Phosphatidyl Inositol 3-Kinase Signaling in Hypothalamic Proopiomelanocortin Neurons Contributes to the Regulation of Glucose Homeostasis

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Abstract

Recent studies demonstrated a role for hypothalamic insulin and leptin action in the regulation of glucose homeostasis. This regulation involves proopiomelanocortin (POMC) neurons because suppression of phosphatidyl inositol 3-kinase (PI3K) signaling in these neurons blunts the acute effects of insulin and leptin on POMC neuronal activity. In the current study, we investigated whether disruption of PI3K signaling in POMC neurons alters normal glucose homeostasis using mouse models designed to both increase and decrease PI3K-mediated signaling in these neurons. We found that deleting p85α alone induced resistance to diet-induced obesity. In contrast, deletion of the p110α catalytic subunit of PI3K led to increased weight gain and adipose tissue along with reduced energy expenditure. Independent of these effects, increased PI3K activity in POMC neurons improved insulin sensitivity, whereas decreased PI3K signaling resulted in impaired glucose regulation. These studies show that activity of the PI3K pathway in POMC neurons is involved in not only normal energy regulation but also glucose homeostasis.

Diabetes rates along with obesity are rising in the United States (1,2), causing substantial life span reductions (3,4,5). Recent studies implicate insulin and leptin in the central regulation of glucose homeostasis. Hypothalamic insulin signaling suppresses hepatic glucose production (HGP), which is elevated in type 2 diabetes (6,7,8). Likewise, central leptin alters peripheral glucose uptake and hepatic glucose output (9,10,11) and rescues hyperphagia-induced hepatic insulin resistance (12). Leptin-deficient mice have impaired insulin and glucose homeostasis (13) that is normalized by leptin administration (14). Furthermore, reexpressing leptin receptors in the arcuate nucleus of leptin receptor null mice reduces hyperglycemia (15,16,17).

Both insulin and leptin activate the phosphatidyl inositol 3-kinase (PI3K) intracellular signaling pathway (18). PI3K consists of an 85-kDa regulatory (p85) and a 110-kDa catalytic (p110) subunit (19), each having several isoforms. p85 binds insulin receptor substrate (IRS) molecules and localizes catalytic activity to the cell membrane, In which p110 phosphorylates phospatidylinositol 4,5-biphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3), activating downstream molecules that bind PIP3 such as Akt. Reduced gene dosage of the catalytic subunit, as occurs in p110α inactivated cells or tissues, causes a severe reduction in insulin-stimulated PI3K activity, pAkt, and the phosphorylation levels of downstream components such as Forkhead box O-transcription factors (20,21). However, genetic inactivation of the regulatory subunit paradoxically increases signaling downstream of PI3K (22). This increase seems to result from both a compensatory increase in p85β expression (23) and reduced phosphatase and tensin homolog deleted from chromosome 10 (PTEN) activity because p85α forms part of the PTEN-activating complex (24). PTEN dephosphorylates PIP3, antagonizing the action of PI3K. Therefore, the loss of p85-induced Akt activation is due, in part, to decreased PTEN activity protecting the PIP3 pool produced by the remaining p110-p85β heterodimers.

Hypothalamic proopiomelanocortin (POMC) neurons are essential for normal body weight homeostasis (25,26,27,28,29) and may be important for glucose homeostasis as well. Centrally administered melanocortin agonists inhibit insulin release and alter glucose uptake and production (10). Furthermore, POMC-specific deletion of suppressor of cytokine signaling (SOCS)-3, a negative regulator of both leptin and insulin signaling, results in improved glucose homeostasis and insulin sensitivity (30). We recently showed that deleting both p85α and p85β from POMC neurons eliminates insulin and leptin effects on POMC neuronal activity (31). We therefore investigated whether normal glucose homeostasis requires POMC PI3K signaling using mouse models designed to either increase or decrease PI3K activity in these neurons. Using the cre/loxP system, we investigated mice lacking p85α in POMC neurons (up-regulation of PI3K mediated signaling) and mice lacking p110α in POMC neurons (down-regulation of PI3K mediated signaling).

Materials and Methods

Animal care

Care of all animals and procedures was approved by the University of Texas Southwestern Medical Center (*pik3CA POMCKO* studies) and the Beth Israel Deaconess Medical Center (*pik3r1 POMCKO* studies) Institutional Animal Care and Use Committees. Mice were housed in a temperature-controlled environment in groups of two to four at 22–24 C using a 14-h light, 10-h dark cycle. The mice were fed either standard chow (4% fat mouse/rat diet 7001; Harlan-Teklad, Madison, WI) or high-fat diet (88137 Western diet; Harlan Teklad), and water was provided *ad libitum* unless noted otherwise. Mice were killed by CO_2 narcosis.

Generation of mouse lines

Pomc-Cre mice [FVB background (26)] were mated with *pik3r1^{loxP/loxP}* mice [129/Sv-C57BL/6-FVB mixed background (32)] or *pik3CA ^{loxP/loxP}* mice [129/SvJ-C57BL/6 mixed background (21)]. Breeding colonies were maintained by mating *pik3r1^{loxP/loxP}* mice with *pik3r1 POMCKO* mice and *pik3CA ^{loxP/loxP}* with *pik3CA POMCKO* mice. Thus, littermate controls with the same genetic background as experimental animals except for POMC-cre expression were used for all experiments. Any mouse that tested positive for deletion of the *pik3r1* or *pik3CA* gene in tail tissue was excluded from all studies. Genotyping was performed according to protocols described previously (21,31).

Immunohistochemistry and *in situ* hybridization

Fed male 10-wk-old mice were perfused with 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO), and frozen coronal sections were cut at 25 μm (1:5 series) on a microtome. Sections were processed as reported previously from our laboratory (33).

To create the p85α and p110α cRNA probes (34), we used RT-PCR amplification. The first-strand cDNA was obtained using total rat brain RNA (Ambion, Inc., Austin, TX) as a template. For the p85α probe, the cDNA was then amplified by PCR using the 5′ primer: 5′-AGA ACG GCT ATC GAA GCA-3′ corresponding to nt 1997-2015 and the 3′ primer: 5′-GAC GCA ATG CTT GAC TTC-3′corresponding to nt 2552-2569 of p85α. The region bounded by these primers is 572 bases and corresponds to the COOH-terminal Src homology 2 domain of p85α. For the p110α probe, the cDNA was then amplified by PCR using the 5′ primer: 5′-TGC GCT GGG TAC TGC GTG GC-3′ corresponding to nt 2701-2720 and the 3′ primer: 5′-TAC GTT CAA AGC ATG CT-3′corresponding to nt 3191-3207 of p110α. The region bounded by these primers is 506 bases. The amplification products were gel purified and cloned into pCR4-TOPO vector (Invitrogen, Carlsbad, CA), by using standard techniques according to the manufacturer's protocol. The inserts of positive clones were verified by DNA sequencing with primers specific to the vector, T7 Sequenase (Amersham Life Science, Inc., Arlington Heights, IL), and the [35]Sequetide system from NEN Life Science Products (Boston, MA). To generate antisense ^{35}S-labeled cRNA, the plasmids were linearized by digestion with *Pst*I (p85α) or *Not*I (p110α) and subjected to *in vitro* transcription with T7 (p85α) or T3 (p110α) RNA polymerase according to the manufacturer's protocol (Promega, Madison, WI). For generation of sense ^{35}S-labeled cRNA, the plasmids were linearized by digestion with *Not*I (p85α) or *Pme*I (p110α) and subjected to *in vitro* transcription with T3 (p85α) or T7 (P110α) RNA polymerase according to the manufacturer's protocol.

The ^{35}S-labeled probes were diluted to 10^6 cpm/ml and used for *in situ* hybridization as previously described (33). Where applicable, after free-floating *in situ* hybridization histochemistry (ISHH), tissue was stained with a β-endorphin antibody (H-022-33; Phoenix Pharmaceuticals, Burlingame, CA) following established protocols. Tissue was mounted on slides for exposure to film and analysis by microscope. Very stringent criteria were used to assess coexpression to minimize false positives; coexpression was considered present if silver grains clustered around the outline of the cell beneath.

Feeding studies and body composition

Mice were housed individually for all body weight and food intake measurements. Body weight and chow consumption were measured once every 7 d. Food pellets were replaced weekly to reduce spillage. Body composition was determined using quantitative magnetic resonance (Minispec MQ10; Bruker, Houston, TX) for the *pik3CA POMCKO* mice by dual-energy x-ray absorptiometry (DEXA) for the *pik3r1 POMCKO* mice and controls. Mice were ketamine anesthetized for DEXA (MEC Lunar Corp., Minster, OH) using the facilities of the Metabolic Physiology Core of the Diabetes Endocrinology Research Center at Beth Israel Deaconess Medical Center.

Hormone measurements

For leptin and insulin assays, tail vein blood was obtained from *ad libitum* chow-fed mice at 12 wk of age unless otherwise specified. Serum was collected by centrifugation and assayed by commercially available ELISA kits (Crystal Chem Inc., Downers Grove, IL).

Corticosterone concentrations were measured from tail vein blood collected 30 min after an ip glucose injection (2 g/kg) to mimic experimental conditions in which differing corticosterone levels might alter glucose levels. Serum was measured by ELISA (correlate-EIA corticosterone kit; Assay Designs, Ann Arbor, MI) according to the manufacturer's instructions.

Evaluation of glucose homeostasis

Glucose in tail blood was assayed using a glucometer (One-Touch Basic; Lifescan, Milpitas, CA), with postprandial measurements taken at approximately 1000 h. Where indicated, blood was obtained from mice fasted for 16 h. For insulin tolerance tests, 3 h-fasted animals were injected ip with 1 mU/g human insulin (Humulin R; Eli Lilly Corp., Indianapolis, IN), and blood glucose values were measured immediately before and 15, 30, 45, 60, 90, and 120 min after injection. For glucose tolerance tests, overnight-fasted mice were injected with D-glucose (1 mg/g body weight), and blood glucose was measured immediately before and 15, 30, 45, 60, and 120 min after injection. Study mice (12–16 wk of age) were maintained on chow and matched for body weight and adiposity for analysis.

Metabolic chambers

Locomotor activity, respiratory quotients, and oxygen consumption were measured in *pik3r1 POMCKO* mice in a Columbus Instruments CLAMS by the Beth Israel Deaconess Medical Center Animal Physiology Core as previously described (15). Physical activity, energy expenditure, and heat production were monitored in *pik3CA POMCKO* mice using a combined indirect calorimetry system (TSE Systems GmbH, Bad Homburg, Germany) (35). For the latter, mice were housed individually at room temperature (22 C) under an alternating 12-h light, 12-h dark cycle. After adaptation for 6 d, home-cage physical activity was determined for 4 d using a multidimensional infrared light beam system with beams installed on cage bottom and cage top levels. Ambulatory movement was defined as breaks of any two different light beams at cage bottom level. Simultaneously O_2 consumption, CO_2 production, and heat production were measured to determine energy expenditure. In addition, food intake was determined continuously.

Hyperinsulinemic euglycemic clamps

At 3–5 d before clamp experiments, mice were anesthetized, and a silicone catheter was inserted in the femoral vein as previously described (36) and externalized through skin at the nape of the neck. The catheter was flushed daily with sterile saline until the animal returned to its preoperative weight. Mice were then fasted for 16 h and then subjected to a 120-min basal clamp followed by a 120-min hyperinsulinemic-euglycemic clamp study in an awake and unrestrained state. During 2 h of basal clamping, the animals were infused with (3-^{3}H) glucose solution (6 μCi total at a rate of 0.05 μCi per 3.3 μl/min) to measure HGP. Blood (30 ml) was sampled from the tail vein 5 min before the end of this period. Then mice received a prime dose of insulin (Humulin R, regular human insulin; Lilly) followed by a continuous infusion of insulin (15 pmol/kg body weight) at a rate of 3 μl/min. A 40% glucose solution was also infused at variable rates and periodically adjusted to maintain the plasma glucose levels at about 6.5 mM. The plasma glucose level was monitored using a glucometer every 10 min (5 ml). A (3-^{3}H) glucose solution was infused through the jugular vein catheter (10 μCi bolus, followed by 0.1 μCi/3 μl/min). Forty-five minutes before the end of study, a bolus of 2-deoxy-D-(1-^{14}C) glucose [2-(^{14}C) DG] was injected through the catheter to estimate insulin-induced glucose uptake in individual tissues (10 μCi per 10 μl). Blood was collected from the tail vein (25 μl at 5, 15, 25, 35, and 45 min after the deoxyglucose injection) to estimate whole-body glucose flux and the disappearance curve of the 2-(^{14}C) DG from blood. After termination of the experiment, white adipose and brown adipose tissues, heart, skeletal muscle, liver, and brain were collected, and 2-(^{14}C) DG content in the tissues was measured.

Western blot analysis

Samples were obtained from the liver 10 min after a 5 U/kg ip injection of insulin and immediately frozen on dry ice. Whole-cell protein extraction was performed. Equal amounts of the samples were separated by SDS-PAGE and visualized after electrophoretical transfer via binding of specific primary and horseradish peroxidase-conjugated secondary antibodies followed by chemiluminescent detection using West Pico chemiluminescent substrate (Thermo Fisher Scientific Inc., Waltham, MA). The primary

antibodies were a 1:1,000 dilution of the antibody against phospho-Akt (serine-473; Cell Signaling Technology, Beverly, MA), and a 1:10,000 dilution of the antibody against β-actin (Santa Cruz Biotechnology, Santa Cruz, CA).

Statistics

The data are reported as mean ± SEM. Comparisons between two groups were made by unpaired two-tailed t tests. Comparisons over time were carried out by calculating area under the curve for blood glucose levels or by repeated-measure ANOVA for body weight curves. $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using Prism software (version 5.0; GraphPad Software, San Diego, CA).

Results

Previous studies have shown that ablation of *pik3r1*, the gene encoding p85α, leads to increased PIP3 levels and subsequent activation of signaling pathways downstream of PI3K (22,23,37,38,39). To generate mice with increased PI3K signaling in POMC neurons, we crossed mice carrying a floxed allele of *pik3r1* gene (40) with transgenic mice expressing Cre-recombinase driven by *Pomc* regulatory elements (26) to create *pik3r1 POMCKO* mice. To generate a second group of mice with reduced PI3K signaling in POMC neurons, we crossed mice carrying a floxed allele of the *pik3CA* gene encoding p110α (21) with *Pomc*-Cre transgenic mice to create *pik3CA POMCKO* mice. For all experiments, mice were compared with littermates not expressing POMC-cre (*pik3r1[loxP/loxP]* or *pik3CA[loxP/loxP]* mice).

Using ISHH, we first examined arcuate nucleus expression of pik3r1 and pik3ca. We found that both genes have extensive expression throughout the arcuate nucleus with a characteristic pattern of hypothalamic mRNA localization (Fig. 1, A and B). We previously demonstrated that deletion of the loxP-flanked region of *pik3r1* in the brains of *pik3CA POMCKO* mice is limited to POMC-expressing areas (31). Likewise, deletion of the loxP-flanked region of pik3CA was restricted to expected regions (supplemental Fig. S1A published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). A subsequent immunohistochemistry analysis of β-endorphin in these POMC neurons revealed that p85α and p110α are expressed in POMC neurons (Fig. 1, C and D). In *pik3r1 POMCKO* or *pik3CA POMCKO* mice, POMC mediated Cre expression efficiently ablated *pik3r1* or *pik3ca* in POMC neurons (Fig. 1, C and D and supplemental Fig. S1B).

We next examined body weight changes on normal chow in males and females of all genotypes. Mice with POMC neuron-specific ablation of p110α displayed a modest increase in body weight. (Fig. 2, A and B). No change was seen in body length (data not shown). To examine the mechanism underlying the weight gain in *pik3CA POMCKO* mice, we placed weight-matched cohorts of *pik3CA POMCKO* and their littermate controls in metabolic chambers. Whereas no change was observed in food intake or activity levels in these mice (Fig. 2, C and D), female *pik3CA POMCKO* mice had significantly lower energy expenditures than weight- and gender-matched controls, as measured by decreased O_2 consumption as well as reduced heat production (Fig. 2, E and G). No difference was seen in the mass of brown adipose tissue from *pik3CA POMCKO* mice (68.17 mg ± 5.07 controls *vs.* 62.67 mg ± 6.87 *pik3CA POMCKO*). The respiratory exchange ratio did not differ between groups arguing against any change in preference for carbohydrate or lipid as substrates. (Fig. 2F). These results suggest that decreased energy expenditure, at least in females, contributes to the increased body weight of *pik3CA POMCKO* mice.

We next examined whether susceptibility to diet-induced obesity changed in mice with altered PI3K signaling in POMC neurons. We found that *pik3r1 POMCKO* females failed to gain weight on high-fat chow to the same extent as their littermate controls (Fig. 3B). In addition, these females exhibited significantly lower fat mass and leptin levels than control mice (Fig. 3, D and H). Conversely, *pik3CA POMCKO* mice showed increased body weight on high-fat chow accompanied by an increased fat mass (Fig. 3, A–D).

As previously mentioned, POMC neurons have been shown to regulate glucose homeostasis (10,30). We therefore went on to examine the effects of altered PI3K activity in POMC neurons on glucose homeostasis in mice with POMC-specific loss of p85α or p110α. Because glucocorticoids can cause glucose intolerance and insulin resistance (41,42,43), we first looked for evidence of changes in the hypothalamus-pituitary-adrenal axis downstream of POMC-expressing corticotrophs by examining corticosterone production under experimental conditions (described in *Materials and Methods*). We found that levels of corticosterone did not differ between the experimental animals and littermate controls (Fig. 4A). These results are in agreement with our previous findings of normal corticosterone production in mice lacking both *pik3r1* and *pik3r2* in POMC neurons (31).

We next examined basal glucose and insulin levels in chow-fed male mice. All measurements of glucose homeostasis were made in chow-fed body weight-matched and fat mass-matched mice to avoid the confounding effects of differing adiposity levels between mutant mice and littermate controls. Male *pik3r1 POMCKO* mice exhibited lower basal insulin levels with no obvious changes in glucose levels, suggesting that ablation of p85α in POMC neurons increased insulin sensitivity (Fig. 4, B and C). In contrast, *pik3CA POMCKO* mice showed both elevated insulin and glucose levels, indicating that POMC neuron-specific loss of p110α resulted in impaired insulin sensitivity. However, no evidence of glucose intolerance was seen in *pik3r1 POMCKO* mice or *pik3CA POMCKO* mice in response to a glucose bolus (Fig. 4D). We then subjected mice of all genotypes to an insulin tolerance test. The *pik3CA POMCKO* mice showed a significant impairment in blood glucose suppression in response to insulin stimulation (Fig. 4E). To investigate whether altered PI3K signaling in POMC neurons affects liver insulin sensitivity, we examined Akt activation in liver tissue taken 10 min after a bolus of insulin (5 U/kg). Akt phosphorylation was clearly reduced in tissue from *pik3CA POMCKO* mice (Fig. 5A); however, a change in pAkt levels could not be clearly shown in *pik3r1 POMCKO* hepatic tissue (data not shown).

For a more sensitive measure of hepatic and peripheral insulin sensitivity of *pik3r1 POMCKO* mice, we subjected them to hyperinsulinemic, euglycemic clamps. Basal HGP was significantly decreased in *pik3r1 POMCKO* mice (Fig. 5A). Given that during the basal period endogenous insulin acts chiefly on liver rather than muscle, this result suggests increased hepatic insulin sensitivity in these mice. In the hyperinsulinemic state, the glucose infusion rate tended to be higher in *pik3r1 POMCKO* mice, although the values did not reach statistical significance (Fig. 5B). However, the extent of suppression of HGP was significantly greater than controls in response to a fixed elevated insulin level (Fig. 5C). We went on to investigate hepatic insulin sensitivity under basal and clamp steady-state conditions by measuring expression levels of protein tyrosine phosphatase-1B (PTP1B). PTP1B dephosphorylates the insulin receptor and blunts its ability to transduce signal, thus mediating insulin resistance (44). We found that the expression of PTP1B was significantly suppressed in *pik3r1 POMCKO* hepatic tissue after hyperinsulinemic clamping (Fig. 5F), suggesting that loss of p85α in POMC neurons improved insulin sensitivity in the liver. Taken together, these data suggest that PI3K activity in POMC neurons can suppress glucose production by the liver and support the model that PI3K signaling in POMC neurons indirectly regulates hepatic insulin signaling.

Discussion

Recent studies suggest that insulin acting on central pathways can exert a potent effect on peripheral glucose metabolism, including HGP (8,10). In addition, leptin may play a critical role in glucose homeostasis, as restoration of leptin receptors to the arcuate nucleus or transgenic overexpression of the POMC gene reduces hyperglycemia in leptin receptor null animals (15,45). Thus, we hypothesized that PI3K signaling in POMC neurons is involved in whole-body glucose homeostasis.

Taking advantage of the specialized roles of the PI3K regulatory and catalytic subunits, we now used targeted deletion of p85α or p110α to selectively up-regulate or down-regulate PI3K-mediated activity,

respectively, in POMC neurons. We found that although both groups maintained glucose tolerance, *pik3r1 POMCKO* displayed an increase in insulin sensitivity, and *pikCA POMCKO* mice displayed a decrease in insulin sensitivity as determined by basal insulin levels, insulin tolerance testing, and/or hyperinsulinemic euglycemic clamping.

Previous studies using targeted perturbation in POMC neurons largely supported a role for POMC PI3K signaling in glucose homeostasis, although definitive evidence has been lacking. Mice lacking 3-phosphoinositide-dependent protein kinase-1, an enzyme downstream of PI3K, in POMC neurons initially displayed impaired glucose metabolism (46). However, these results were confounded by severe hypocortisolism caused by loss of POMC-expressing corticotrophs in the pituitary. In addition, selective deletion of SOCS-3, a negative regulator of leptin and insulin signaling, in POMC neurons led to a significant improvement in glucose homeostasis and insulin sensitivity without any apparent impact on the adrenal axis (30). However, mice lacking insulin receptors in POMC neurons did not show any defect in glucose or energy homeostasis (47). Mice with POMC-specific disruption of PTEN, expected to result in constitutively high levels of PI3K signaling, were apparently not evaluated for insulin levels or glucose regulation (48). We previously examined the effect of simultaneous deletion of both regulatory subunits, p85α and p85β, from POMC neurons by crossing *pik3r1 POMCKO* mice with mice germline knockout for pik3r2 (31). We found that concurrent loss of these two p85 subunits in POMC neurons abolishes the ability of these neurons to respond to leptin or insulin stimulation, but any effect on glucose homeostasis could not be evaluated due to increased insulin sensitivity in the global p85β-null mice. Our current data establish that alteration of PI3K activity in POMC neurons can modulate hepatic insulin sensitivity and glucose regulation, providing a mechanistic link between the response of POMC neurons to leptin or insulin and their effects on glucose homeostasis (30,45).

In the current experiments, we also observed an effect of POMC neuronal PI3K activity on body weight regulation. This finding extends our prior study of the effect of PI3K regulatory subunits on body weight (30). Whereas ablation of both p85α and p85β subunits in POMC neurons was required to impair POMC neuronal activity in response to insulin and leptin as well as acute leptin-induced feeding, no obvious impact on long-term body weight regulation was observed in those mice (31). The contrast to the current results may arise from the fact that global deletion of p85β has been reported to reduce mouse size and affect insulin sensitivity (49). Thus, it is possible that the experimental animals were protected from developing obesity in the initial study. This interpretation is in agreement with results from mice lacking SOCS-3 in POMC neurons showing reduction in weight gain on a high-fat diet (30). In addition, Plum *et al.* (48) have shown that POMC-specific disruption of PTEN results in hyperphagia, male obesity on normal chow, and female obesity on high-fat chow. However, interpretation of the latter results is complicated by morphological changes in POMC neurons and the existence of other downstream targets of PTEN, including MAPK and Shc (50,51,52,53,54).

Our results show that deletion of a single p85 isoform results in resistance to diet-induced obesity in female mice, possibly because the well-known anorexigenic effects of estrogens may be enhanced through increased PI3K signaling in POMC neurons (55,56,57). In addition, mice with increased PI3K activity in POMC neurons diverged from control values over time, whereas the body weight of mice with reduced PI3K signaling in these neurons tended to converge with control values. This observation is consistent with the hypothesis that high-fat feeding promotes weight gain in part by reducing PI3K signaling in POMC neurons.

These studies focused on the actions of the p110α catalytic subunit, although a p110β isoform also exists in the brain (58,59,60). p110α is selectively recruited and activated by the IRS signaling complex and is required for leptin and insulin-induced IRS-1/2-associated PI3K activity in the hypothalamus (20,61). Mice heterozygous for a kinase-inactive p110α mutation display hyperinsulinemia, glucose intolerance, hyperphagia, and increased adiposity without compensatory up-regulation of the activity of p110β (20).

In contrast, p110β does not appear to play a significant role in insulin-stimulated Akt activation (61,62), although interesting kinase-independent actions have recently been demonstrated in liver (67). Thus, it would be intriguing to examine the metabolic and glucoregulatory impact of p110β deletion as well.

In conclusion, these studies demonstrate that the PI3K pathway in POMC neurons plays a role in hepatic insulin sensitivity and glucose homeostasis. Moreover, PI3K signaling in POMC neurons has the ability to influence body weight regulation, especially in response to a high-fat dietary challenge.

Supplementary Material
[Supplemental Data]

Acknowledgments

We thank Michelle Choi, Charlotte Lee, Frank Marino, Brad Lampe, Sarah Greeley, Damalie Namponye, and the Mouse Metabolic Phenotyping Core at University of Texas Southwestern Medical Center at Dallas (supported by Grant PL1 DK081182-01) for assistance in performing studies described in this paper.

Footnotes

Disclosure Summary: The authors have nothing to declare.

First Published Online October 9, 2009

Abbreviations: 2-(^{14}C) DG, 2-Deoxy-D-(1-^{14}C) glucose; DEXA, dual-energy x-ray absorptiometry; HGP, hepatic glucose production; IRS, insulin receptor substrate; ISHH, *in situ* hybridization histochemistry; p85, 85-kDa regulatory subunit; p110, 110-kDa catalytic subunit; PI3K, phosphatidyl inositol 3-kinase; PIP3, phosphatidylinositol-3,4,5-trisphosphate; POMC, proopiomelanocortin; PTEN, phosphatase and tensin homolog deleted from chromosome 10; PTP1B, protein tyrosine phosphatase-1B; SOCS, suppressor of cytokine signaling.

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Figures and Tables

Figure 1
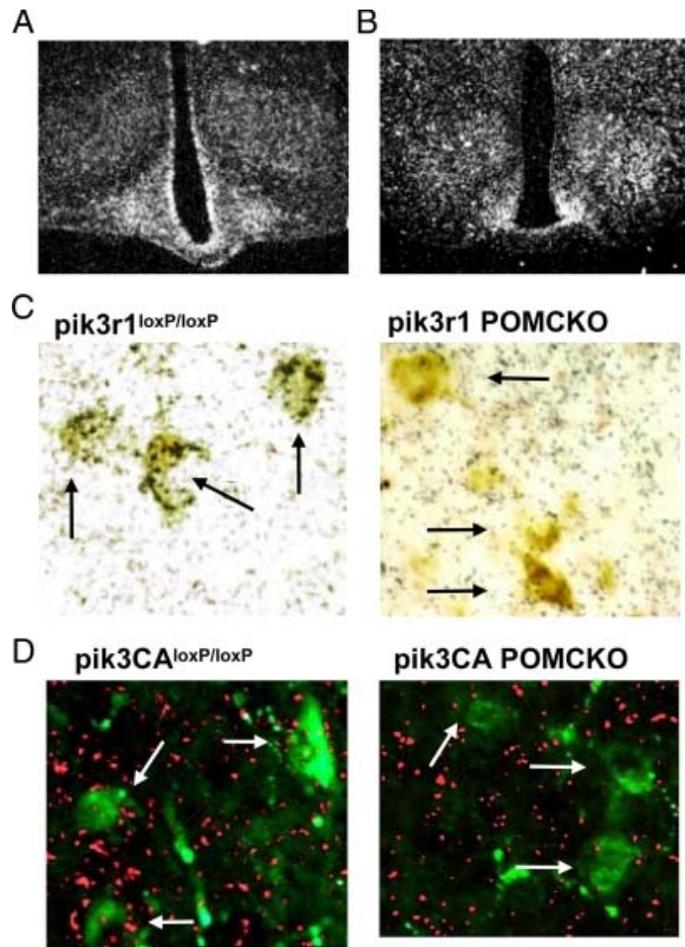
Targeted p85α or p110α deletion in POMC neurons. Photomicrographs of hypothalami from 3-month-old wild-type mice hybridized with probes for p85α (A) or p110α (B). Mice expressing Cre recombinase (Cre) under *Pomc* promoter control were mated with mice carrying loxp-flanked p85α or p110α alleles. Immunohistochemistry for β-endorphin was performed after ISHH for p85α (C) or p110α (D) in brain slices from targeted knockouts and littermate controls. *Green*, β-Endorphin immunofluorescence; *fuchsia*, p110α precipitated silver grains. *Arrows* indicate neurons in which double labeling is present (*pik3r1^{loxP/loxP}* and *pik3CA^{loxP/loxP}* mice) or absent (*pik3r1 POMCKO and pik3CA POMCKO mice). *, P* < 0.05; **, *P* < 0.01.

Figure 2
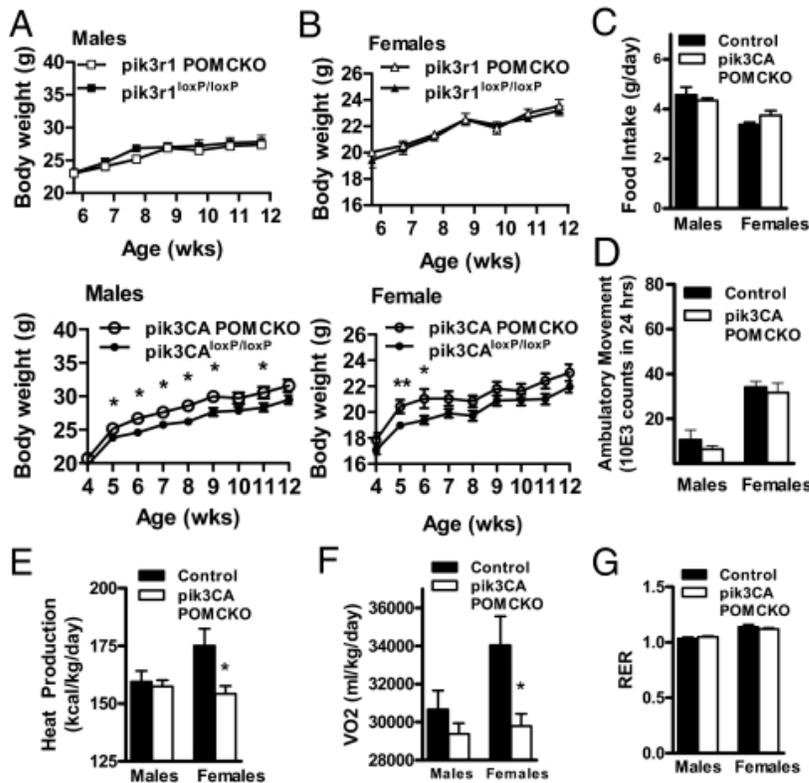
Deletion of p85α or p110α in POMC neurons impacts body weight and metabolic parameters. A, Weekly weight gain in male *pik3r1 POMCKO* mice or *pik3CA POMCKO* mice and littermate controls on normal chow ($P = 0.024$, n = 7–11). B, Weight gain in female *pik3r1 POMCKO* mice or *pik3CA POMCKO* mice and littermate controls on normal chow ($P = 0.42$, n = 7–17). Food intake (C), ambulatory movement (D), heat production (E), O_2 consumption (F), and respiratory exchange ratio (G) of 3-month-old chow-fed female *pik3r1 POMCKO* mice, *pik3CA POMCKO* mice, and their littermate controls were measured by the TSE metabolic system over a 32-h time period (n = 6–9 all groups). *, $P < 0.05$.

Figure 3
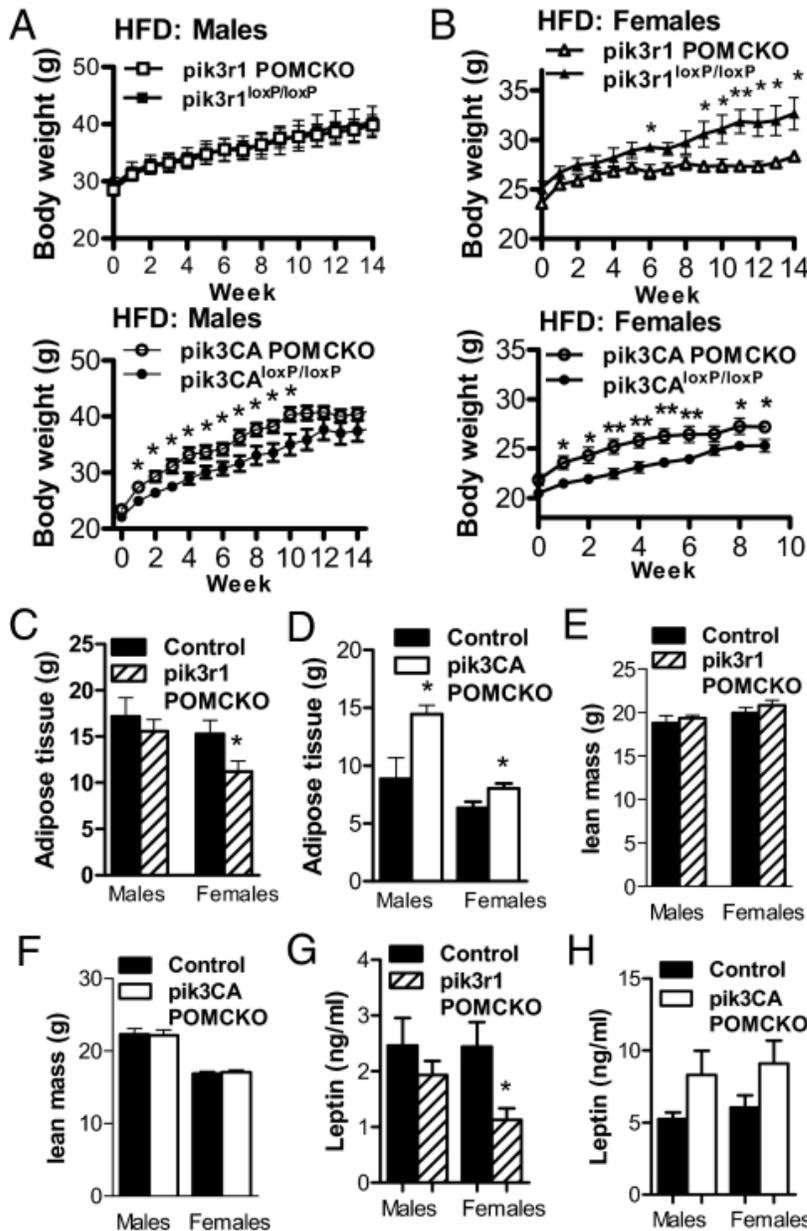
Altered body weight and adiposity in high-fat chow-fed (HFD) mice lacking p85α or p110α in POMC neurons. Weight gain in male (A) and female (B) *pik3r1 POMCKO* or *pik3CA POMCKO* mice and littermate controls were measured after introduction of high-fat chow at 3 months of age (P = 0.030 for *pik3CA POMCKO* males, P = 0.015 for *pik3r1 POMCKO* females and P = 0.008 for *pik3CA POMCKO* females; n = 7–8 male and 10–12 female cohorts). C–F, Fat and lean mass in 5-month-old *pik3r1 POMCKO*, *pik3CA POMCKO*, and littermate controls were analyzed by DEXA (*pik3r1 POMCKO* cohorts) or nuclear magnetic resonance (*pik3CA POMCKO* cohorts) (n = 6–12). G and H, Serum leptin levels were assessed by ELISA in 4-month-old *pik3r1 POMCKO, pik3CA POMCKO*, and littermate controls (n = 6–7). *, P < 0.05; **, P < 0.01.

Figure 4
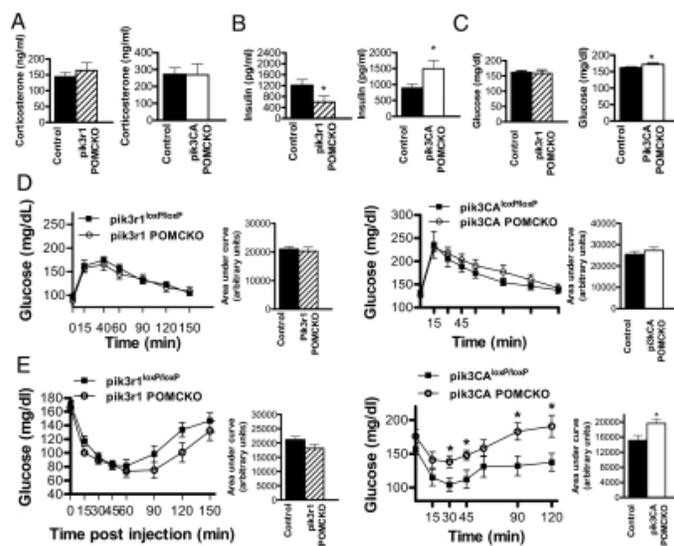
Impaired insulin responsiveness in mice lacking p110α in POMC neurons A, Corticosterone levels (nanograms per milliliter) of *pik3r1 POMCKO*, *pik3CA POMCKO*, and littermate control mice (n = 6–8), while undergoing a glucose tolerance test. Tail vein blood samples were drawn 30 min into the test. B, Serum insulin levels were measured by ELISA after removal of food for 2 h in male *pik3r1 POMCKO*, *pik3CA POMCKO*, and Cre-negative littermate controls (n = 6–10). C, Blood glucose levels were measured by glucometer after removal of food for 2 h in male *pik3r1 POMCKO*, *pik3CA POMCKO*, and littermate controls (n = 6–8). Blood glucose response curve for glucose (D) and insulin (E) tolerance tests in *pik3r1 POMCKO*, *pik3CA POMCKO*, and littermate control mice (n = 6–8) is shown. Calculated areas under the curves are displayed adjacent to each. Tolerance tests were performed on male mice with 2 g glucose/kg or 1 U of insulin/kg body weight. For all of these experiments, the mice were 3 months of age; body weight of control and experimental mice did not differ significantly. Results are expressed as mean blood glucose concentration ± SEM. *, $P < 0.05$.

Figure 5
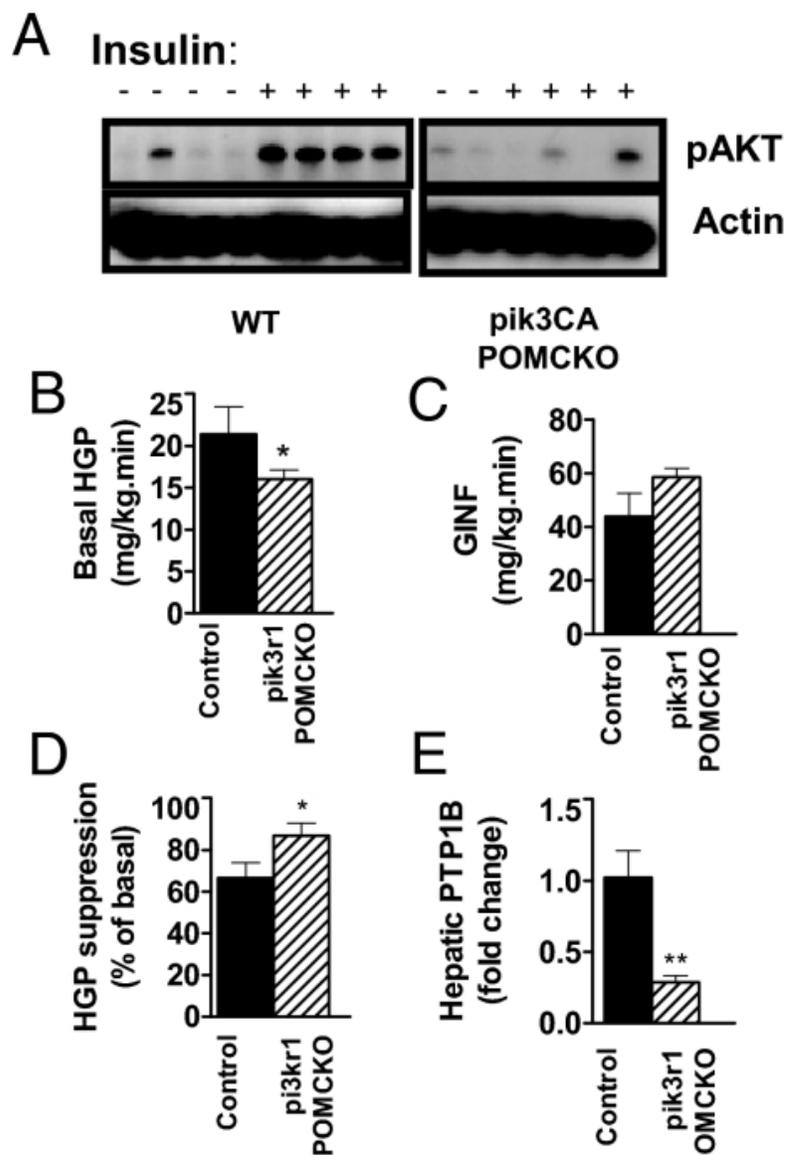
Corresponding changes in hepatic insulin sensitivity result from altered PI3K signaling in POMC neurons. A, Western blot analysis showed that phosphor-Akt (Ser-473) levels were dramatically reduced in *pik3CA POMCKO* hepatic tissue after insulin stimulation (5 U/kg ip, 10 min time point) compared with controls. Basal HGP (B) glucose infusion rate (C) and (D) suppression of HGP *in vivo* during hyperinsulinemic-euglycemic clamps in awake mice at 12–16 wk of age (*pik3r1 POMCKO* and controls, n = 8) are shown. E, PTP1B gene expression in male *pik3r1 POMCKO* and littermate controls after hyperinsulinemic-euglycemic clamp (n = 5–7). Body weights of control and experimental mice did not differ significantly. *, $P < 0.05$; **, $P < 0.01$. WT, Wild type; GINF, glucose infusion rate.