Short-Term Food Restriction and Refeeding Alter Expression of Genes Likely Involved in Brain Glucosensing

JUN ZHOU,*[,1] DAVID S. ROANE,† XIAOCHUN XI,* IWONA BOGACKA,* BING LI,* DONNA H. RYAN,* AND ROY J. MARTIN*

Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana 70808 and †Department of Basic Pharmaceutical Sciences, University of Louisiana at Monroe, Monroe, Louisiana 71209–0470

Several genes involved in glucosensing of the endocrine pancreas have been proposed to serve a similar function in the brain. These genes include the glucose transporter-2 (Glut-2) and glucokinase (GK). In addition, the glucagon-like peptide 1 receptor, which serves as a downstream signal modulator in pancreatic glucosensing and centrally alters feeding, is also of interest. We used quantitative real-time RT-PCR to measure changes in hypothalamic and brainstem Glut-2, GK, and Glp-1R expression of these genes induced by food restriction and refeeding. Sprague-Dawley rats were 50% food restricted for 1 day; one-half of the food-restricted rats were refed with chow for 1 hr before sacrifice. In both hypothalamus and brainstem, gene expression of Glut-2, GK, and Glp-1R was significantly lower in refed rats compared with food-restricted rats. The measures of gene expression in two feeding control groups (*ad libitum* and voluntarily overfed animals) were intermediate between the food-restricted and refed groups, but were not significantly different from each other. The results indicate that putative glucosensing (GK, Glut-2, and Glp-1R) gene expression in the hypothalamus and brainstem is reduced in response to food intake, depending on prior nutritional status. Exp Biol Med 228:943–950, 2003

Key words: glucose transport 2; glucokinase; glucagon-like peptide-1 receptor; feeding

Hypothalamic and brainstem regions have long been implicated as glucose-sensing brain areas important to the control of energy balance (1–6). Electrophysiological studies have identified glucose-responsive and glucose-sensitive neurons in both areas (7–10). The application of glucose, or glucoprivic agents, in these brain regions alters multiple facets of energy balance, including peripheral glucose homeostasis and feeding (11–13). The precise mechanism by which the brain senses and responds to alterations in glucose availability is not well understood. Multiple studies suggest that glucose-sensing mechanisms in pancreatic β cells provide a model for central glucose-sensing processes (14–18). Several genes implicated in the regulation of pancreatic β cell glucosensing and insulin secretion are expressed in the hypothalamus and brainstem. These genes include the glucose transporter-2 (Glut-2), glucokinase (GK), and glucagon-like peptide-1 receptor (Glp-1R) (19–27). Glut-2 is a high-capacity glucose transporter that permits rapid intracellular and extracellular equilibration of glucose, whereas the hexokinase, GK, possesses a K_m in the range of physiological glucose concentrations (28). The physical properties of GK and Glut-2 facilitate proportional cellular responses to alterations in glucose availability over a range of physiological concentrations. As such, Glut-2 and GK have been proposed to optimize "gatekeeper" functions for glucose entry and metabolism during glucosensing processes (14, 29, 30). Glp-1R is a receptor-recognizing hormone synergistic with glucose in controlling insulin release in pancreatic β cells and is important in determining cellular sensitivity to glucose (31).

Previous studies have shown Glut-2, GK, and Glp-1R mRNA to be colocalized in discrete brain regions associated with the regulation of food intake (20, 21, 32). Several *in vivo* studies indicate a role for Glut-2, GK, and Glp-1R in the control of feeding. Intracerebroventricular injection of antisense against Glut-2 reduces glucoprivic hyperphagia (33); the injection of possible GK inhibitors into the third cerebral ventricle disrupts normal feeding (34); and centrally administered Glp-1R agonists and antagonists reciprocally affect feeding (20, 35, 36). Collectively, these findings support the hypothesis that Glut2, GK, and Glp-1R participate in central glucosensing related to the regulation of food intake.

This work was supported by a U.S. Army Grant DAMD 17-97-2-7013.
[1] To whom requests for reprints should be addressed at Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808. E-mail: Zhouj@pbrc.edu

Received December 2, 2002.
Accepted April 22, 2003.

1535-3702/03/2288-0943$15.00

In the pancreas and liver, the expression of Glut-2 and GK genes is altered by nutritional status; fasting reduces the expression of both genes in both organs, whereas refeeding rapidly enhances GK expression and more slowly increases Glut-2 expression (37). It is not known whether such treatments affect the brain expression of these genes. Here, we hypothesized that short-term food restriction and refeeding alter the expression of Glut-2, GK, and Glp-1R genes in selected regions of the rat brain. Highly sensitive, quantitative real-time RT-PCR was used to measure hypothalamic and brainstem Glut-2, GK, and Glp-1R mRNA levels in food-restricted rats and refed rats. We also examined gene expression in liver as a peripheral tissue control and for methodological validation. Brain cortex was used as a negative tissue control. Two additional feeding control groups were included in the attempt to account for changes in expression that may occur due to food restriction and also due to food-intake independent of prior feeding status (Fig. 1).

Our results show that Glut-2, GK, and Glp-1R expression in the hypothalamus and brainstem were lower in refed rats compared with food-restricted rats. Expression levels were not changed by short-term food restriction or voluntary hyperphagia. The data provide evidence that changing nutritional status can acutely alter the expression of genes proposed to be involved in brain glucosensing.

Materials and Methods

Forty adult male, Sprague-Dawley rats weighing 250 g were obtained from Harlan Sprague-Dawley (Houston, TX) and were individually housed in wire mesh cages in a humidity- and temperature-controlled room (22° ± 2°C, 65%–67% humidity) on a 12:12-hr light:dark cycle with lights on at 0700 hr. All rats had free access to regular chow (Rodent Laboratory Chow 5001; Purina Mills, St. Louis, MO) and tap water, and were handled daily. The food intakes were measured daily for 7 days. One day before the start of experiment, rats were randomly divided into two groups: control and food restricted. Control rats had *ad libitum* access to food; food-restricted rats were fed with 50% of their daily chow intake for 1 day, starting at 0800 hr. The 50% value was based on individual animals' average daily intake for the previous 3 days. At 0800 hr of the experimental day, the food-restricted rats were subdivided into two groups labeled as food restricted (FR) and refed. Animals in the FR group were continued with no access to food, whereas the animals in the refed group were given *ad libitum* access to chow for a 1-hr period. Control rats were also subdivided into two groups labeled as "control 1" and "control 2." Both control 1 and control 2 rats had continued *ad libitum* access to chow throughout the experimental period. The rats in the control 2 group were additionally provided *ad libitum* access to cookies (Jack's Vanilla Wafers; Murray Biscuit Company, Elmhurst, IL, caloric content: 4.3 kcal/g, composition: 11.1% fat, 81.5% carbohydrate, and 7.4% protein) for the 1-hr period of refeeding. Cookies were chosen based on the results of preliminary studies that showed that non-FR rats voluntarily consume similar gram quantities of cookies compared with grams of chow eaten by FR rats in a similar experimental period. A variety of other palatable foods yielded inferior responses (Zhou J, unpublished observations). All rats were decapitated at the end of the 1 hr refeeding period. The feeding portion of the experimental design is schematically displayed in Figure 1.

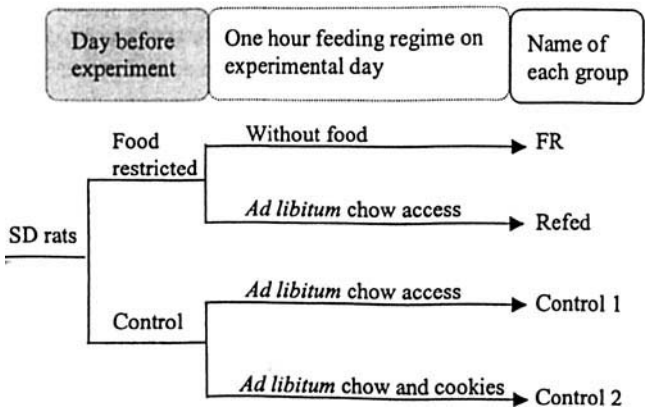

Figure 1. Schematic illustration of the experimental design of the feeding portion of this study. For the food-restricted period, rats were provided with 50% amount of their daily consumed chow for 1 day. Refeeding began at 0800 hr on the following morning. There were 10 rats in each group.

The postmortem measurements included 1 hr of food intake corrected for spillage, blood glucose (measured with a glucose testing meter, Accu-Chek Advantage; Roche Diagnostics, Indianapolis, IN); serum insulin (Rat Insulin RIA kit; Linco Research, St. Louis, MO); and Glut-2, GK, and Glp-1R gene expression in hypothalamus, brainstem, cortex, and liver. The animal protocol was previously approved by the Pennington Biomedical Research Center Institutional Animal Use and Care Committee.

The brains and livers were rapidly removed and frozen on dry ice (brain) or liquid nitrogen (liver) after decapitation. The hypothalamus, brainstem, and cortex were grossly dissected from the whole brain as tissue blocks on ice.

Dissection of the Hypothalamus, Brainstem, and Cortex. A 7-mm coronal section of hypothalamic area was made from the optic chiasm (corresponding approximately to 0.6–0.7 mm posterior to bregma in the atlas of Paxinos and Watson [38]) and extended posteriorly to the mamillary bodies. The resulting section was laid on a plate with the rostral surface facing up. A horizontal cut was made at the top of the third ventricle. The resulting lower portion was cut parasaggitally at the hypothalamic sulci.

Medial hindbrain, including area postrema, nucleus of the solitary tract, and dorsal motor nucleus of the vagus were collected as a contiguous tissue block. The brain was placed on a cool surface, dorsal side up. The cerebellum was gently retracted and a coronal cut was made approximately 1.0 mm caudal to the anterior-most edge of the visible area postrema. A second coronal cut was made approximately 1.0 mm rostral to this landmark.

The brain cortex was collected bilaterally at the frontal cortex, motor area.

Total RNA Extraction from Hypothalamus, Brainstem, Cortex, and Liver. Trizol reagent (GIBCO, Gaithersburg, MD) was used for total RNA extraction, with the exception that an Atlas Glass Total RNA Isolation kit (Clontech Laboratories, Palo Alto, CA) was used for the hypothalamus. Total RNA from each sample was quantified spectrophotometrically and was confirmed by ethidium bromide staining of the 18S and 28S bands under UV light after electrophoresing samples on a 1% agarose/formaldehyde gel.

Real-Time RT-PCR. Glut-2, GK, and Glp-1R mRNA levels were determined by quantitative real-time RT-PCR. Cyclophilin mRNA levels from each sample were used as internal controls to normalize the mRNA levels (38). TaqMan probes and primers were designed using Primer Express Software, version 1.0 (Applied Biosystems, Branchburg, NJ) following the manufacturer's guidelines. The specificity of the designed primers and probes for their respective target genes was confirmed by nucleotide BLAST search on the NCBI database. There were no sequence homologies of probes or primers for any genes other than the target genes in the same species. Primers of GK were designed to nonselectively amplify both pancreatic and liver forms of mRNA. The sequences of primers and probes for cyclophilin (GenBank no. M15933), Glut-2 (GenBank no. NM_012879), GK (GenBank no. M25807), and Glp-1R (GenBank no. NM_012728) are listed in Table I.

Real-time RT-PCR was performed in a 50-μl final reaction volume using a TaqMan 100Rxn PCR core reagent kit (Applied Biosystems). The forward primer concentrations were 500 n*M* for Glut-2, GK, and Glp-1R and 100 n*M* for cyclophilin. The reverse primer concentrations were 500 n*M* for Glut-2, GK, and Glp-1R and 200 n*M* for cyclophilin. The probe concentrations were 250 n*M* for Glut-2, GK, and Glp-1R and 100 n*M* for cyclophilin. Real-time RT-PCR conditions were as follows: 48°C for 30 min, then 95°C for 10 min for one cycle, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min on an ABI PRISM 7700 sequence detector (Applied Biosystems). The standard curves were generated from corresponding tissues from normal, untreated Sprague-Dawley rats. The data within the linear region of the amplification curve was analyzed according to ABI's user bulletin no. 2. The specificity of PCR product for each tested gene was confirmed by sequencing.

Statistic Analysis. Blood glucose and serum insulin data were analyzed by one-way analysis of variance (ANOVA) with adjusted Tukey's *post hoc* pair-wise comparison. For analysis of the differences in the means of blood glucose and serum insulin values specifically in FR/refed groups and in control 1/control 2 groups, a linear function of random variables model was used according to the method of Freund and Wilson (40). All of the gene expression data were analyzed by one-way ANOVA. For comparisons between FR and refed groups, we used FR rats as the control. For comparisons between the control 1 and control 2 groups, we use control 1 as the control. Data are presented as means ± SE.

Results

One day of 50% food restriction resulted in lower body weights compared with control rats (273 ± 4 g vs 291 ± 3 g; $P < 0.01$). One-hour calorie intake during the experimental period is shown in Figure 2. Refed rats ate more chow than control 1 rats and ate a similar amount of food (grams) compared with control 2 rats (data not shown). The calorie intake of the control 2 group was slightly higher than the refed group; 99% of food eaten by control 2 rats was cookies. Blood glucose and serum insulin values are shown in Table II. Blood glucose was significantly lower in FR animals and was significantly higher in the refed and control 2 groups compared with control 1 rats (Table II; $P < 0.01$). The serum insulin levels of FR rats were significantly lower compared with the control 1 group; refed rats' serum insulin values were similar to those of the control 1 group. The insulin levels of the control 2 (cookie-fed) group were significantly elevated compared with control 1 rats (Table II; $P < 0.01$). The magnitude of change in blood glucose and

Table I. The Sequences of Primers and Probes for Cyclophilin (GenBank no. M15933), Glut-2 (no. NM_012879), GK (no. M25807), and Glp-1R (no. NM_012728)

Name	Sequence (5′-3′)
Cyclophilin	Forward primer: CCCACCGTGTTCTTCGACAT
	Reverse primer: TGCAAACAGCTCGAAGCAGA
	Probe: CAAGGGCTCGCCATCAGCCG
GK	Forward primer: CAAGCTGCACCCGAGCTT
	Reverse primer: TGATTCGATGAAGGTGATTTCG
	Probe: TCAGCCTGCGCACACTGGCG
Glut-2	Forward primer: GTCCAGAAAGCCCCAGATACC
	Reverse primer: TGCCCCTTAGTCTTTTCAAGCT
	Probe: TTGCCCTGACTTCCTCTTCCAAATTTAGGTAA
GLp-1R	Forward primer: CTGCATCGTGATAGCCAAGCT
	Reverse primer: GGACTTCGCGAGTCTGCATT
	Probe: AGGCTAATCTCATGTGTAAGACC

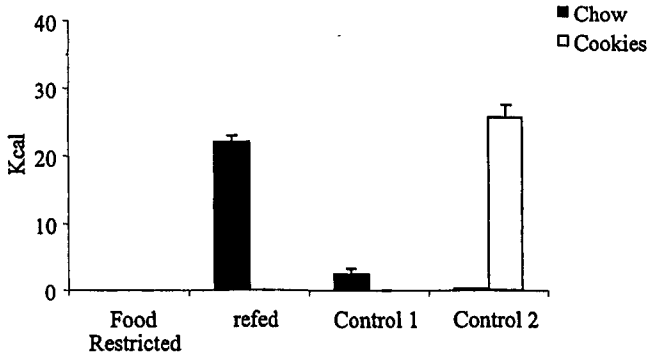

Figure 2. Food intakes during 1-hr experimental period. Refed rats ate more chow than control 1 rats and had slightly lower calorie intakes than control 2 rats. Ninety-nine percent of food consumed by control 2 rats came from cookies. Data are means ± SE.

serum insulin in FR versus refed animals compared with the changes occurring in control 1 versus control 2 groups is shown in Table II. The data indicate that the extent to which blood glucose rose in the refed rats compared with the FR animals was significantly greater than the rise in blood glucose in the control 2 group compared with control 1 ($P < 0.01$). The magnitude of change in serum insulin reveals no difference between FR and refed animals compared with the difference between controls 1 and 2 ($P > 0.05$).

The results of Glut-2, GK, and Glp-1R expression in the hypothalamus and brainstem for all four treatment groups are illustrated in Figure 3A (hypothalamus) and Figure 3B (brainstem). In both the hypothalamus and brainstem, gene expression of Glut-2, GK, and Glp-1R was significantly lower in refed rats compared with FR animals ($F_{(1, 16)} = 4.78, P < 0.05$; $F_{(1, 16)} = 11.5, P < 0.01$; $F_{(1, 16)} = 19.5, P < 0.01$ for Glut-2, GK, and Glp-1R, respectively, in the hypothalamus; $F_{(1, 15)} = 7.4, P < 0.05$; $F_{(1, 15)} = 4.55, P = 0.05$; $F_{(1, 15)} = 6.16, P < 0.05$ for Glut-2, GK, and Glp-1R, respectively, in the brainstem). Although the changes in Glut-2 and Glp-1R for FR and refed rats were small, there was still a 35% reduction in Glut-2 and a 16% reduction in Glp-1R. Gene expression in the control 2 group, which had similar food intake as the refed group during the 1-hr refeeding period, did not show the changes compared with control 1 (non-FR) rats. The measures of gene expression in control groups 1 and 2 (non-FR or cookie-fed) were intermediate between the FR and refed groups, but were not significantly different from each other or from FR rats.

In the liver (Fig. 4), changes in Glut-2 gene expression were similar to those observed in the brain: 1 hr of refeeding significantly reduced Glut-2 gene expression in liver compared with FR rats ($P < 0.05$). The two control groups had intermediate Glut-2 gene expression between the FR and refed groups, but the values were not significantly different from each other or the other experimental groups. The changes of GK expression in liver showed a distinctly different pattern compared with brain GK expression. FR reduced GK expression compared with control 1 ($P < 0.05$). Refed animals showed significantly increased liver GK expression compared with FR rats ($P < 0.05$), and similar levels as the control 1 rats. GK expression in the control 2 (cookie-fed) group was 400% higher than the control 1 group ($P < 0.01$).

GK and Glp-1R expression could not be detected in samples from the brain cortex. Glut-2 was detected in cortex, although the levels were very low compared with the other brain areas and the liver. In normal, untreated Sprague-Dawley rats, ratios of Glut-2 to cyclophilin were 10.89 ± 1.89, 0.37 ± 0.19, 0.19 ± 0.05, and 0.09 ± 0.02 for liver, brainstem, hypothalamus, and cortex, respectively. Glp-1R was not detectable in the liver, consistent with previous reports (32, 41–43).

Discussion

This study was conducted to determine the effects of food restriction and refeeding on changes in Glut-2, GK, and Glp-1R gene expression in proposed glucosensing regions of the rat brain (2, 11, 12, 43). The two control groups were designed to isolate the effects of food restriction (control 1) and the effects of feeding independent of prior restriction (control 2). Our results indicate that feeding, after a mild caloric restriction, reduces putative glucosensing gene expression in the hypothalamus and brainstem, whereas overfeeding from a nonrestricted state does not produce such changes. However, we cannot rule out the possibility that overfeeding on cookies failed to alter gene expression due to unidentified dietary constituents absent in vanilla wafers but present in chow.

Table II. Blood Glucose and Serum Insulin of Fasted and Refed Rats

	Food restricted	Refed	Control 1	Control 2	Difference	
					Food restricted versus refed	Control 1 versus control 2
Blood glucose (mg/dl)	102 ± 2^a	134 ± 3^b	118 ± 3^c	132 ± 3^b	32 ± 3^A	14 ± 4^B
Serum insulin (ng/ml)	3.4 ± 0.4^a	16.2 ± 1.0^c	13.2 ± 1.1^c	25.9 ± 2.2^b	12.9 ± 0.3	12.7 ± 1.3

Note. Data are means ± SE for groups 9 to 10 rats measured at the end of a 1-hr feeding period. Statistical significance was determined by one-way ANOVA with Tukey's *post hoc* pair-wise comparison ($P < 0.05$). Values for a given parameter that do not share a common superscript are significantly different. For the differences between fasted versus refed and control 1 versus control 2, serum insulin levels were not different, whereas the magnitude of changes in glucose level were significantly higher in fasted/refed group compared with control 1/control 2 group.

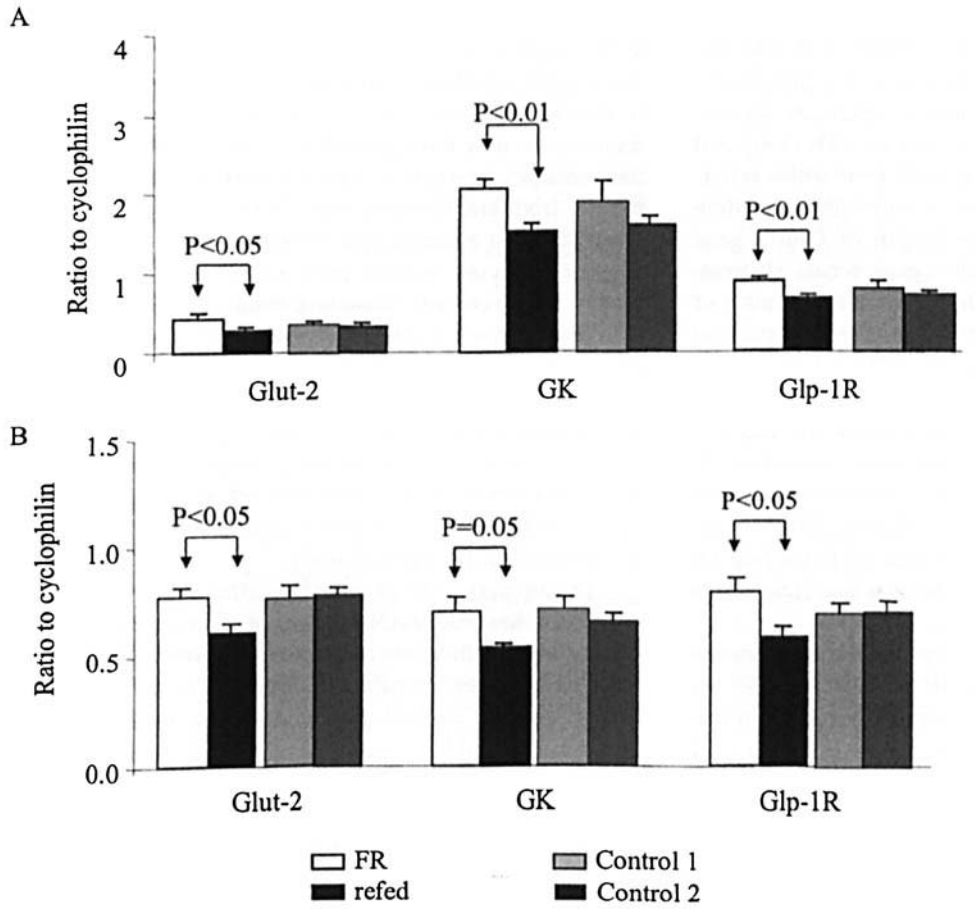

Figure 3. Glut-2, GK, and Glp-1R expression in hypothalamus (A) and brainstem (B) measured at the end of experiment (after 1 hr of refeeding). Data are means ± SE for groups of seven to nine rats.

The level of mRNA from all three genes (GK, Glut-2, and Glp-1R) was reduced, approximately to the same extent in both brain regions during refeeding, consistent with regulation by a coordinated response or a similar response to a common factor, as has been suggested for Glut-2 and GK in βTC6-F7 cells (43). The most likely candidate, as a common factor affecting the expression, is glucose, which has been shown to initiate the expression of GK and Glut-2 in pancreas (45–47) and Glp-1R in cell lines (32, 48). Extracellular brain glucose has been shown to parallel blood glucose over a wide range of concentrations (49). We assume that, with respect to the refed and control 2 rats, the cells of the brain regions tested had all been acutely exposed to changes in glucose availability immediately before sampling. The magnitude of change of blood glucose in the FR/refed animals was significantly greater than the change in the control 1/control 2 groups (Table II). Thus, the gene expression changes in brain might have resulted from either the extent or rate of change in glucose availability. These results support Bray's proposed glucodynamic theory, which suggests that it is the dynamic change of glucose, not the absolute level of blood glucose, that is responsible for control food intakes (1).

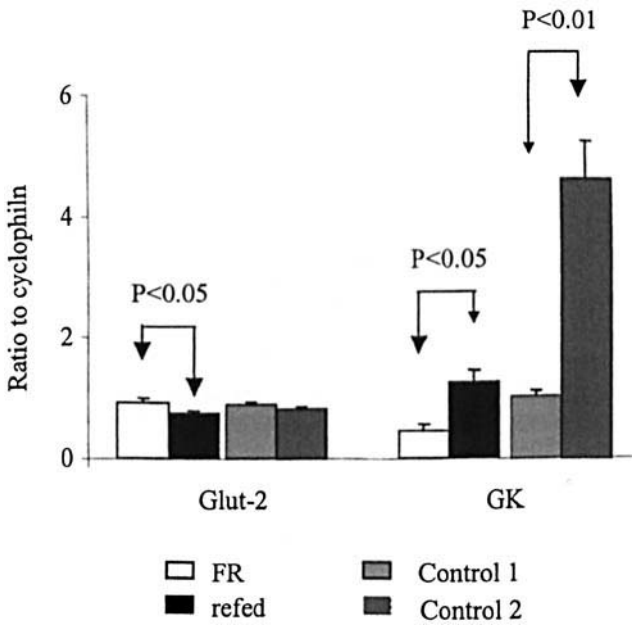

Figure 4. Glut-2 and GK expression in liver measured at the end of experiment (after 1 hr of refeeding). Data are means ± SE for groups of eight to nine rats.

The observed changes in hepatic GK expression confirm the previous conclusion that the dietary manipulations had strong effects on the expression of GK in liver (47, 50, 51). Also, across all feeding groups, the relative levels of liver GK expression coincide with the dietary-induced changes in serum insulin levels (Table II), and support pre-

vious reports that insulin is an important regulator of GK expression in the liver (37, 50). Compared with GK, the changes in hepatic Glut-2 expression were less profound.

In the hypothalamus and brainstem, refed rats showed lower mRNA levels of GK, Glut-2, and Glp-1R compared with FR animals. Our data indicate both similarities (Glut-2) and differences (GK) in gene expression after refeeding between the brain and liver. The pattern of Glut-2 gene expression is similar between both organs across all treatment groups, suggesting a possible common mechanism of control in both brain and liver. In contrast, the hepatic and brain expression of GK are distinctly different from each other. In the liver, food restriction decreased, and refeeding increased GK expression, whereas refeeding reduced GK expression in the brain, and food restriction showed no effect. Thus, factors regulating GK expression in the brain are presumed to be different from those affecting GK expression in the liver. A reliable comparison of brain Glp-1R gene expression with other organ systems is less clear at this time.

The primary hypothesis of study is that the mechanisms of glucosensing in pancreatic β cells provide a model for glucosensing in the brain. Previous reports have shown that glucose, or glucose dynamics, control the expression of Glut-2 and GK in pancreatic β cells (45–47), and similarly regulate Glp-1R expression in MIN6 cells (32). These reports consistently show upregulation of gene expression in response to increasing concentrations of glucose availability. Our results indicate the opposite situation in the brain where Glut-2, GK, and Glp-1R were downregulated in the face of presumed increasing glucose concentrations. These results imply that either elevated glucose differentially regulates the expression of Glut-2 and GK in pancreas and brain, or that other unidentified local factors are responsible for the brain effects. Given the diversity of receptor and postreceptor-mediated events affecting brain cells, together with the lack of the individual identity of the cells measured in the current study, we cannot make firm conclusions about the mechanisms responsible for the observed changes. It is likely that fasting optimizes brain cell sensitivity to feeding and is required to produce measurable changes in gene expression. However, we cannot exclude the alternative possibilities that the differences in expression may have resulted from differences in nutrient composition (chow versus cookies), or that hedonic or other sensory inputs from feeding on highly palatable food might have interfered with glucose-associated influences on gene expression.

The quantitative real-time RT-PCR technique used in this study has several advantages over alternative mRNA assessment techniques. Compared with *in situ* hybridization and RNase protection assays, real-time RT-PCR is more specific and more sensitive for detecting small changes in the RNA expression, and eliminates the need for electrophoresis (52, 53). To further confirm the specificity of real-time PCR products, we tested for the possibility of genomic DNA contamination, and the PCR products have been sequenced to ensure that the PCR amplification was specific to the target genes. However, real-time RT-PCR has a low anatomical specificity compared with *in situ* hybridization. It should be noted that by using grossly dissected brain regions, we may have missed measuring small, but important changes in specific brain nuclei due to dilution by mRNA from unaffected tissues. Nevertheless, our data represent the first evidence that refeeding after fasting reduces proposed glucose-sensing gene expression in brain areas related to the control of feeding behavior.

Our ability to assign physiological relevance of the present findings is limited because mRNA levels may not reflect the levels of protein or protein activities (54–57). The levels of proteins coded by our target genes in the brain are extremely low, and current protein detection methods are much less sensitive than real-time PCR. Thus, we cannot successfully detect comparable changes of protein in the tissues used in the present study.

In summary, 1 hr of refeeding after 1 day of 50% food restriction lowered mRNA levels of Glut-2, GK, and Glp-1R in the hypothalamus and brainstem. Similar food intake induced by access to palatable food had no measurable effect in non-FR animals. The changes in hepatic gene expression induced by fasting and refeeding, and by overfeeding, were consistent with previous reports. The pattern of changes in hepatic GK expression is dissimilar to the changes seen in the brain. Although the mechanism underlying the nutritionally related changes in gene expression are not understood at this time, we found lower expression of the glucosensing gene in hypothalamic and brainstem in response to food intake, depending on prior nutritional status.

1. Bray GA. Static theories in a dynamic world: a glucodynamic theory of food intake. Obes Res **4**:489–492, 1996.
2. Campfield LA, Smith FJ. Transient declines in blood glucose signal meal initiation. Int J Obes **14**(Suppl 3):15–31; discussion 31–34, 1990.
3. Louis-Sylvestre J, Le Magnen J. Fall in blood glucose level precedes meal onset in free-feeding rats. Neurosci Biobehav Rev **4**(Suppl 1):13–15, 1980.
4. Mayer J. Glucostatic mechanism of regulation of food intake. N Engl J Med. **249**:13–16, 1953.
5. Mayer J. Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. Ann N Y Acad Sci **63**:15–34, 1955.
6. Oomura Y, Yoshimatsu H. Neural network of glucose monitoring system. J Auton Nerv Syst **10**:359–372, 1984.
7. Anand BK, Chhina GS, Sharma KN, Dua S, Singh B. Activity of single neurons in the hypothalamus feeding centers: effect of glucose. Am J Physiol **207**:1146, 1964.
8. Oomura Y, Ono T, Ooyama H, Wayner MJ. Glucose and osmosensitive neurons of the rat hypothalamus. Nature **222**:282–284, 1969.
9. Mizuno Y, Oomura Y. Glucose responding neurons in the nucleus tractus solitarius of the rat: in vitro study. Brain Res **307**:109–116, 1984.
10. Oomura Y, Kimura K, Ooyama H, Maeo T, Iki M, Kuniyoshi N. Reciprocal activities of the ventromedial and lateral hypothalamic area of cats. Science **143**:484, 1964.
11. Bird E, Cardone CC, Contreras RJ. Area postrema lesions disrupt food

intake induced by cerebroventricular infusions of 5-thioglucose in the rat. Brain Res **270:**193–196, 1983.
12. Ritter S, Dinh TT, Zhang Y. Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. Brain Res **856:**37–47, 2000.
13. Borg MA, Sherwin RS, Borg WP, Tamborlane WV, Shulman GI. Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. J Clin Invest **99:**361–365, 1997.
14. Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic β cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes **50:**1–11, 2001.
15. Yang XJ, Kow LM, Funabashi T, Mobbs CV. Hypothalamic glucose sensor: similarities to and differences from pancreatic β cell mechanisms. Diabetes **48:**1763–1772, 1999.
16. Penicaud L, Leloup C, Lorsignol A, Alquier T, Guillod E. Brain glucose sensing mechanism and glucose homeostasis. Curr Opin Clin Nutr Metab Care **5:**539–543, 2002.
17. Levin BE. Arcuate npy neurons and energy homeostasis in diet-induced obese and resistant rats. Am J Physiol **276:**R382–R387, 1999.
18. Roane DS, Bounds JK. ATP-sensitive K^+ channels in the regulation of feeding: a hypothesis. Nutr Neurosci **2:**209–255, 1999.
19. Roncero I, Alvarez E, Vazquez P, Blazquez E. Functional glucokinase isoforms are expressed in rat brain. J Neurochem **74:**1848–1857, 2000.
20. Navarro M, Rodriquez de Fonseca F, Alvarez E, Chowen JA, Zueco JA, Gomez R, Eng J, Blazquez E. Colocalization of glucagon-like peptide-1 (glp-1) receptors, glucose transporter glut-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of glp-1 receptor agonists as an inhibitory signal for food and water intake. J Neurochem **67:**1982–1991, 1996.
21. Alvarez E, Roncero I, Chowen JA, Thorens B, Blazquez E. Expression of the glucagon-like peptide-1 receptor gene in rat brain. J Neurochem **66:**920–927, 1996.
22. Leloup C, Arluison M, Lepetit N, Cartier N, Marfaing-Jallat P, Ferre P, Penicaud L. Glucose transporter 2 (glut 2): expression in specific brain nuclei. Brain Res **638:**221–226, 1994.
23. Brant AM, Jess TJ, Milligan G, Brown CM, Gould GW. Immunological analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. Biochem Biophys Res Commun **192:**1297–1302, 1993.
24. Lynch RM, Tompkins LS, Brooks HL, Dunn-Meynell AA, Levin BE. Localization of glucokinase gene expression in the rat brain. Diabetes **49:**693–700, 2000.
25. Maekawa F, Toyoda Y, Torii N, Miwa I, Thompson RC, Foster DL, Tsukahara S, Tsukamura H, Maeda K. Localization of glucokinase-like immunoreactivity in the rat lower brain stem: four possible location of brain glucose-sensing mechanisms. Endocrinology **141:**375–384, 2000.
26. Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. J Comp Neurol **403:**261–280, 1999.
27. Shughrue PJ, Lane MV, Merchenthaler I. Glucagon-like peptide-1 receptor (glp1-r) mRNA in the rat hypothalamus. Endocrinology **137:**5159–5162, 1996.
28. Meglasson MD, Burch PT, Berner DK, Najafi H, Vogin AP, Matschinsky FM. Chromatographic resolution and kinetic characterization of glucokinase from islets of Langerhans. Proc Natl Acad Sci U S A **80:**85–89, 1983.
29. Matschinsky FM. Glucokinase as glucose sensor and metabolic signal generator in pancreatic β cells and hepatocytes. Diabetes **39:**647–652, 1990.
30. Schuit FC. Is glut2 required for glucose sensing? Diabetologia **40:**104–111, 1997.
31. Holz GGT, Kuhtreiber WM, Habener JF. Pancreatic β cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). Nature **361:**362–365, 1993.
32. Yamato E, Ikegami H, Takekawa K, Fujisawa T, Nakagawa Y, Hamada Y, Ueda H, Ogihara T. Tissue-specific and glucose-dependent expression of receptor genes for glucagon and glucagon-like peptide-1 (glp-1). Horm Metab Res **29:**56–59, 1997.
33. Wan HZ, Hulsey MG, Martin RJ. Intracerebroventricular administration of antisense oligodeoxynucleotide against glut2 glucose transporter mRNA reduces food intake, body weight change and glucoprivic feeding response in rats. J Nutr **128:**287–291, 1998.
34. Fujimoto K, Sakata T, Shiraishi T, Kurata K, Terada K, Etou H. Anorexia induced in rat by D-glucosamine deoxidized at c-1. Am J Physiol **251:**R481–R491, 1986.
35. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature **379:**69–72, 1996.
36. Donahey JC, van Dijk G, Woods SC, Seeley RJ. Intraventricular glp-1 reduces short- but not long-term food intake or body weight in lean and obese rats. Brain Res **779:**75–83, 1998.
37. Tiedge M, Lenzen S. Effects of glucose refeeding and glibenclamide treatment on glucokinase and glut2 gene expression in pancreatic β cells and liver from rats. Biochem J **308:**139–144, 1995.
38. Paxinos GWC. The Rat Brain in Sterotaxic Coordinates. San Diego: Academic Press, 1997.
39. Medhurst AD, Harrison DC, Read SJ, Campbell CA, Robbins MJ, Pangalos MN. The use of taqman RT-PCR assays for semiquantitative analysis of gene expression in CNS tissues and disease models. J Neurosci Methods **98:**9–20, 2000.
40. Freund RJ, Wilson WJ. Statistical Methods. San Diego: Academic Press, 1996.
41. Blackmore PF, Mojsov S, Exton JH, Habener JF. Absence of insulinotropic glucagon-like peptide-i(7-37) receptors on isolated rat liver hepatocytes. FEBS Lett **283:**7–10, 1991.
42. Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology **137:**2968–2978, 1996.
43. Dunn-Meynell AA, Govek E, Levin BE. Intracarotid glucose selectively increases fos-like immunoreactivity in paraventricular, ventromedial and dorsomedial nuclei neurons. Brain Res **748:**100–106, 1997.
44. Porzio O, Marlier LN, Federici M, Hribal ML, Magnaterra R, Lauro D, Fusco A, Sesti G, Borboni P. Glut2 and glucokinase expression is coordinately regulated by sulfonylurea. Mol Cell Endocrinol **153:**155–161, 1999.
45. Ferrer J, Gomis R, Fernandez Alvarez J, Casamitjana R, Vilardell E. Signals derived from glucose metabolism are required for glucose regulation of pancreatic islet glut2 mRNA and protein. Diabetes **42:**1273–1280, 1993.
46. Chen L, Alam T, Johnson JH, Hughes S, Newgard CB, Unger RH. Regulation of β cell glucose transporter gene expression. Proc Natl Acad Sci U S A **87:**4088–4092, 1990.
47. Tiedge M, Lenzen S. Regulation of glucokinase and glut-2 glucose-transporter gene expression in pancreatic β cells. Biochem J **279:**899–901, 1991.
48. Waeber G, Thompson N, Haefliger JA, Nicod P. Characterization of the murine high kM glucose transporter glut2 gene and its transcriptional regulation by glucose in a differentiated insulin-secreting cell line. J Biol Chem **269:**26912–26919, 1994.
49. Silver IA, Erecinska M. Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. J Neurosci **14:**5068–5076, 1994.
50. Iynedjian PB, Ucla C, Mach B. Molecular cloning of glucokinase cDNA. Developmental and dietary regulation of glucokinase mRNA in rat liver. J Biol Chem **262:**6032–6038, 1987.
51. Iynedjian PB, Pilot PR, Nouspikel T, Milburn JL, Quaade C, Hughes S, Ucla C, Newgard CB. Differential expression and regulation of the glucokinase gene in liver and islets of Langerhans. Proc Natl Acad Sci U S A **86:**7838–7842, 1989.

52. Walker NJ. Tech.Sight. A technique whose time has come. Science **296:**557–559, 2002.
53. Bustin SA. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J Mol Endocrinol **29:**23–39, 2002.
54. Garcia-Flores M, Blazquez E, Zueco JA. Effects of triiodothyronine and bovine growth hormone on glucose transporter isoform-2 (glut-2) and glucokinase (gk) gene expression in pancreatic islets of fetal and adult rats. Pflugers Arch **442:**662–667, 2001.
55. Garcia Flores C, Aguilar R, Reyes de la Cruz H, Albores M, Sanchez de Jimenez E. A maize insulin-like growth factor signals to a transduction pathway that regulates protein synthesis in maize. Biochem J **358:**95–100, 2001.
56. Garcia-Flores M, Zueco JA, Alvarez E, Blazquez E. Expression of glucagon-like peptide-1 (glp-1) receptor and the effect of glp-1-(7-36) amide on insulin release by pancreatic islets during rat ontogenic development. Eur J Biochem **268:**514–520, 2001.
57. Villanueva-Penacarrillo ML; Puente J, Redondo A, Clemente F, Valverde I. Effect of glp-1 treatment on glut2 and glut4 expression in type 1 and type 2 rat diabetic models. Endocrine **15:**241–248, 2001.