

# Evidence From *Glut2*-Null Mice That Glucose Is a Critical Physiological Regulator of Feeding

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**A role for glucose in the control of feeding has been proposed, but its precise physiological importance is unknown. Here, we evaluated feeding behavior in *glut2*-null mice, which express a transgenic glucose transporter in their  $\beta$ -cells to rescue insulin secretion (*ripglut1;glut2*<sup>-/-</sup> mice). We showed that in the absence of GLUT2, daily food intake was increased and feeding initiation and termination following a fasting period were abnormal. This was accompanied by suppressed regulation of hypothalamic orexigenic and anorexigenic neuropeptides expression during the fast-to-refed transition. In these conditions, however, there was normal regulation of the circulating levels of insulin, leptin, or glucose but a loss of regulation of plasma ghrelin concentrations. To evaluate whether the abnormal feeding behavior was due to suppressed glucose sensing, we evaluated feeding in response to intraperitoneal or intracerebroventricular glucose or 2-deoxy-D-glucose injections. We showed that in GLUT2-null mice, feeding was no longer inhibited by glucose or activated by 2-deoxy-D-glucose injections and the regulation of hypothalamic neuropeptide expression by intracerebroventricular glucose administration was lost. Together, these data demonstrate that absence of GLUT2 suppressed the function of central glucose sensors, which control feeding probably by regulating the hypothalamic melanocortin pathway. Furthermore, inactivation of these glucose sensors causes overeating. *Diabetes* 55:988–995, 2006**

**T**he control of body weight depends on the balance between food intake and energy expenditure. The current epidemic of obesity, which represents a major risk factor for the development of type 2 diabetes and cardiovascular diseases, is

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2-DG, 2-deoxyglucose; AgRP, agouti-related peptide; CART, cocaine- and amphetamine-related transcript; CRH, corticotropin-releasing hormone; LH, lateral hypothalamus; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular hypothalamic nucleus; TRH, thyrotropin-releasing hormone.

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caused by a dysregulation of this homeostatic process (1). Both internal and environmental signals cooperate to trigger or terminate food intake and to stimulate anabolic or catabolic pathways. The internal signals are hormones derived from the gut, such as ghrelin, cholecystokinin, glucagon-like peptide-1, or peptide YY<sub>3–36</sub>, from adipocytes (leptin), and pancreatic  $\beta$  cells (insulin) but also nutrients such as glucose and lipids. These signals are integrated by the central nervous system to control feeding and energy expenditure (2,3). In this integrative function, the melanocortin pathway of the hypothalamus plays a critical role, as it is directly regulated by hormones and nutrients (4–9). This pathway consists of neurons of the arcuate nucleus, which synthesize either orexigenic (neuropeptide Y [NPY] and agouti-related peptide [AgRP]) or anorexigenic (proopiomelanocortin [POMC] and cocaine- and amphetamine-related transcript [CART]) neuropeptides. These then regulate second-order neurons, in particular those located in the paraventricular hypothalamic nucleus (PVN) or the lateral hypothalamus (LH). Whereas the PVN neurons express anorexigenic peptides such as thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone (CRH), LH neurons express the orexigenic peptides orexins and melanin concentrating hormone (10). Thus, the balance between the output from the PVN and LH plays a critical role in regulating feeding and energy homeostasis.

A role for glucose in the regulation of food intake was proposed based on various studies (11). For instance, it was demonstrated that initiation of feeding was preceded by a small drop in glycemia, which, if prevented, suppressed food intake (12,13). In many other studies, the administration of 2-deoxyglucose (2-DG), an antimetabolite that reduces glucose metabolism and ATP production, was used to induce a state of cellular glucoprivation (14) to trigger physiological responses similar to those induced by hypoglycemia. It was shown that both central (15,16) and peripheral (17) administration of 2-DG induced food intake.

These experiments, however, did not provide information on the role of glucose in regulating feeding behavior in physiological conditions, since 2-DG is a nonnatural substrate and only acute responses were recorded. There is therefore a need for a better understanding of the importance of glucose in the regulation of feeding and how glucose may interact with the other hormonal signals to control, in particular, the melanocortin pathway.

The best-described system for glucose sensing is that of the pancreatic islet  $\beta$ -cells, where stimulated insulin secretion requires glucose uptake by GLUT2 and its subsequent metabolism (18,19). Inactivation of the *glut2* gene led to severe impairment of  $\beta$ -cell glucose sensing and early

death due to impaired insulin secretion (20). Transgenic expression of GLUT1 in the pancreatic  $\beta$ -cells of *glut2*<sup>-/-</sup> mice restored normal dose-dependent glucose-stimulated insulin secretion and rescued the mice from early lethality (21). Using these rescued mice (*ripplut1;glut2*<sup>-/-</sup>), we previously demonstrated that glucose sensors located in the portal vein and which control glucose utilization by muscle and fat were also GLUT2 dependent (22) and that GLUT2- and astrocyte-dependent sensors controlled some aspects of the counterregulatory response to hypoglycemia (10).

We now show that *ripplut1;glut2*<sup>-/-</sup> mice have increased daily food intake and present defects in both feeding initiation and termination following a fast. This was associated with a loss of regulated expression of hypothalamic orexigenic and anorexigenic neuropeptides. Furthermore, we show that absence of GLUT2 causes a failure of the mice to respond to intraperitoneal or intracerebroventricular injections of glucose or 2-DG by an inhibition or stimulation of feeding, respectively. The suppressed regulation of hypothalamic neuropeptide expression was also directly linked to a defect in central glucose sensing. Together, our data demonstrate that central glucose sensors are critical physiological regulators of feeding.

## RESEARCH DESIGN AND METHODS

We used 12- to 16-week-old *ripplut1;glut2*<sup>-/-</sup> mice (21) backcrossed for seven generations with C57BL/6 mice and C57BL/6 mice as controls. All experimental protocols were accepted by the Service Vétérinaire Cantonal (Lausanne, Switzerland).

**Surgical procedures.** For intracerebroventricular injections of glucose or 2-DG, anesthetized mice were stereotactically implanted in the right lateral ventricle with stainless steel cannulas secured to the skull with dental acrylic. Mice were single-housed following surgery and were allowed to recover for 2–5 days before 2-DG or glucose administration. Correct implantation of the cannulas was checked at the end of the experiments by histological examination of frozen brain sections. Only data from mice with correct implantations were considered.

**Feeding test.** Mice were individually housed and fed with standard powder diet for at least 3 days before the experiments. Feeding tests were conducted between 08:00 A.M. and 1:00 P.M. For glucose administration, mice were fasted overnight and received an intraperitoneal injection of saline (0.9%, 40  $\mu$ l/10 g body wt) or of glucose (10% solution, 400 mg/kg) or a 5  $\mu$ l i.c.v. injection of glucose (20 mg/ml solution) or of saline at a rate of 2.5  $\mu$ l/min. Food was given immediately after glucose administration, and food consumption was measured by weighing the food remaining in the cages 2 h after the intraperitoneal injection and 4 h after the intracerebroventricular administration. For 2-DG intraperitoneal administration, mice were fed ad libitum and injected between 8:00 and 9:00 A.M. with 2-DG (150 mg/kg) or saline (0.3%, 10  $\mu$ l/g body wt). For intracerebroventricular injection of 2-DG, 10  $\mu$ l of a 50-mg 2-DG/ml or a saline (0.9%) solution were injected at the rate of 2.5  $\mu$ l/min. Feeding was determined as above. Rat ghrelin (10  $\mu$ g; Bachem, Bubendorf, Switzerland) was injected intraperitoneally in fed mice, and food intake was measured as above.

Daily food intake and cumulative food consumption during fast-to-refed transition were determined using metabolic cages (Feed-Scale; Columbus Instruments). Mice were habituated to the cages for 2 days; they were then fasted for 24 h, and refeeding started at 8:00 A.M. and was recorded for a period of 48 h.

**Hypothalamus RNA preparation and blood sampling.** Control and *ripplut1;glut2*<sup>-/-</sup> mice were fasted for 24 h. Half of the mice were refed for 6 h. Cannula-implanted mice were fasted overnight and had no access to food during the 2 h following the intracerebroventricular administration of 5  $\mu$ l saline (0.9%) or glucose (20 mg/ml) at the rate of 2.5  $\mu$ l/min. Blood glucose concentrations were determined using a Glucometer (Roche Diagnostics, Rotkreuz, Switzerland). Mice were then anesthetized using isoflurane and killed by decapitation. Blood and brain were immediately sampled. Hypothalamus dissection was performed in cold and oxygenated artificial cerebrospinal fluid and were frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until mRNA extraction.

**Analytical procedures and determination.** Blood was collected in the presence of 1  $\mu$ g/ml aprotinin and 1 mmol/l EDTA, immediately centrifuged, and the plasma stored at  $-70^{\circ}\text{C}$  until assayed. Plasma leptin and ghrelin concentrations were assessed by radioimmunoassay and plasma insulin concentrations by enzyme-linked immunosorbent assay (Linco, St. Louis, MO). **Determination of mRNA neuropeptides expression.** Quantification of hypothalamic neuropeptide mRNAs was by real-time RT-PCR analysis using a LightCycler Instrument (Roche Applied Science). Total hypothalamic mRNA was extracted using the peqGOLD TriFast method (Peqlab, Erlangen, Germany), and single-strand cDNA was synthesized from 2.5  $\mu$ g total RNA with random hexamer (Applied Biosystems) and Superscript II reverse transcriptase (Invitrogen).

RT-PCR were prepared with a LightCycler kit (Eurogentec, Belgium) in a final volume of 20  $\mu$ l containing 250 ng of reverse-transcribed total RNA and 0.5  $\mu$ l of SYBR Green and in presence of the following forward (F) and reverse (R) primers: F 5'-ATGGGGCTGTGTGGACTGAC-3' and R 5'-AAGTTTCATTCCCACACCAC-3' for NPY, F 5'-CAGAGTTC(C,T)CAGGTCTAAGTC-3' and R 5'-TTGAAGAAGCGGCAGTAGCAC-3' for AgRP, F 5'-TACGGCCAAAGTCCCA TGTG-3' and R 5'-GGGAACGCAAACCTTTATTGTTG-3' for CART, and F 5'-TGGGTATTGGACCACTGCAC-3' and R 5'-TGGTTACCGTTGGCCTGAAG G-3' for orexin. Glyceraldehyde-3-phosphate dehydrogenase mRNA was used as invariant control and was amplified using the following primers: F 5'-GTCGGTGTGAACGGATTTGG-3' and R 5'-GACTCCACGACATACTCAGC-3'. The amplification of POMC and TRH mRNA required the use of tagged-extended primers in order to eliminate primer-dimer accumulation. The primer was F 5'-GCGTACTAGCGTACCACGTCGACT-GAGCGTGGCAGGAGAG-3' and R 5'-GCGTACTAGCGTACCACGTCGACT-TTGAAGAGCGTCCACCAGG G-3' for POMC and F 5'-GCGTACTAGCGTACCACGTCGACT-TCACAAAA CGCCAGCATCC-3' and R 5'-GCGTACTAGCGTACCACGTCGACT-TGACA TCAGACTCCATCCAG-3' for TRH.  $\beta$ -Actin mRNA was used as invariant control (F 5'-GCGTACTAGCGTACCACGTCGACT-CACGAAACTCACTTCA ACTCC-3' and R 5'-GCGTACTAGCGTACCACGTCGACT TCATACTCCTGC TGCTTGCTGATC-3'). The real-time PCR were prepared as above in presence of the reverse and forward primer and of the homo-tag primer (5'-GCGTAC TAGCGTACCACGTCGACT-3'). The relative amount of all mRNAs was calculated using the LightCycler analysis software Version 3.5.

## RESULTS

**Abnormal feeding behavior in *ripplut1;glut2*<sup>-/-</sup> mice.** *Ripplut1;glut2*<sup>-/-</sup> mice have an inactivated *glut2* gene and express a transgenic *glut1* in their  $\beta$ -cells. This restores normal glucose-induced insulin secretion and allows the mice to grow, live, and reproduce normally (21). Their body weight is the same as control mice ( $29.6 \pm 2.9$  vs.  $28.1 \pm 2.5$  g for control and mutant mice used in this study, respectively), and they have the same percentage of lean and fat mass ( $87.4 \pm 1.4\%$  vs.  $86.7 \pm 1.2\%$  lean mass in mutant and control mice, respectively, and  $12.5 \pm 1.4\%$  vs.  $13.3 \pm 1.2\%$  fat mass in mutant and control mice, respectively).

To evaluate feeding behavior of the *ripplut1;glut2*<sup>-/-</sup> mice, we first measured their daily food intake as well as that of control mice. Mice were housed individually and habituated to their new environment for at least 3 days before measuring food intake over 24 h. Figure 1A shows that the mutant mice ate 27% more than the controls. Next, we measured refeeding after a fast by control or *ripplut1;glut2*<sup>-/-</sup> mice placed in metabolic cages and previously habituated to this environment for 2 days. Figure 1B shows that refeeding for 6 h after the 24-h fast was reduced in mutant compared with control mice. However, cumulative food intake by the same mice measured for 48 h after initiation of refeeding showed that the mutant mice ate more than their controls (Figure 1B). Together, these data show increased feeding in ad libitum fed mutant mice and a defect in both feeding initiation and feeding termination following a fast.

**Regulation of hypothalamic neuropeptide gene expression.** The regulation of feeding behavior is very complex and incompletely understood. However, numer-

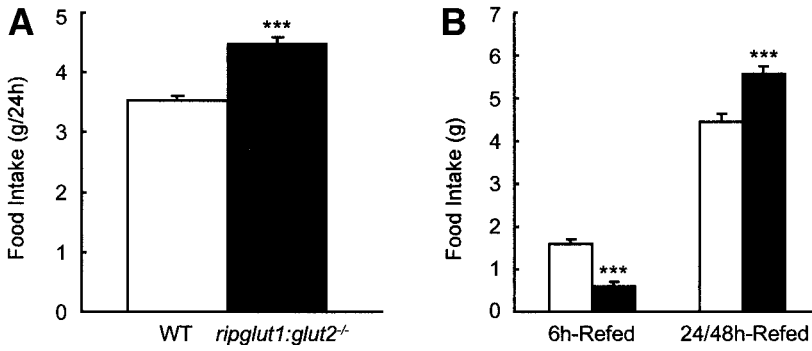


FIG. 1. Increased food intake in *ripglut1:glut2<sup>-/-</sup>* mice and loss of feeding regulation during fast-to-refed transition. **A:** The daily food intake was determined in control and in mutant mice. Mutant mice ate 27% more than control mice. **B:** Mice were fasted for 24 h and refed for 48 h. The cumulative food intake was determined for a 6-h period of refeeding and between 24 and 48 h after the refeeding. Results are mean  $\pm$  SE ( $n = 6-12$ ). Statistical analysis by Student's *t* test. \*\*\* $P < 0.001$ . □, wild-type (WT) mice; ■, *ripglut1:glut2<sup>-/-</sup>* mice.

ous studies have proposed an important role of the hypothalamic melanocortin pathway in integrating internal signals of body energy levels with the control of feeding and energy expenditure. To evaluate the activity of these circuits in the mutant mice, we measured by quantitative RT-PCR the level of expression of hypothalamic neuropeptide mRNAs at the end of a 24 h fast and after 6-h of refeeding. Figure 2A and B shows that expression of the mRNAs for the orexigenic peptides NPY and AgRP was significantly decreased during the fast to refed transition in control mice. In contrast, there was no regulation of expression of these mRNAs in *ripglut1:glut2<sup>-/-</sup>* mice, which remained at the level found in fed control animals. The mRNAs for the anorexigenic peptides POMC and CART were increased upon refeeding in control mice (Fig. 2C); again, no regulated expression of these neuropeptide

mRNA was observed in *glut2-null* mice (Fig. 2D) and their level remained at a comparatively low level.

These arcuate nucleus neuropeptides modulate the activity of neurons located in the PVN and the LH, expressing, respectively, TRH and CRH, and orexins and melanin concentrating hormone. The expression of melanin concentrating hormone mRNA was not altered during the fast-to-fed transition in control or *ripglut1:glut2<sup>-/-</sup>* mice (data not shown). Similarly, no change in orexin mRNA expression occurred during the fast to refed transition (Fig. 2E), but its expression level was significantly higher in absence of GLUT2. TRH mRNA expression was markedly induced after refeeding in control mice (Fig. 2F). In contrast to control mice, mRNA expression of TRH in *ripglut1:glut2<sup>-/-</sup>* mice was not regulated by the fast-to-refed transition and remained at a comparatively elevated

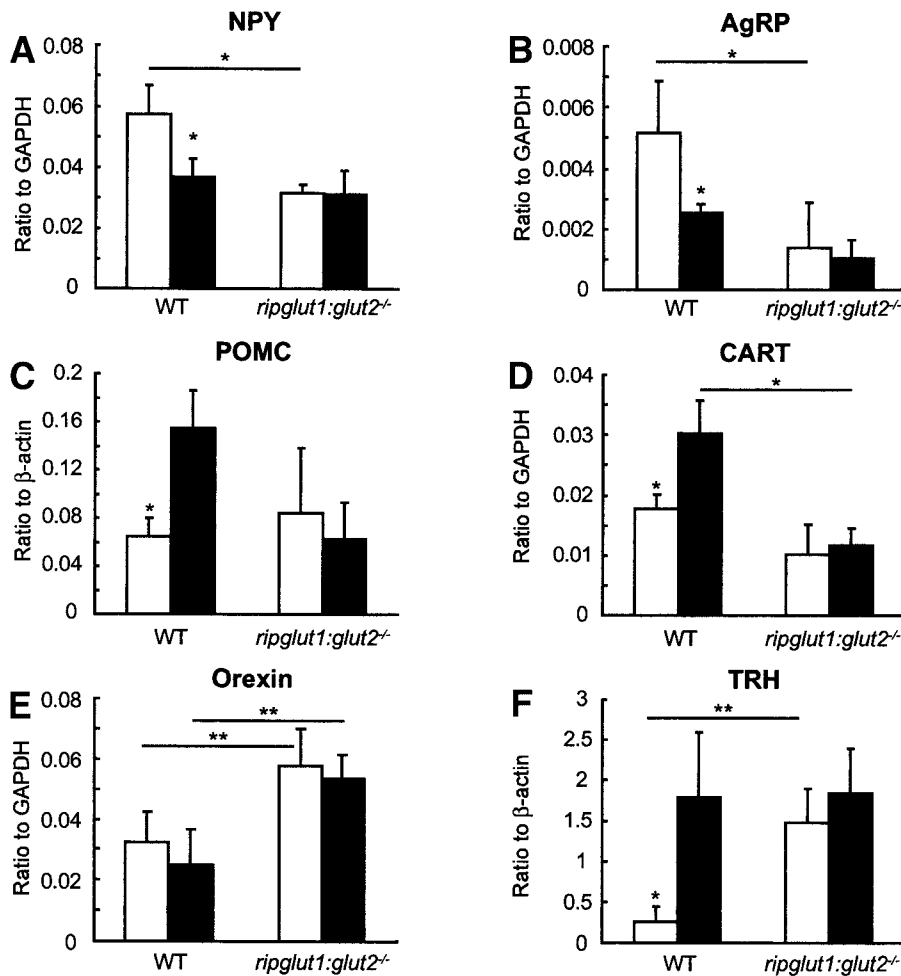
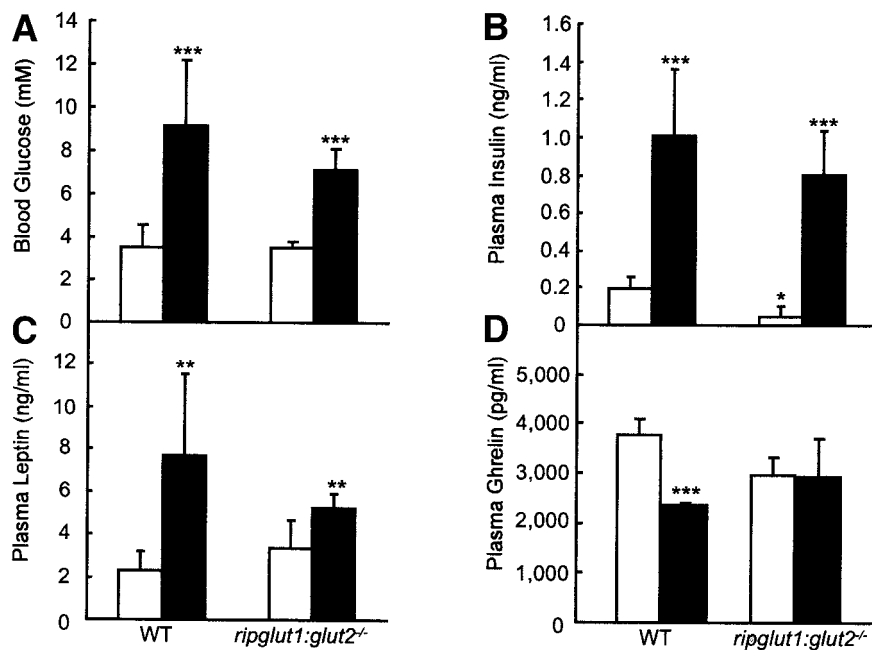


FIG. 2. Analysis of hypothalamic neuropeptide mRNA expression by quantitative RT-PCR during the fast-to-refed transition in control and *ripglut1:glut2<sup>-/-</sup>* mice. **A:** NPY. **B:** AgRP. **C:** POMC. **D:** CART. **E:** Orexin. **F:** TRH. □, 24-h fasted; ■, after 6 h of refeeding. All data are means  $\pm$  SD,  $n = 6-8$ . Statistical analysis by Student's *t* test \* $P < 0.05$ ; \*\* $P < 0.01$ . GAPDH, glyceraldehyde-3-phosphate dehydrogenase; WT, wild type.



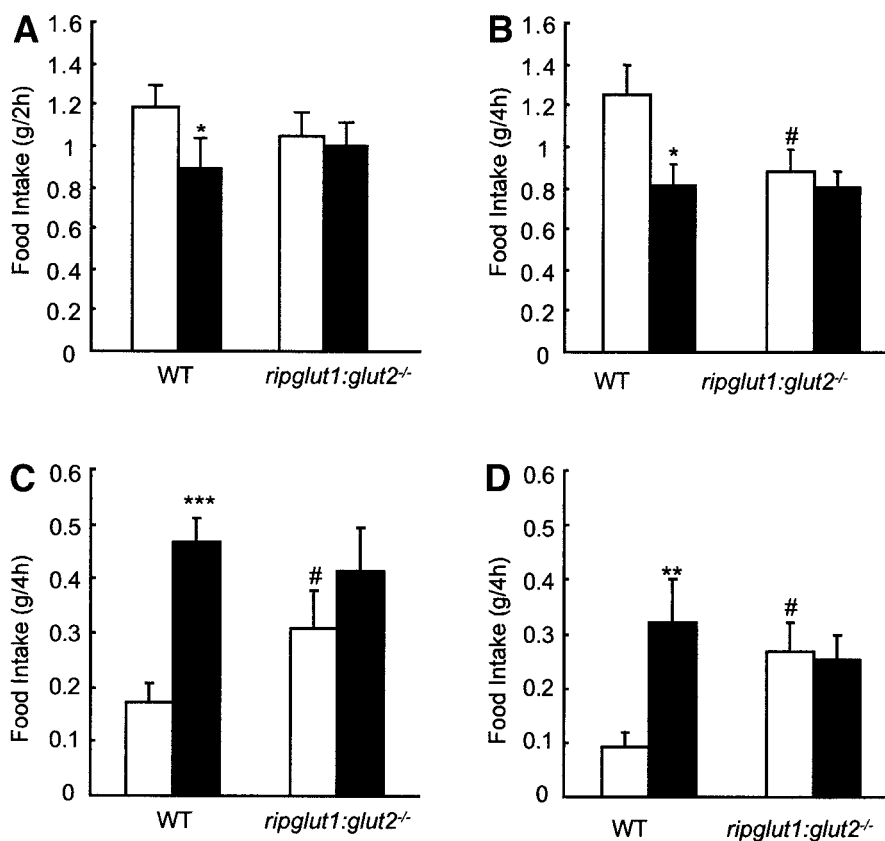


**FIG. 3.** Blood glucose and plasma hormone concentrations during the fast-to-refed transition. Blood glucose (A), plasma insulin (B), plasma leptin (C), and plasma ghrelin (D) levels during the fast-to-refed transition in wild-type (WT) and in *ripglut1; glut2<sup>-/-</sup>* mice. □, measurements after 24 h of fast; ■, after 6 h of refeeding. All results are means  $\pm$  SD,  $n = 6-8$ . Statistical analysis by Student's *t* test. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; #comparison with control mice,  $P < 0.005$ .

level. Thus, in the absence of GLUT2, the normal regulated expression of the hypothalamic neuropeptides during the fast-to-refed transition was lost.

**Blood glucose and plasma hormone concentrations.** Regulation of hypothalamic nuclei controlling food intake is under the control of hormones such as insulin, leptin and ghrelin. We thus assessed these hormone plasma levels as well as blood glucose concentrations during the fast-to-refed transition in control and mutant mice. Figure 3A shows that the blood glucose levels were similarly

regulated in *ripglut1; glut2<sup>-/-</sup>* and control mice, with similar glycemia in the fasted and fed periods. The insulin plasma levels were similarly regulated in the control and mutant mice. The fasted levels were however lower in fasted *ripglut1; glut2<sup>-/-</sup>* compared with control mice ( $0.19 \pm 0.07$  vs.  $0.04 \pm 0.06$  ng/ml [mean  $\pm$  SD] for control and mutant mice, respectively), as previously described (21) (Fig. 3B). After refeeding, the insulinemic levels were not different between the *ripglut1; glut2<sup>-/-</sup>* and control mice ( $1.01 \pm 0.35$  vs.  $0.81 \pm 0.23$  ng/ml in control and



**FIG. 4.** Loss of feeding regulation by glucose or 2-DG in *ripglut1; glut2<sup>-/-</sup>* mice. A and B: Suppression of refeeding by glucose administration. Fasted mice received an intraperitoneal or an intracerebroventricular administration of saline or glucose (400 mg/kg i.p. or 0.1 mg i.c.v.), and cumulative food intake was measured over 2 h (A) or 4 h (B). Glucose failed to suppress feeding in the mutant mice. A: □, intraperitoneal saline; ■, intraperitoneal glucose. B: □, intracerebroventricular saline; ■, intracerebroventricular glucose. C and D: Stimulation of feeding by 2-DG. Mice fed ad libitum received saline or 2-DG i.p. (150 mg/kg) (C) or i.c.v. (0.5 mg) (D), and the 4-h cumulative food intake was recorded. 2-DG did not stimulate feeding in the *ripglut1; glut2<sup>-/-</sup>* mice. C: □, intraperitoneal saline; ■, intraperitoneal 2-DG. D: □, intracerebroventricular saline; ■, intracerebroventricular 2-DG. These data show that glucoregulation of feeding is lost in mutant mice. Results are means  $\pm$  SE ( $n = 8-12$ ). Statistical analysis by Student's *t* test. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; #comparison with control mice,  $P < 0.05$ . WT, wild type.

mutant mice, respectively). In mutant mice, leptin was also significantly regulated during the fast-to-refed transition but to a smaller extent than in control mice (Fig. 3C). Strikingly, however, the ghrelin plasma levels were no longer regulated by the fast-to-refed transition and were at a level intermediate between the fasted and refed values of control mice (Fig. 3D). Thus, dysregulation of hypothalamic neuropeptide expression was not related to absence of regulation of plasma insulin and leptin levels or of blood glucose but was associated with a loss of regulation of plasma ghrelin levels. This therefore suggested that GLUT2 expression is required for normal regulation of the melanocortin pathway and that this regulation may, at least in part, be caused by regulation of ghrelin plasma levels.

**Loss of feeding response to glucose and 2-DG in GLUT2-null mice.** The above data suggest that absence of GLUT2 causes dysregulated feeding and hypothalamic neuropeptide expression because of suppression of GLUT2-dependent glucose sensing mechanisms. To directly evaluate this hypothesis, we first measured refeeding in mice fasted overnight and refed after receiving a bolus injection of glucose, either intraperitoneally (400 mg/kg) or intracerebroventricularly (0.1 mg/mice) or a corresponding saline injection. Figure 4A and B shows that intraperitoneal glucose injection reduced food intake over the first 2 h of refeeding in control mice but had no effect on refeeding of the mutant mice. Similarly, intracerebroventricular glucose injection markedly reduced refeeding in control but not in the mutant mice. Next, we evaluated whether the glucoprivic signal induced by 2-DG administration would trigger feeding in mutant as well as in control mice. The data from Fig. 4C and D show that either intraperitoneal or intracerebroventricular 2-DG administration in fed control mice markedly increased feeding over the 4-h time period. No response was however observed in the mutant mice, which ate more than the fed control mice. Thus, together, the above data indicate that inactivation of the *glut2* gene

indeed led to suppression of GLUT2-dependent sensors, some of which are centrally located, and which control inhibition of food intake in response to glucose or stimulation of feeding in response to a glucoprivic signal.

**Suppressed regulation of hypothalamic neuropeptide expression by glucose.** To directly evaluate whether glucose could regulate neuropeptide expression, we performed intracerebroventricular glucose injections in fasted mice and measured NPY and POMC mRNA expression. Figure 5 shows that 2 h following intracerebroventricular glucose injections (100  $\mu$ g/mouse), in conditions in which the peripheral glycemic levels did not increase (Fig. 5C), the level of NPY mRNA was reduced in control mice compared with mice receiving a saline solution. Simultaneously, the mRNA for the anorexigenic neuropeptide POMC was markedly increased. In contrast, in mutant mice the NPY and POMC mRNAs were not regulated by intracerebroventricular glucose. In these same experiments, ghrelin plasma levels were decreased in control but not in the mutant mice (not shown). This therefore showed that in the absence of GLUT2-dependent central glucose sensing, the regulated expression of these neuropeptides was lost, implicating glucose as a key regulator of these neuronal circuits.

**Preserved feeding response to ghrelin.** Finally, to evaluate whether absence of GLUT2 in the mutant mice could lead to abnormal development and function of the melanocortin pathway, which could explain the absence of regulated expression of the neuropeptides, we tested whether ghrelin could still induce feeding in the mutant mice. Figure 6 shows that intraperitoneal administration of ghrelin strongly and similarly stimulated feeding in fed control and mutant mice. As ghrelin stimulates feeding mostly by a direct action on the arcuate nucleus neurons, mainly the NPY but also the POMC neurons (23–26), this demonstrated that absence of GLUT2 did not lead to abnormal development of these neuronal circuits.

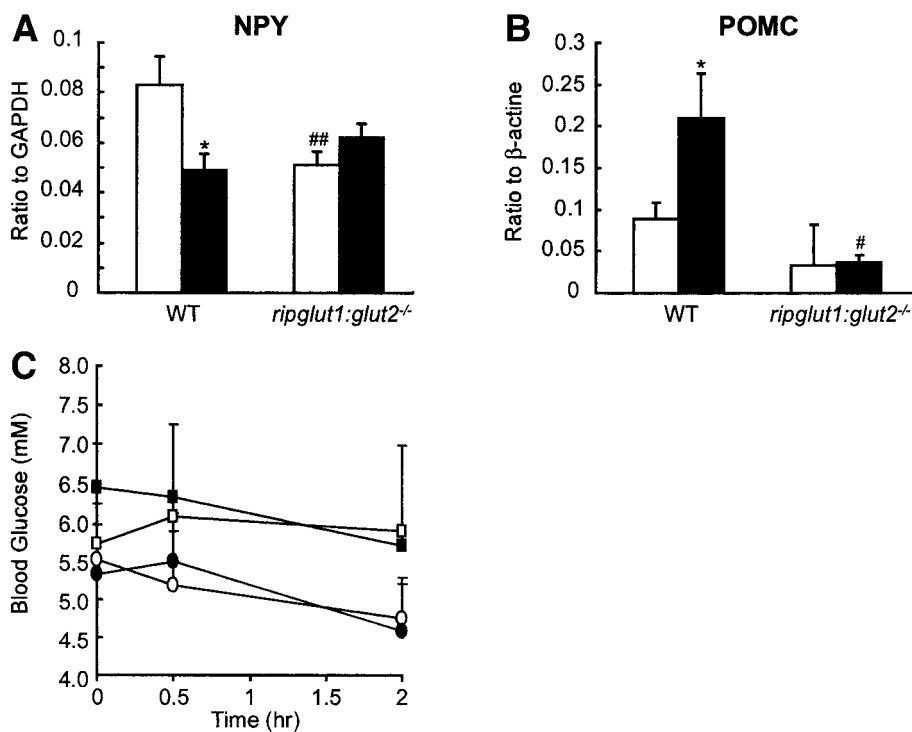


FIG. 5. Loss of regulated expression by glucose of NPY and POMC in *ripglut1;glut2<sup>-/-</sup>* mice. Overnight-fasted mice received an intracerebroventricular administration of saline or glucose (100 mg/mouse). Hypothalami were sampled 2 h later. Relative mRNA expression of NPY (A) and POMC (B) was determined by quantitative RT-PCR. All results are means  $\pm$  SE,  $n = 7$ –12. Statistical analysis by Student's  $t$  test. \* $P < 0.05$  for comparison vs. saline-injected mice. # $P < 0.05$ ; ## $P < 0.01$  for comparison with wild-type (WT) mice with same treatment. □, intracerebroventricular saline; ■, intracerebroventricular glucose. C: Peripheral blood concentration was not increased by intracerebroventricular glucose administration. □, WT intracerebroventricular saline; ■, WT intracerebroventricular glucose; ○, *ripglut1;glut2<sup>-/-</sup>* intracerebroventricular saline; ●, *ripglut1;glut2<sup>-/-</sup>* intracerebroventricular glucose.

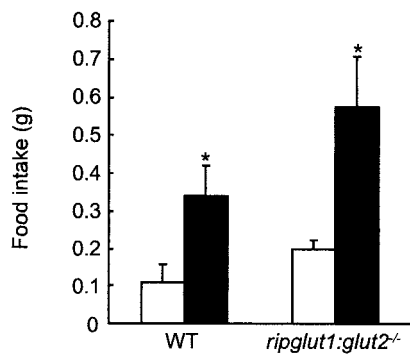


FIG. 6. Normal stimulation of feeding by ghrelin in *ripglut1;glut2<sup>-/-</sup>* mice. Fed control and *ripglut1;glut2<sup>-/-</sup>* mice received an intraperitoneal injection of saline or ghrelin solution (10  $\mu$ g/mouse). Food intake was determined 1 h after the intraperitoneal injection. Results are means  $\pm$  SE ( $n = 5-6$ ). Statistical analysis by Student's  $t$  test. \* $P < 0.05$ .  $\square$ , intraperitoneal saline;  $\blacksquare$ , intraperitoneal ghrelin. WT, wild type.

## DISCUSSION

In the present study, we provide evidence that GLUT2-dependent glucose sensors are critical physiological regulators of feeding. Inactivation of these sensors leads to higher daily food intake and abnormal feeding initiation and termination after a fast and is associated with suppressed regulated expression of the hypothalamic orexigenic and anorexigenic neuropeptides.

Daily food intake by mutant mice was 27% greater than by control mice and, after a fast, the mutant mice showed a 63% reduction in food intake over the first 6 h of refeeding, but cumulative food intake measured over a 48-h period was higher in the mutant compared with control mice. Thus, in the absence of GLUT2, there was overeating and a defect in feeding initiation and termination. We showed that this abnormal regulation of feeding in the absence of GLUT2 expression was caused by a loss of glucose sensing since intraperitoneal or intracerebroventricular glucose injections failed to reduce food intake. Similarly, a glucoprivic signal induced by intraperitoneal or intracerebroventricular 2-DG administration did not increase feeding. These observations suggested that central, but possibly also peripheral, GLUT2-dependent glucose sensors were involved in the regulation of feeding.

The higher daily food intake of *ripglut1;glut2<sup>-/-</sup>* mice could have been expected to cause obesity, which was, however, not the case. Preliminary experiments indicated that preservation of a normal body weight could not be explained by an increase in energy expenditure or by an increase in locomotor activity. The most likely explanation for the lack of weight gain is the absence of GLUT2 from the proximal convoluted tubules that prevents renal glucose reabsorption and thus leads to an elimination of glucose in the urine. The mutant mice have daily urine volumes of  $\sim 6$  ml and excrete  $\sim 3.2$  g/dl glucose per day, which corresponds to  $\sim 770$  calories/day. This accounts for  $\sim 82\%$  of the 940-calorie excess in food intake. GLUT2 is, however, also expressed in the basolateral membrane of the enterocytes. Previous studies have shown that glucose was absorbed with a normal kinetics in the mutant mice, as assessed in glucose tolerance tests and using an in situ perfused intestine system (27). Nevertheless, we cannot exclude some defects in intestinal nutrient absorption.

Hypothalamic neuronal circuits play an important integrative role in the control of feeding and are regulated by internal hormonal and nutrient signals. These signals act,

at least in part, by regulating the expression of hypothalamic orexigenic and anorexigenic neuropeptides. Regulation of these hypothalamic circuits is observed during the fast-to-refed transition and is accompanied by a decreased expression of the orexigenic and an increased expression of the anorexigenic neuropeptides. It was thus striking to observe that during the fast-to-refed transition, expression of these neuropeptides was no longer regulated in the *ripglut1;glut2<sup>-/-</sup>* mice. This could not be explained by abnormal regulation of blood glucose or of plasma levels of insulin or leptin but was associated with an absence of regulated ghrelin plasma levels. Absence of central glucose sensing was clearly the cause of the absence of regulation of NPY or POMC mRNAs as shown after intracerebroventricular glucose injection. However, after intracerebroventricular glucose injection, plasma ghrelin levels in the mutant mice were no longer regulated in the mutant mice. Therefore, the failure of intracerebroventricular glucose or of refeeding to regulate hypothalamic neuropeptide expression may be secondary to a defect in ghrelin regulation in addition to a direct regulation of the melanocortin pathway by central glucose sensing cells. Further experiments will be required to evaluate the contributions of each of these regulatory mechanisms.

An interesting observation was also that in the mutant mice the level of expression of the arcuate nucleus orexigenic and anorexigenic peptides was similar to or lower than the lowest level present in the hypothalamus of control mice. This suggests that the role of the GLUT2-dependent sensors is to stimulate NPY and *AgRP* expression in the fasted state and POMC and CART in the fed state. Furthermore, in the mutant mice, the level of expression of the second-order neuropeptides TRH and orexin was at or above the highest level of expression found in the control mice. This suggests that in physiological situations, the arcuate neurons predominantly suppress the expression of the PVN and LH neuropeptides.

The location of the GLUT2-dependent sensors that we have functionally identified is still unclear. Previous studies have shown that glucose-excited (or glucose-responsive) and glucose-inhibited (or glucose-sensitive) neurons are present in the hypothalamus, in particular in the arcuate, the ventromedial and lateral hypothalamic nuclei (28-30), and in the brainstem, in particular in the nucleus of the tractus solitarius (31,32). GLUT2 has been reported to be present in several of these structures by RT-PCR analysis (33-35). Immunohistochemical detection studies at the light and electron microscopic levels (36,37) found GLUT2 to be present in different hypothalamic and brainstem nuclei and in many cerebral structures but in dispersed cells rather than associated with specific nuclei or groups of cells. At the cellular level, GLUT2 was found in neurons, astrocytes, endothelial cells, and tanycytes of the third ventricles (38-40). Thus, regulation of orexigenic and anorexigenic peptide expression by GLUT2-dependent sensors may be controlled indirectly, possibly even by sensors located in another brain region. In support of this possibility, it was shown that food intake could be stimulated by direct injections of 5-thio-D-glucose into specific brainstem nuclei (41). Furthermore, destruction by immunotoxins of brainstem neurons projecting to the hypothalamus, in particular to the PVN and the arcuate nucleus, suppressed the effect of intraperitoneal 2-DG administration on food intake (42) and on the regulation of NPY and *AgRP* expression in the arcuate nucleus (43). Thus, the hindbrain may be a critical site of glucodetection in



physiological conditions to control food intake. It cannot be excluded however that peripheral injections of 2-DG may also activate the hepatoportal sensor (17), which requires the presence of GLUT2 for its normal function (22) and which participates in the overall regulation of feeding through its vagal afferent connections to the nucleus of the tractus solitarius and the hypothalamus (44,45).

Our data indicate that the GLUT2-dependent sensors are responsive to both increases in glucose and to glucoprivation. Whereas the  $\beta$ -cell model of glucose sensing would apply to the sensors controlled by high glucose, it is not clear how glucoprivation could stimulate feeding in a GLUT2-dependent manner. We previously reported that in *ripplut1;glut2<sup>-/-</sup>* mice (in a mixed genetic background), the glucagon response to both hypoglycemic and hyperglycemic clamps was lost (46). We proposed that these results could be explained by the presence of two sets of glucose sensors, both GLUT2-dependent, one which stimulates glucagon production and which is progressively inhibited when glucose concentrations are increased and the other that generates an inhibitory signal for glucagon secretion, which increases when glucose concentrations rise. Suppression of GLUT2 expression would thus lead to a permanent activation of the stimulatory sensor and suppressed activation of the inhibitory sensor (47). For the control of food intake, a similar involvement of two distinct GLUT2-dependent sensors could be compatible with our observations. This hypothesis will nevertheless require further investigation, initiated by an unambiguous identification of the GLUT2-expressing cells in the central nervous system.

In conclusion, our data provide evidence that glucose sensing is critically involved in the physiological control of feeding. The sensors involved are, at least in part, located centrally and depend on the expression of the glucose transporter GLUT2. These sensors control the expression of hypothalamic orexigenic and anorexigenic peptides. Dysregulated expression of these neuropeptides during the fast-to-refed transition is the likely cause of the abnormal feeding behavior observed in the *glut2*-null mice. Defects in glucose sensing causes overeating, and we hypothesize that they may underlie the development of obesity and may represent a link between obesity and type 2 diabetes, a disease characterized by impaired glucose sensing of the pancreatic  $\beta$ -cells.

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