose at multiple time points following oral glucose administration. The elevations in blood glucose excursion were even greater in male null mice, compared with age-matched female null mice (Fig. 1*b*). The elevations in fasting glucose in male null mice have not consistently been observed in all null mice (see Fig. 1*e*), but fasting glucose elevations were more common in *GLP1R*$^{-/-}$ mice, and the increase in fasting blood glucose was often statistically significant (Fig. 1*h*).

As GLP1 is one of the most potent stimulators of insulin gene expression and secretion both *in vitro* and *in vivo*[5,18], we measured plasma insulin levels in both fasting mice and mice after an oral glucose challenge. Fasting insulin levels in *GLP1R*$^{-/-}$ mice were similar to values observed in wild-type controls; the levels of circulating insulin were comparable to values in control wild-type mice 60 minutes after challenge with oral glucose (Fig. 1*c*). In contrast, insulin levels were clearly lower (compared with wild-type controls) in *GLP1R*$^{-/-}$ mice 30 minutes after glucose challenge (Fig. 1*c*, $P < 0.05$). Furthermore, no statistically significant differences in the levels of circulating plasma glucagon were detected; fasting plasma glucagon levels were 63.1 ± 8.2 versus 82.9 ± 9.4, and 30 minutes after OGTT, glucagon levels were 78.6 ± 4.2 versus 73.6 ± 9 pg/ml, in wild-type and null mice, respectively. These observations strongly suggest that an intact GLP1/GLP1R axis is an essential component of glucose-dependent insulin secretion following enteral glucose administration.

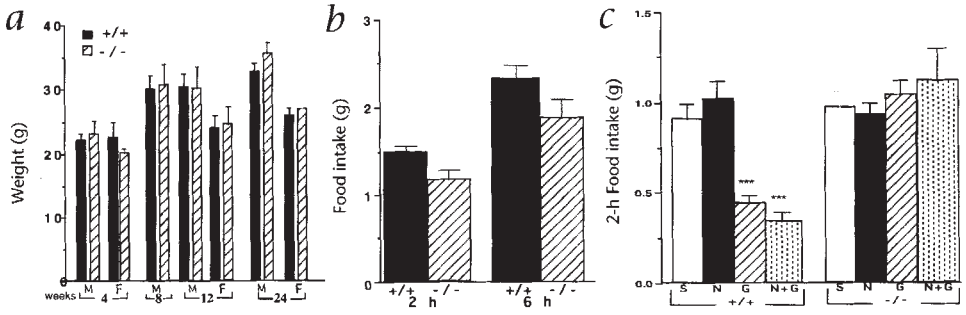

Fig. 2 Body weight, food intake and feeding behavior in *GLP1R*$^{-/-}$ mice. Values are means ± s.e.m. *a*, Body weight measurements of 4-, 8-, 12- and 24-week-old male (M) and female wild-type (F) and null mice. Body weights were measured in grams for 4 weeks (n = 14), 8 weeks (n = 24), 12 weeks (n = 17) and 24 weeks (n = 17) male and female wild-type (solid bars) and *GLP1R*$^{-/-}$ (hatched bars) mice. *b*, Food intake during 2 h and 6 h in control and *GLP1R*$^{-/-}$ mice. Wild-type (+/+, n = 12) and null mice (–/–, n = 12) mice were fasted for 20 h, after which they were placed in individual cages containing preweighed rodent diet. After 2 and 6 h of feeding, the remaining diet was removed and reweighed, and the total amount of diet (in grams) consumed by each mouse was calculated. *c*, Food intake during 2 h in wild-type and null mice fasted for 20 h followed by ICV injection of 5 µl 0.9% saline (S), 5 µg NPY (N), 5 µg GLP1 (G) or NPY plus GLP1 (N+G). Six- to 8-week-old wild-type (+/+) and *GLP1R* knockout (–/–) male mice (20–25 g) were fasted for 20 h and placed in individual cages. Test agents were administered in 0.9% saline. Total food intake (in grams) is depicted as the mean ± s.e.m. for four separate experiments. Statistical significance between groups was compared by analysis of variance. ***$P < 0.0001$. The attenuated NPY induction of food intake is likely attributable to the stress-associated liberation of endogenous NPY because of the anesthesia and experimental manipulation[36].

Glucagon-like peptide 1 secretion from enteroendocrine cells normally increases to meet the demands of a rising blood glucose after nutrient ingestion. Current evidence supports a potential role for GLP1 in the regulation of glucose clearance[13,14,19], possibly through an insulin-dependent mechanism[15]. Alternatively, GLP1 may exert its glucose-lowering action in part through inhibition of glucagon secretion[8,20]. If the GLP1/GLP1R axis is physiologically important for control of blood glucose, we reasoned that glucose handling should be abnormal irrespective of the site of glucose entry. To assess the response of *GLP1R*$^{-/-}$ mice to a non-enteric glucose load and hence to avoid the effects of GLP1 on gastric emptying, we injected control and null mice with glucose intraperitoneally. These experiments clearly demonstrated that mice lacking GLP1 receptors exhibit increased levels of blood glucose, independent of whether glucose is absorbed through the gut or bypasses the enteroinsular axis entirely following absorption through the peritoneum (Fig. 1, *d* and *e*).

To address the possibility that a second class (perhaps exhibiting a different affinity for ligand) of GLP1 receptors is expressed in the periphery and may mediate GLP1 action, we repeated the OGTTs with and without the administration of a pharmacological dose of GLP1. In wild-type mice, concomitant GLP1 administration markedly attenuated the increase in glucose excursion after oral glucose administration (Fig. 1*f*). In contrast, no effect of exogenous GLP1 on glucose levels was observed in null mice (Fig. 1*g*). These data provide clear evidence for the functional elimination of the GLP1 response in *GLP1R*$^{-/-}$ mice. Taken together, these data demonstrate that an intact GLP1 signaling system is important for control of glycemia *in vivo*, irrespective of the site of glucose entry into the circulation.

The expression of GLP1 receptors in the central nervous system[21–24] raised the possibility that GLP1 functions as a neuropeptide *in vivo*. Injection of GLP1 into the cerebral ventricles of fasted rats inhibited feeding, and this effect was blocked by the GLP1 receptor antagonist, exendin (9–39) (ref. 6). Furthermore, injection of exendin (9–39) alone doubled food intake in satiated rats. These findings prompted the suggestion that GLP1 is a potent physiological regulator of satiety[6]. To determine whether *GLP1R*$^{-/-}$ mice exhibit abnormalities in body weight, we compared the weights of male and female wild-type and *GLP1R*$^{-/-}$ mice from 4 to 24 weeks of age. No significant differences in body weight (compared with age-matched controls) were detected in male or female null mice of various ages, suggesting that the GLP1/GLP1R axis is not a key determinant of body mass under basal conditions (Fig. 2*a*). We next studied the feeding behavior of null and wild-type mice after a 20-hour fast, after which the amount of food ingested was determined. In four separate experiments, the mean 2-hour food intake was 1.48 ± 0.78 versus 1.16 ± 0.11 g in wild-type and null mice, respectively. The mean 6-hour food intake was 2.33 ± 0.15 versus 1.88 ± 0.22 g in wild-type and null mice, respectively. No difference in 2-hour or 6-hour food intake was observed in any experiment with either male or female GLP-IR$^{-/-}$ mice (Fig. 2*b*). Taken together, these data

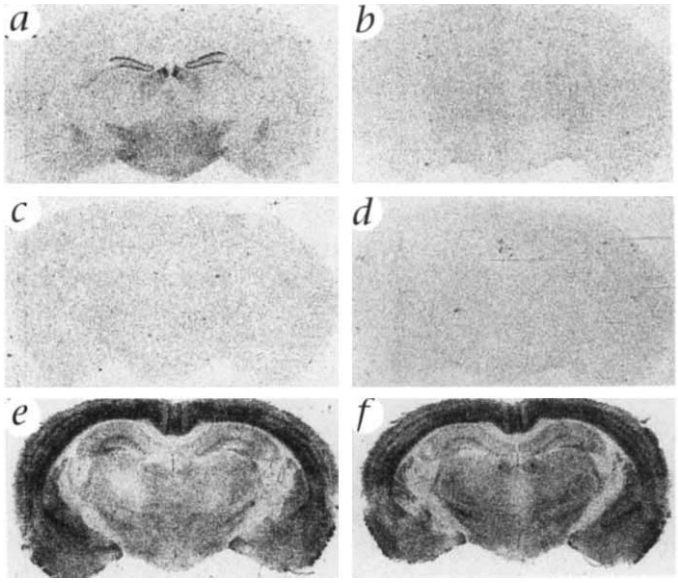

Fig. 3 Binding of ^{125}I-labeled GLP1 (*a–d*) and ^{125}I-labeled CCK-8 (*e* and *f*) in the brains of wild-type (*a*, *c* and *e*) and *GLP1R*$^{-/-}$ (*b*, *d* and *f*) mice. Sections were incubated with ^{125}I-labeled GLP1 (labeled using chloramine-T) in the same buffer, with (*b* and *d*) or without (*a* and *c*) addition of 200 nM unlabeled GLP1.

demonstrate that disruption of the mouse GLP1 receptor is not associated with disturbances in body weight or appetite.

As ICV administration of GLP1 in rats was associated with a potent inhibition of feeding[6], we assessed whether ICV GLP1 also modulated food intake in mice. GLP1 (5 μg) markedly inhibited feeding in wild-type mice, in the presence or absence of NPY. In contrast, the GLP1-mediated inhibition of food intake was completely abolished in null mice (Fig. 2*c*). These observations confirm the potent effects of ICV-administered GLP1 in rodents and suggest that the satiety-regulating effects of GLP1 are mediated through the common "pancreatic" GLP1 receptor. To exclude the possibility that the normal feeding behavior and lack of obesity in *GLP1R*$^{-/-}$ mice are attributable to the existence of a second putative GLP1 receptor, we studied the presence and distribution of GLP1 binding sites in the central nervous system of wild-type and null male mice. A distinctive pattern of distribution of ^{125}I-labeled GLP1 was observed in wild-type mice, including labeling of regions of the mediobasal and lateral hypothalamus, the zona incerta, the central and lateral nuclei of the amygdala, the medial and lateral habenula and the granule cell layer of the hippocampal dentate gyrus (Fig. 3*a*). Binding of ^{125}I-labeled GLP1 was abolished by coincubation with 200 nM unlabeled GLP1 (Fig. 3*b*). No saturable binding of ^{125}I-labeled GLP1 was observed in sections from *GLP1R*$^{-/-}$ mice (Fig. 3, *c* and *d*). In contrast, labeling with ^{125}I-labeled CCK was unaffected in adjacent sections from null mice (Fig. 3, *e* and *f*). Taken together, these observations provide clear evidence demonstrating the loss of both GLP1 bioactivity and GLP1 binding sites in the CNS of *GLP1R*$^{-/-}$ mice. The normal body weights and feeding behavior of *GLP1R*$^{-/-}$ mice suggest that the mouse GLP-I receptor may not be a single critical genetic determinant of appetite and feeding behavior. Furthermore, genetic redundancy in the control of feeding behavior appears to be increasingly complex, as mice with a targeted inactivation of the NPY gene were not obese and exhibited normal food intake but increased sensitivity to leptin administration[25].

The finding that *GLP1R*$^{-/-}$ mice exhibit clear abnormalities in glucose-dependent insulin secretion suggests that GLP1 is a physiologically important incretin. The abnormal glucose tolerance tests observed in *GLP1R*$^{-/-}$ mice after both oral and intraperitoneal glucose administration emphasizes the importance of GLP1 for glycemic control, irrespective of the site of glucose entry, and strongly supports the hypothesis that GLP1 exerts important effects both through the islet beta cell and also peripherally, possibly through regulation of glucose disposal[13,14].

In keeping with the peripheral effects of GLP1, GLP1 receptors have been detected by RT-PCR in adipocytes and skeletal muscle[26], and GLP1 has been shown to enhance insulin-stimulated glucose metabolism and to increase glucose incorporation into glycogen in adipocytes and skeletal muscle, respectively[26,27]. GLP1 improves glucose tolerance in human subjects by increasing glucose disappearance and glucose effectiveness in the absence of changes in insulin or glucagon[13,14], and GLP1 infusion has been associated with a reduction in hepatic glucose production in human subjects[28]. The abnormal increase in blood glucose observed in *GLP1R*$^{-/-}$ mice after oral and intraperitoneal glucose challenge emphasizes the physiological importance of GLP1 for the control of blood glucose and supports the contention that GLP1 may prove to be a valuable therapeutic agent for patients with both non-insulin-dependent and insulin-dependent diabetes mellitus.

Methods

Targeting vector. A 16.2-kb *GLP1R* gene fragment was cloned by plaque hybridization to the rat *GLP1R* cDNA (ref. 29) from a genomic mouse ES cell 129 I Dash library. pPNT (ref. 30) was used to construct a targeting vector by replacing two exons encoding the first and third transmembrane domains and intervening peptide sequence with a PGK-neo cassette in the same transcription orientation along with 4.8 and 3.5 kb of *GLP1R* sequences 5′ and 3′ to the PGK-neo sequence, respectively. The linearized construct was electroporated into ES cell line R1 (ref. 31, 32), and ES cell lines with one disrupted *GLP1R* allele were aggregated with CD1 morulae[33] to generate germline chimeras. *GLP1R*$^{+/-}$ mice from separate litters were mated to obtain mice homozygous for the *GLP1R* mutation.

Glucose tolerance and insulin determinations. Different groups of age- and sex-matched 6- to 8-week-old mice were used for the OGTT and intraperitoneal glucose tolerance testing (IPGTT) experiments shown in Fig. 1. Mice were housed under a light/dark cycle of 12 h, and food was removed at 3 p.m. of the afternoon before each glucose challenge experiment (a minimum of 18 h fasting) after which males (n = 7), and females (n = 10, experimental and control) were given 1.5 mg glucose per gram body weight orally through a gavage tube or intraperitoneally. Blood was withdrawn from a tail vein at 0, 10, 20, 30, 60, 90 and 120 min (seven samples per mouse), and blood glucose levels were measured with a Glucometer Elite monitor (Bayer, Inc., Etobicoke, Ontario). Values are means ± s.e.m. Insulin levels were determined for venous blood samples from separate groups of male mice after an 18-h fast (n = 6) and oral glucose load. Blood for insulin radioimmunoassay (1 ml) was withdrawn (by cardiac puncture) at 30 min (n = 7 per group) or 60 min (n = 11 per group) after oral glucose. Plasma insulin levels were determined in duplicate using an insulin radioimmunoassay kit (Linco) with rat insulin as a standard. Values are means ± s.e.m. *P < 0.05.

Glucagon levels were measured as previously described[34]. Different groups of mice were used for all experiments depicted in Fig. 1.

Intracerebroventricular (ICV) injections. Fasted (18 h) groups of mice were lightly anesthetized by methoxyflurane (Metofane, Janssen Pharmaceutica, Mississauga, Ontario), after which compounds were administered by ICV injection using a 2.5 mm × 30 gauge needle attached to a Hamilton syringe. Each mouse was given only a single ICV injection (either saline or peptide but not both). The injection site was 0.5–1.0 mm posterior and 1.0 mm lateral to bregma. Dye injections performed in 11 mice verified that this needle placement consistently and accurately targeted the lateral ventricle. After injection, mice were placed in cages containing preweighed rodent diet. Mice recovered from anesthesia and were actively eating by 20 min after ICV injection. After 2 h, the remaining diet was removed and reweighed.

***In situ* autoradiography.** Mice were killed and their brains immediately removed and frozen at –80 °C. Serial coronal sections (20 μm) were cut on a Jung Frigocut 2800E cryostat (Leica Instruments, Willowdale, Ontario) maintained at –25 °C and thaw-mounted on slides coated with poly-L-lysine (Peninsula Laboratories, Belmont, California). GLP1 autoradiography was performed essentially as described[6]. Sections were preincubated at 25 °C for 20 min with 25 mM HEPES buffer (pH 7.4) containing 2 mM $MgCl_2$, 1% BSA, 1 mM dithiothreitol, 0.05% Tween 20 and 0.1% bacitracin, then incubated for 1.5 h at 25 °C. Slides were rinsed 3 times for 1 min each in assay buffer at 4 °C, dipped in ice-cold distilled water, then dried under an air stream at 4 °C. CCK-8 autoradiography (Fig. 3, *e* and *f*) was performed as described previously[35]. Slides from both sets of incubations were exposed against Hyperfilm-^{3}H (Amersham, Oakville, Ontario), for either 1 week (CCK-8) or 2 weeks (GLP1), and developed in Kodak D-19 developer. Regional labeling with ^{125}I-labeled CCK-8 was completely abolished by coincubation with 200 nM unlabeled CCK (data not shown).

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1. Creutzfeldt, W. & Ebert, R. New developments in the incretin concept today. *Diabetologia* **28**, 565–573 (1985).
2. Mojsov, S., Weir, G.C. & Habener, J.F. 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 (1987).
3. Kreymann, B., Ghatei, M.A., Williams, G. & Bloom, S.R. Glucagon-like peptide-1 7–36: A physiological incretin in man. *Lancet* **ii**, 1300–1304 (1987).
4. Holst, J.J., Orskov, C., Nielsen, O.V. & Schwartz, T.W. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett.* **211**, 169–174 (1987).
5. Fehmann, H.-C., Goke, R. & Goke, B. Cell and molecular biology of the incretin hormones glucagon-like peptide 1 and glucose-dependent releasing polypeptide. *Endocrine Rev.* **16**, 390–410 (1995).
6. Turton, M.D. *et al.* A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379**, 69–72 (1996).
7. Nauck, M.A. *et al.* Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J. Clin. Invest.* **91**, 301–307 (1993).
8. Nauck, M.A. *et al.* Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* **36**, 741–744 (1993).
9. Gutniak, M., Orskov, C., Holst, J.J., Ahrén, B. & Efendic, S. 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 (1992).
10. Dupré, J. *et al.* Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. *Diabetes* **44**, 626–630 (1995).
11. Thorens, B. Expression cloning of the pancreatic B cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc. Natl. Acad. Sci. USA* **89**, 8641–8645 (1992).
12. Willms, B. *et al.* 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 (1996).
13. D'Alessio, D.A., Kahn, S.E., Leusner, C.R. & Ensinck, J.W. Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J. Clin. Invest.* **93**, 2263–2266 (1994).
14. D'Alessio, D.A., Prigeon, R.L. & Ensinck, J.W. Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes — A physiological role of glucagon-like peptide I. *Diabetes* **44**, 1433–1437 (1995).
15. Toft-Nielsen, M., Madsbad, S. & Holst, J.J. 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 (1996).
16. Thorens, B. *et al.* Cloning and functional expression of the human islet GLP-1 receptor: Demonstration that exendin-4 is an agonist and exendin-(9–39) an antagonist of the receptor. *Diabetes* **42**, 1678–1682 (1993).
17. Gremlich, S. *et al.* Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. *Diabetes* **44**,1202–1208 (1995).
18. Drucker, D.J., Philippe, J., Mojsov, S., Chick, W.L. & Habener, J.F. 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 (1987).
19. D'Alessio, D.A. *et al.* 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 (1996).
20. Ritzel, R., Orskov, C., Holst, J.J. & Nauck, M.A. Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7–36 amide] after subcutaneous injection in healthy volunteers: Dose-response-relationships. *Diabetologia* **38**, 720–725 (1995).
21. Hoosein, N.M. & Gurd, R.S. Human glucagon-like peptides 1 and 2 activate rat brain adenylate cyclase. *FEBS Lett.* **178**, 83–86 (1984).
22. Kanse, S.M., Kreymann, B., Ghatei, M.A. & Bloom, S.R. Identification and characterization of glucagon-like peptide-1 7–36 amide-binding sites in the rat brain and lung. *FEBS Lett.* **241**, 209–212 (1988).
23. Campos, R.V., Lee, Y.C. & Drucker, D.J. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* **134**, 2156–2164 (1994).
24. Wei, Y. & Mojsov, S. 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 (1995).
25. Erickson, J.C., Clegg, K.E. & Palmiter, R.D. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* **381**, 415–418 (1996).
26. Egan, J.M., Montrose-Rafizadeh, C., Wang, Y., Bernier, M. & Roth, J. Glucagon-like peptide-1(7–36) amide (GLP-1) enhances insulin-stimulated glucose metabolism in 3T3-L1 adipocytes: One of several potential extrapancreatic sites of GLP-1 action. *Endocrinology* **135**, 2070–2075 (1994).
27. Villanueva-Peñacarrillo, M.L., Alcántara, A.I., Clemente, F., Delgado, E. & Valverde, I. Potent glycogenic effect of GLP-1(7–36)amide in rat skeletal muscle. *Diabetologia* **37**, 1163–1166 (1994).
28. Hvidberg, A., Nielsen, M.T., Hilsted, J., Orskov, C. & Holst, J.J. Effect of glucagon-like peptide-1 (proglucagon 78–107 amide) on hepatic glucose production in healthy man. *Metabolism* **43**, 104–108 (1994).
29. Wheeler, M.B. *et al.* Functional expression of the rat glucagon-like peptide-I receptor: Evidence for coupling to both adenylyl cyclase and phospholipase-C. *Endocrinology* **133**, 57–62 (1993).
30. Tybulewicz, V.L.J., Crawfor, C.E., Jackson, P.K., Bronson, R.T. & Mulligan, R.C. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-*abl* proto-oncogene. *Cell* **65**, 1153–1163 (1991).
31. Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W. & Roder, J.C. Derivation of completely cell culture-derived mice from early passage embryonic stem cells. *Proc. Natl. Acad. Sci. USA* **90**, 8424–8428 (1993).
32. Nagy, A. & Rossant, J. Production of completely ES cell-derived fetuses. in *Gene Targeting: A Practical Approach.* (ed. Joyner, A.L.) 147–178 (Oxford Univ. Press, Oxford, 1993).
33. Wurst, W. & Joyner, A.L. Production of targeted embryonic stem cell clones. in *Gene Targeting: A Practical Approach.* (ed. Joyner, A.L.) 33–61 (Oxford Univ. Press, Oxford, 1993).
34. Brubaker, P.L., Lee, Y.C. & Drucker, D.J. Alterations in proglucagon processing and inhibition of proglucagon gene expression in glucagon-SV40 T antigen transgenic mice. *J. Biol. Chem.* **267**, 20728–20733 (1992).
35. Akesson, T.R., Mantyh, P.W., Mantyh, C.R., Matt, D.W. & Micevych, P.E. Estrous cyclicity of ^{125}I- cholecystokinin octapeptide binding in the ventromedial hypothalamic nucleus. Evidence for downmodulation by estrogen. *Neuroendocrinology* **45**, 257–262 (1987).
36. Heinrichs, S.C. *et al.* Endogenous corticotropin releasing factor modulates feeding induced by neuropeptide Y or a tail-pinch stressor. *Peptides* **13**, 879–884 (1992).