

Growth Hormone Regulation of p85 α Expression and Phosphoinositide 3-Kinase Activity in Adipose Tissue

Mechanism for Growth Hormone–Mediated Insulin Resistance

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Phosphoinositide (PI) 3-kinase is involved in insulin-mediated effects on glucose uptake, lipid deposition, and adiponectin secretion from adipocytes. Genetic disruption of the p85 α regulatory subunit of PI 3-kinase increases insulin sensitivity, whereas elevated p85 α levels are associated with insulin resistance through PI 3-kinase–dependent and –independent mechanisms. Adipose tissue plays a critical role in the antagonistic effects of growth hormone (GH) on insulin actions on carbohydrate and lipid metabolism through changes in gene transcription. The objective of this study was to assess the role of the p85 α subunit of PI 3-kinase and PI 3-kinase signaling in GH-mediated insulin resistance in adipose tissue. To do this, p85 α mRNA and protein expression and insulin receptor substrate (IRS)-1-associated PI 3-kinase activity were measured in white adipose tissue (WAT) of mice with GH excess, deficiency, and sufficiency. Additional studies using 3T3-F442A cells were conducted to confirm direct effects of GH on free p85 α protein abundance. We found that p85 α expression 1) is decreased in WAT from mice with isolated GH deficiency, 2) is increased in WAT from mice with chronic GH excess, 3) is acutely upregulated in WAT from GH-deficient and –sufficient mice after GH administration, and 4) is directly upregulated by GH in 3T3-F442A adipocytes. The insulin-induced increase in PI 3-kinase activity was robust in mice with GH deficiency, but not in mice with GH excess. In conclusion, GH regulates p85 α expression and PI 3-kinase

activity in WAT and provides a potential explanation for 1) the insulin hypersensitivity and associated obesity and hyperadiponectinemia of GH-deficient mice and 2) the insulin resistance and associated reduced fat mass and hypo adiponectinemia of mice with GH excess. *Diabetes* 56:1638–1646, 2007

Growth hormone (GH) has well-known diabetogenic effects. As early as 1931, Houssay and Biasotti (1) showed that injection of crude pituitary extracts induced diabetogenic effects in dogs. By 1949, it was confirmed that these effects were caused by GH (2). There is a 30–56% prevalence of overt diabetes in humans with acromegaly, a disease characterized by excessive GH production (3,4). The molecular mechanism(s) of GH-induced insulin resistance is not clear.

A large number of studies demonstrate that adipose tissue plays a critical role in the antagonistic effects of GH on insulin action on carbohydrate and lipid metabolism (rev. in 5). Studies showing direct actions of GH in adipose cells or tissue include: kinetic studies in pigs, which demonstrated that GH-induced insulin resistance results predominantly from reduced incorporation of glucose into adipose tissue (6,7); studies in 3T3-L1 and 3T3-F442A adipocytes showing that GH inhibits insulin action on glucose transport and lipid accumulation (5,8); and studies in human adipose tissue and adipocytes showing that GH reduces the incorporation of glucose into triglycerides (9). It has been proposed that GH-induced insulin resistance in adipocytes is caused by a mechanism involving the phosphoinositide (PI) 3-kinase signaling pathway (10).

The PI 3-kinase pathway has an essential role in insulin-induced glucose uptake and lipid metabolism in adipocytes (11), and it is also involved in the insulin-mediated increase in secretion of the adipocyte-derived hormone adiponectin (12–14). PI 3-kinase is a heterodimer consisting of one p85 regulatory and one p110 catalytic subunit, each of which occurs in multiple isoforms. p85 α and the p55 α and p50 α splice variants represent 70–80% of the total PI 3-kinase regulatory subunits in insulin-sensitive tissues, and they are encoded by the *Pik3r1* gene (15). In addition to its role as part of the PI 3-kinase dimer, the p85 α regulatory subunit is also recognized as an important

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DMEM, Dulbecco's modified Eagle's medium; GH, growth hormone; IRS, insulin receptor substrate; PI, phosphoinositide; SOCS, suppressor of cytokine signaling; TBST, Tris-buffered saline with Tween; WAT, white adipose tissue.

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negative regulator of PI 3-kinase and insulin action. This has been shown in mouse models with partial or total knockout of p85 α , which demonstrate the paradoxical phenotype of increased insulin sensitivity (16–18). The inverse correlation between p85 levels and insulin sensitivity is also applicable when this regulatory subunit is expressed at greater-than-normal levels. For instance, p85 overexpression in cells inhibited PI 3-kinase signaling and glucose uptake (19). This mechanism has been recently supported by studies of the insulin-resistant states induced by human placental GH (20), and in obesity and type 2 (21) and gestational diabetes (22).

Because studies with inhibitors of RNA and protein synthesis suggest that many (perhaps all) of the anti-insulin effects of GH involve mRNA transcription and protein synthesis (5,23,24), we investigated the effects of GH on the p85 α subunit of PI 3-kinase and its potential involvement in GH-mediated insulin resistance. Our results strongly support that GH modulates insulin action through regulation of the expression of the p85 α subunit of PI 3-kinase in adipose tissue.

RESEARCH DESIGN AND METHODS

Generation of giant transgenic mice expressing bovine GH (bGH mice) under the regulation of the metallothionein I promoter was previously described (25). Mice with this transgene show high serum bovine GH levels (25). C57BL/6J lit/lit and lit/+ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). The little (lit) autosomal recessive mutation of the GH-releasing hormone receptor causes isolated GH deficiency in lit/lit mice, whereas heterozygous lit/+ mice are GH sufficient (26). lit/lit and lit/+ mice received a subcutaneous bolus (120 ng/g body wt) of rat recombinant GH (gift from Genentech, San Francisco, CA) or vehicle (0.9% saline, 100 μ l) 4 h before death. bGH and lit mice were killed at 3 months of age using halothane. White adipose tissue (WAT) depots were snap-frozen and stored at -80°C . Male mice were used in all of the studies. Animal procedures were approved by the institutional animal care and use committees of the University of Virginia, Ohio University, and the University of Colorado Health Sciences Center.

Measurement of blood parameters. Blood was obtained by cardiac puncture. Plasma glucose and insulin were measured after a 6-h fast in lit/lit and lit/+ mice using the glucose oxidase method (YSI, Yellow Springs, OH) and a sensitive rat insulin radioimmunoassay kit (Linco Research, St. Charles, MO), respectively. Adiponectin concentrations were measured in serum from nonfasted lit/lit and bGH mice and their respective controls using a mouse adiponectin radioimmunoassay kit (Linco Research). The insulin resistance index was calculated by multiplying the fasting blood glucose level (mg/dl) by the fasting insulin level (ng/ml) (27).

Determination of adipocyte size in lit/lit and lit/+ mice. Epididymal adipose tissue removed from lit/lit and lit/+ mice was fixed in a 4% paraformaldehyde/PBS solution, embedded in paraffin, cut into 5- μ m sections, and stained with hematoxylin and eosin. Cross-sectional areas of at least 100 adipocytes per mouse, from at least seven microscopic fields, were measured using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The mean area value from each mouse was subsequently used for comparisons between groups ($n = 5$ mice per group).

Cells. 3T3-F442A cells were provided by Dr. P.J. Bertics (University of Wisconsin) and maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% calf serum and a mixture of penicillin and streptomycin. Confluent cells were differentiated by adding DMEM containing 10% fetal bovine serum (Invitrogen) and 172 nmol/l insulin (Eli Lilly, Indianapolis, IN). Medium was renewed every 2nd day. After 10 days, differentiated cells were incubated overnight in a serum-free media (DMEM with 0.1% BSA) and then treated with media with or without recombinant human GH (200 ng/ml; Genentech) at 37 $^{\circ}\text{C}$ for 3, 6, and 24 h. In experiments performed to determine the effects of GH on the abundance of free p85 α protein, 3T3-F442 adipocytes were incubated with and without 500 ng/ml rat recombinant GH for 24 h.

Real-time RT-PCR. Total RNA was extracted using Tri Reagent (Molecular Research Center, Cincinnati, OH) followed by further purification using an RNeasy mini kit (Qiagen, Valencia, CA) with on-column DNA digestion with an RNase-free DNase set (Qiagen). RNA concentrations were measured using a RiboGreen quantitation kit (Molecular Probes, Eugene, OR). Primers for the mouse Pik3r1 gene targeted exons 14 (5'-CTTGTCGGGAGAGCAGTAAGCA-3') and 15 (5'-CAGGTTGTAGGGCTCGGCAAG-3') because the COOH-termi-

nal region, encoded by nine exons (E7–E15), is common to all variants (p85 α , p55 α , and p55 α) of the Pik3r1 gene (28). Primers targeted exon 5 of the Pik3r1 gene (5'-TCATCAGTGTGGCTTACGCTTCAGTA-3') for p85 α , exon E1–55 (5'-CAGGTTTGGACTATGGAAGACCTG-3') for p55 α , and exon E1–50 (5'-TTCTCTGTGGGATACTGGCAGTTCAA-3') for p50 α . A reverse primer common to the three splice variants targeted the COOH-terminal region on exon 7 (5'-TTGTTGGCTACAGTAGTGGCTTGG-3'). Primers for the IGF-1 gene and 18S rRNA were as previously described (29).

Plasmids were constructed with the PCR products generated with the above-mentioned specific primer pairs and were cloned into the pGEM-T vector (Promega, Madison, WI) and introduced in *Escherichia coli* JM109 (Promega). From a selected transformant containing the desired construct, plasmid DNA was isolated using a Qiaprep Spin Miniprep kit (Qiagen). A serial dilution of each plasmid was used to make a standard curve for quantification. RNA reverse transcription and real-time PCR were performed as previously described (29). The obtained values (copy number) were normalized to 18S rRNA.

Western blots. Polyclonal anti-p110 α (07-658) and anti-p110 β (06-568) antibodies used for coimmunoprecipitation of p85 α , and a specific anti-p85 α monoclonal antibody (anti-PI 3-kinase p85 N-SH3 clone AB6) used for p85 α detection, were from Upstate. Tissues or 3T3-F442A adipocytes were homogenized in an ice-cold modified radioimmunoprecipitation assay buffer (50 mmol/l Tris-HCl, pH 7.4, 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS) with a protease inhibitor cocktail (Calbiochem, La Jolla, CA), followed by two cycles of centrifugation at 10,000g for 10 min at 4 $^{\circ}\text{C}$. The supernatant was removed from the centrifugation tube. Proteins were measured using a bicinchoninic acid assay reagent kit (Pierce, Rockford, IL). Equal amounts of protein (50 μ g) from each sample were mixed 2:1 with 4 \times Laemmli sample buffer, boiled for 5 min, separated on a 7.5% SDS-PAGE gel, and transferred to nitrocellulose membranes (Protran BA85; Schleicher & Schuell, Keene, NH). Nitrocellulose blots were stained with Ponceau S (0.5% in 1% acetic acid) to confirm equal protein loading, destained with Tris-buffered saline with Tween (TBST; 0.15 mol/l NaCl, 0.1% Tween 20, 50 mmol/l Tris, pH 7.6), and incubated for 1 h at room temperature with blocking buffer (5% BSA in TBST) and then incubated for an additional 1 h at room temperature with anti-p85 α antibody at 1:500 dilution. After washing three times in TBST, membranes were incubated for 2 h with a goat anti-mouse horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Bands were visualized using SuperSignal West Pico chemiluminescence substrate (Pierce) and exposed to CL-XPosure film (Pierce). Band densitometry was analyzed with an Image Master TotalLab (Amersham Pharmacia Biotech, Piscataway, NJ). To assess the effects of GH on free p85 α abundance, p85 α -p110 dimers were immunodepleted by subjecting protein lysates to three rounds of sequential immunoprecipitation with a combination of anti-p110 α (1:100) and anti-p110 β (1:100) antibodies. Immunoprecipitates were captured with ImmunoPure immobilized protein A/G (Pierce). p85 α protein remaining in the supernatant, representing the p85 monomer, was detected with an anti-p85 α antibody by Western blot.

Insulin receptor substrate-1-associated PI 3-kinase activity. PI 3-kinase activity was measured in epididymal fat from mice with 1) chronic GH deficiency (lit/lit) and their littermate controls (lit/+), 2) chronic GH excess (bGH) and their wild-type littermates, and 3) short-term GH excess (C57BL/6 wild-type mice). The latter C57BL/6, at 2–3 months of age, were injected with rat recombinant GH (1 μ g/g; National Hormone and Pituitary Program, Harbor-UCLA Medical Center, Los Angeles, CA) subcutaneously twice daily for 3 days. At the time of death, mice were fasted for 6 h and anesthetized with ketamine (150 mg/kg) and acepromazine (5 mg/kg). Their right-sided epididymal fat pad was rapidly removed and frozen immediately in liquid nitrogen. An insulin bolus of 10 units/kg body wt was then injected into the portal vein. At 5 min after injection, the epididymal fat pad from the opposite side was excised and frozen immediately. The level of insulin receptor substrate (IRS)-1-associated PI 3-kinase activity was determined as previously described (30).

Statistical analysis. Results are the means \pm SE. Unpaired Student's *t* tests and one-way ANOVAs were used for comparisons between groups as appropriate, and a nonparametric (Spearman) method was used for correlation analysis. Statistical significance was considered at $P < 0.05$.

RESULTS

Mice with chronic GH excess showed increased p85 α expression in WAT and reduced serum adiponectin. To test the hypothesis that GH modulates insulin action in WAT through regulation of Pik3r1 expression, we studied bGH transgenic mice. These mice showed increased Pik3r1 mRNA (Fig. 1A) and concomitantly increased p85 α

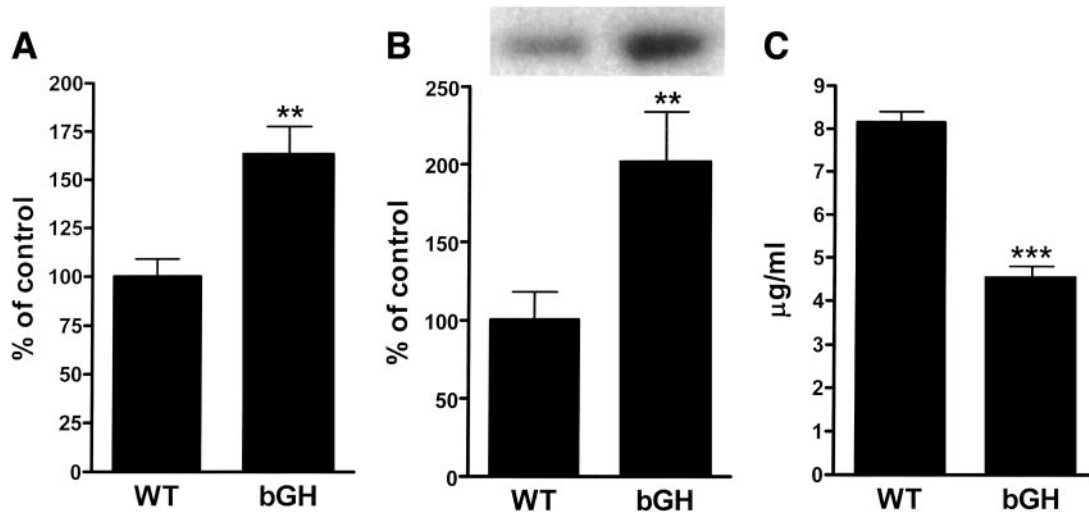


FIG. 1. Increased p85 α expression in WAT and reduced adiponectin in serum of bGH mice. **A:** Pik3r1 mRNA levels in mesenteric fat from bGH and wild-type littermates (WT). Percent values are the means \pm SE ($n = 5-6$ per group). **B:** Western blot of p85 α protein in perirenal fat of bGH mice. Experiments were performed as described in RESEARCH DESIGN AND METHODS. The upper panel shows a representative picture, and the lower panel shows the means \pm SE of arbitrary densitometry units of three independent experiments. **C:** Serum adiponectin concentrations in bGH mice. ** $P \leq 0.01$; *** $P < 0.001$.

protein levels in WAT (Fig. 1B). Circulating adiponectin concentrations are in general negatively correlated with insulin resistance and with fat mass (31). In the bGH mice that are lean but insulin resistant (32,33), adiponectin concentrations were 55% of those in their wild-type littermates (Fig. 1C).

Mice with chronic GH deficiency show reduced p85 α expression in WAT, hypoinsulinemia, hyperadiponectinemia, and adipocyte hypertrophy. p85 α protein in WAT of lit/lit mice was reduced by $\sim 60\%$ compared with lit/+ mice (Fig. 2A). Although plasma glucose concentrations were not significantly different between lit/lit and lit/+ mice (Fig. 2B), fasting insulin concentrations in lit/lit mice were 38% of those in lit/+ mice (Fig. 2C). Estimation of insulin action using the insulin resistance index (fasting blood glucose level [in mg/dl] multiplied by the fasting insulin level [in ng/ml]) (27) supports that lit/lit mice have enhanced insulin sensitivity compared with lit/+ controls ($P = 0.006$) (Fig. 2D). Similarly significant results were obtained with other commonly used formulas for insulin action estimation, including QUICKI (quantitative insulin-sensitivity check index) (34) or HOMA-IR (homeostatic model assessment-insulin resistance) (35) (not shown). The cross-sectional area of adipocytes was significantly higher in lit/lit than in lit/+ mice (947.7 ± 99.28 vs. $456.6 \pm 91.68 \mu\text{m}^2$) (Fig. 2E). Circulating adiponectin has previously been inversely correlated with adipocyte size (31); however, lit/lit mice, despite their enlarged adipocyte size, showed higher serum adiponectin concentrations compared with lit/+ mice (30.33 ± 1.36 vs. $11.28 \pm 0.97 \mu\text{g/ml}$) (Fig. 2F).

Effects of chronic GH deficiency and acute GH replacement on total Pik3r1 and its p85 α , p55 α , and p50 α splice variants in WAT. Because mice with heterozygous disruption of the Pik3r1 gene have shown increased insulin sensitivity, we explored whether the enhanced insulin sensitivity of GH-deficient mice might be related to a decreased expression of the Pik3r1 gene. lit/lit mice showed Pik3r1 mRNA levels (which included the three products, p85 α , p55 α and p50 α , of the gene) that were approximately one-half of those in their GH-sufficient lit/+ littermates (Fig. 3A). Because we found that mice

with chronic excess GH had increased p85 α expression, we analyzed the response at 4 h after an acute dose of rat recombinant GH on Pik3r1 expression. Pik3r1 mRNA levels in GH-treated lit/lit mice were 169% higher than in vehicle-treated lit/lit mice (Fig. 3A). Pik3r1 mRNA levels also increased in GH-treated lit/+ mice (Fig. 3A). To elucidate the response of each of the splice variants of the Pik3r1 gene under different levels of GH action, we measured p85 α , p55 α , and p50 α mRNA levels. p85 α , the most abundant product, showed changes very similar to those found for Pik3r1 (Fig. 3B). In contrast, p55 α (Fig. 3C) and p50 α (Fig. 3D) mRNA levels did not show significant differences between any of the studied groups. Correlation analysis between Pik3r1 and p85 α , p55 α , and p50 α mRNA levels showed, respectively, values of 0.89 ($P < 0.001$), 0.31 ($P = \text{NS}$), and 0.03 ($P = \text{NS}$) (not shown), confirming that the observed effects on total Pik3r1 expression can be accounted for by changes in p85 α expression. Because the IGF-1 gene is an established target of GH, we validated our experimental model by analyzing IGF-1 mRNA levels in the same groups of mice (Fig. 3E), and we obtained results similar to those of the Pik3r1 gene. A strong correlation between IGF-1 and Pik3r1 mRNA levels (Spearman $r = 0.79$) was found, suggesting that GH regulates both Pik3r1 and IGF-1 expression in WAT.

GH increases Pik3r1 mRNA levels in differentiated 3T3-F442A cells. To gain further insight into the possibility of a direct effect of GH on Pik3r1, we analyzed 3T3-F442A adipocytes incubated with or without GH. Pik3r1 mRNA levels increased to 191, 244, and 247% within 3, 6, and 24 h of incubation with GH, respectively (Fig. 4), supporting a direct effect of GH on Pik3r1 expression in adipose cells.

Insulin-stimulated IRS-1 PI 3-kinase activity is increased in WAT of mice with chronic GH deficiency and decreased in WAT of mice with chronic or short-term GH excess. To analyze whether the GH-induced changes in p85 α expression had concomitant effects on PI 3-kinase activity in WAT, epididymal fat from mice with diverse levels of GH action were analyzed. Whereas lit/+ and lit/lit mice showed, respectively, a 57 and 207% increase in IRS-1-associated PI 3-kinase activity in response

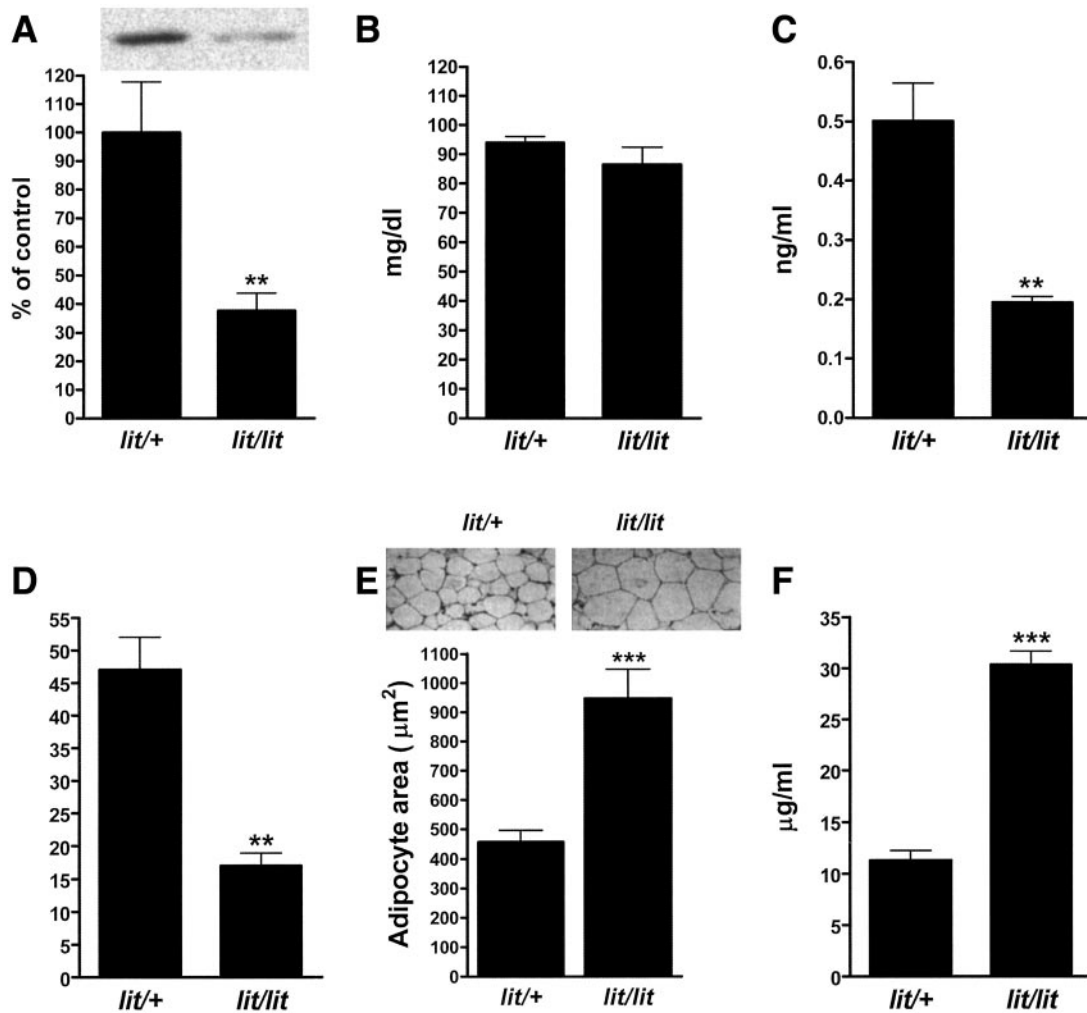


FIG. 2. Reduced p85 α protein, normoglycemia, hypoinsulinemia, hyperadiponectinemia, and increased adipocyte size in GH-deficient mice. A–F: p85 α protein in epididymal fat (A), fasting plasma glucose (B), insulin concentrations (C), insulin resistance index (glucose mg/dl \times insulin ng/ml) (D), cross-sectional adipocyte area (with representative picture in upper panel) (E), and serum adiponectin concentrations (F) in *lit/lit* compared with *lit/+* mice ($n = 5$ /group). p85 α was measured in epididymal fat protein pooled from five mice per group in three independent experiments. Values are the means \pm SE. ** $P < 0.01$; *** $P < 0.001$.

to insulin (Fig. 5A), bGH mice did not have a significant response (Fig. 5B). To assess a shorter-term effect of GH, wild-type mice were exposed to rat recombinant GH or vehicle for a period of 3 days. Although insulin significantly increased PI 3-kinase activity in wild-type mice (Fig. 5C), this effect was abrogated in mice treated for 3 days with rat recombinant GH (Fig. 5C), demonstrating a GH inhibitory effect on insulin action on IRS-1-associated PI 3-kinase activation.

Effects of GH on the abundance of the p85 α monomer. To gain further insight into the effects of GH on the abundance of the p85 α monomer, we measured p85 α protein levels in supernatants of 3T3-F442A adipocyte lysates after immunodepletion of p110 α and p110 β protein, resulting in coimmunoprecipitation of their bound p85 α protein. Free p85 α was increased by $\sim 290\%$ in GH-treated cells (Fig. 6A). Using the same technique, we found increased free p85 α in WAT from bGH mice (Fig. 6B) and decreased free p85 α in WAT from *lit/lit* mice (Fig. 6C).

DISCUSSION

GH can exert divergent actions on carbohydrate and lipid metabolism, showing acute insulin-like effects and delayed

anti-insulin effects. The insulin-like effects are observed under conditions of previous GH deprivation (36); however, these effects are short-lived, and their physiological significance remains unclear (5). In contrast, the insulin-opposing effects of GH are present after a lag period of a few hours and persist chronically if exposure to GH continues (5,36). The main hypothesis in the current study was that GH antagonizes insulin actions through regulation of p85 α expression in adipose tissue. Our results show that GH upregulates the expression of the p85 α -encoding gene *Pik3r1* both directly in adipose cells and in adipose tissue in mice. Several lines of evidence support that p85 α can reciprocally regulate insulin action; PI 3-kinase-dependent and -independent mechanisms have been proposed as explanations for these p85 α -mediated effects (rev. in 37). We found in the current study that PI 3-kinase is involved in the insulin-opposing effects of GH in WAT, as shown by the fact that the normal increase in insulin-stimulated IRS-1-associated PI 3-kinase activity was abrogated in mice exposed to exogenous GH. We observed similar changes in bGH mice, a model of lifelong GH excess, because IRS-1-associated-PI 3-kinase activity did not increase in response to insulin in these mice. The latter result confirms insulin resistance and is consistent

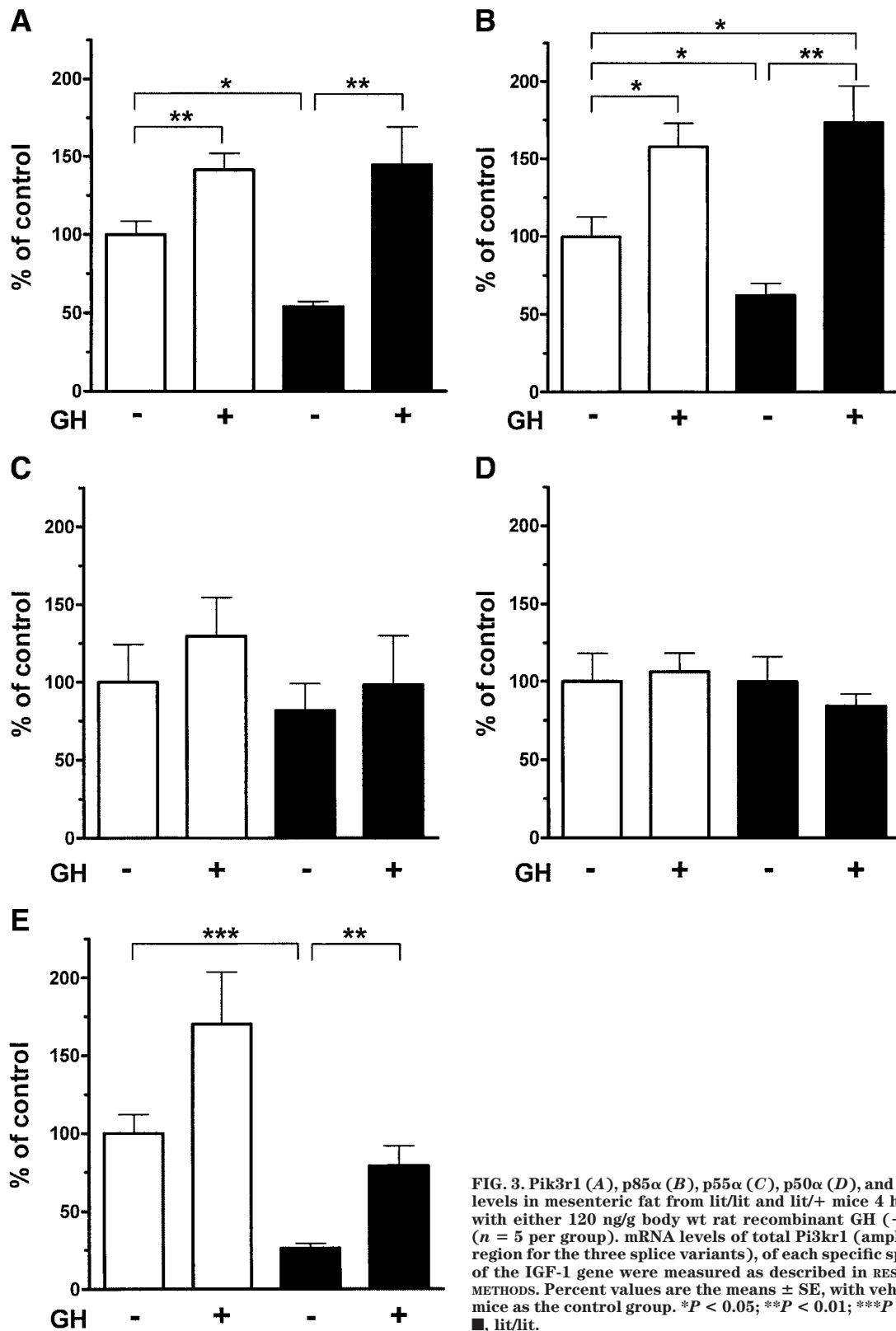


FIG. 3. *Pik3r1* (A), *p85α* (B), *p55α* (C), *p50α* (D), and *IGF-1* (E) mRNA levels in mesenteric fat from *lit/lit* and *lit/+* mice 4 h after treatment with either 120 ng/g body wt rat recombinant GH (+) or vehicle (-) ($n = 5$ per group). mRNA levels of total *Pi3kr1* (amplifying a common region for the three splice variants), of each specific splice variant, and of the *IGF-1* gene were measured as described in RESEARCH DESIGN AND METHODS. Percent values are the means \pm SE, with vehicle-treated *lit/+* mice as the control group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. □, *lit/+*; ■, *lit/lit*.

with a previous analysis of the same strain of bGH mice showing an 80% decrease in insulin-simulated glucose uptake in WAT (33). Mice with GH excess demonstrate dissociation between body mass and insulin sensitivity because, despite being lean (32) and resistant to high-fat diet-induced obesity (38), they are severely insulin resistant (33). Because PI 3-kinase is involved in upregulation

of glucose uptake and lipid deposition in adipocytes (11,39), an increase of *p85α*, a negative regulator of insulin action, specifically in adipose tissue potentially explains the dissociation between body mass and insulin resistance in mice with GH excess.

Insulin hypersensitivity in hypophysectomized animals was described in the 1920s (1). Mice with chronic GH

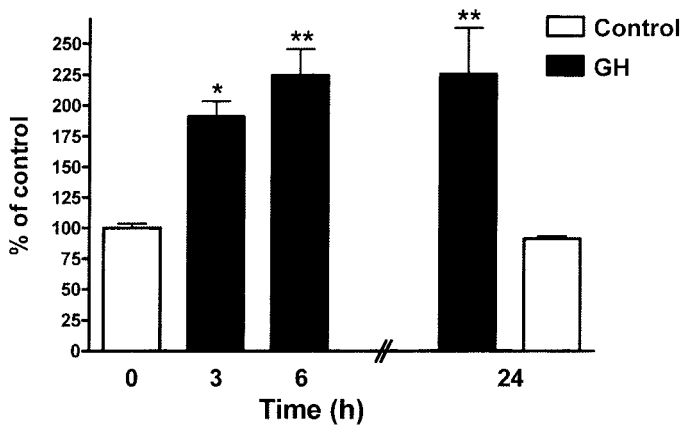


FIG. 4. In vitro regulation of Pik3r1 mRNA levels by GH. 3T3-F442A cells were induced to differentiate in DMEM, 10% fetal bovine serum, and 172 nmol/l insulin. After 10 days, a serum-free media was used overnight, and cells were incubated with or without recombinant human GH (200 ng/ml) for 3, 6, and 24 h. Percent values are the means \pm SE ($n = 3$ per time point) of the time 0 reference control. * $P < 0.05$; ** $P < 0.01$.

deficiency (40,41) and mice with genetically reduced p85 α show insulin hypersensitivity (18). We questioned whether the enhanced insulin sensitivity of GH-deficient mice was related to a decrease in p85 α expression in WAT. mRNA levels of the Pik3r1 gene (in a region amplifying not only p85 α but also the splice forms p55 α and p50 α) were decreased in WAT of lit/lit mice. Further real-time RT-PCR analysis in lit/lit and lit/+ mice treated with rat recombinant GH or vehicle demonstrated that the observed effects of GH action on Pik3r1 were accounted for by changes in its most abundant isoform, p85 α . Consistent with changes in p85 α mRNA, lit/lit mice showed reduced p85 α protein levels and a substantial (>200%) increase in insulin-induced IRS-1-associated PI 3-kinase activity. The increased insulin sensitivity found in mice with GH deficiency occurs, despite an increase in adiposity (32,40,41). A previous study in lit/lit mice showed that body fat accounted for 52% of body weight at age 12 months versus 25% in lit/+ mice (26), and we found that adipocytes of lit/lit mice are hypertrophic. As mentioned above, PI 3-kinase is involved in upregulation of glucose

uptake and lipid deposition in adipose tissue; therefore, a decrease in p85 α in this tissue provides a potential explanation for the dissociation between increased insulin sensitivity and adiposity in GH deficiency. This notion is consistent with results from a recent study where liver IGF-1-deficient (LID) mice—having increased plasma GH because of lack of IGF-1 negative feedback on GH secretion—were crossed with transgenic mice expressing a GH antagonist (GHa) to block GH action. The resulting LID + GHa mice showed improved total body insulin sensitivity and increased insulin-stimulated glucose uptake in WAT and muscle, despite a twofold increase in adipose tissue mass (42). We propose that the dissociation between insulin sensitivity and adiposity in mouse models with altered GH action is best explained by direct effects of GH action in adipose tissue. This concept is consistent with the physiological role of GH in switching energy away from adipose tissue (through decreased lipogenesis and increased lipolysis), resulting in increased lipid oxidation and preservation of body protein (43). Do the adipocytes of GH-deficient animals reach a limit in their capacity to accumulate energy that would result in reduction of insulin sensitivity? We did not investigate this question; however, increased insulin sensitivity, despite obesity, is also observed in children with GH deficiency, an effect that diminishes with increasing age; paradoxically, GH-deficient adults demonstrate insulin resistance even before GH replacement therapy (43,44). Of note, too, is a recent study in lit/lit mice in which insulin sensitivity was analyzed up to 18 months of age; at age 12 months, lit/lit mice still had greater insulin sensitivity than control mice, but by age 18 months, they showed a blunted response to insulin (45).

A larger adipocyte size has been related with reduced serum adiponectin concentrations (31); this was not the case for lit/lit mice because, despite their enlarged adipocyte size, they showed hyperadiponectinemia. Because PI 3-kinase has been implicated in upregulation of adiponectin secretion in 3T3-L1 adipocytes (12–14), it is possible that decreased p85 α in WAT (accompanied by greater responsiveness to insulin in PI 3-kinase activity) contributes to the increased serum adiponectin concentrations found in lit/lit mice. In the case of bGH mice, an increase

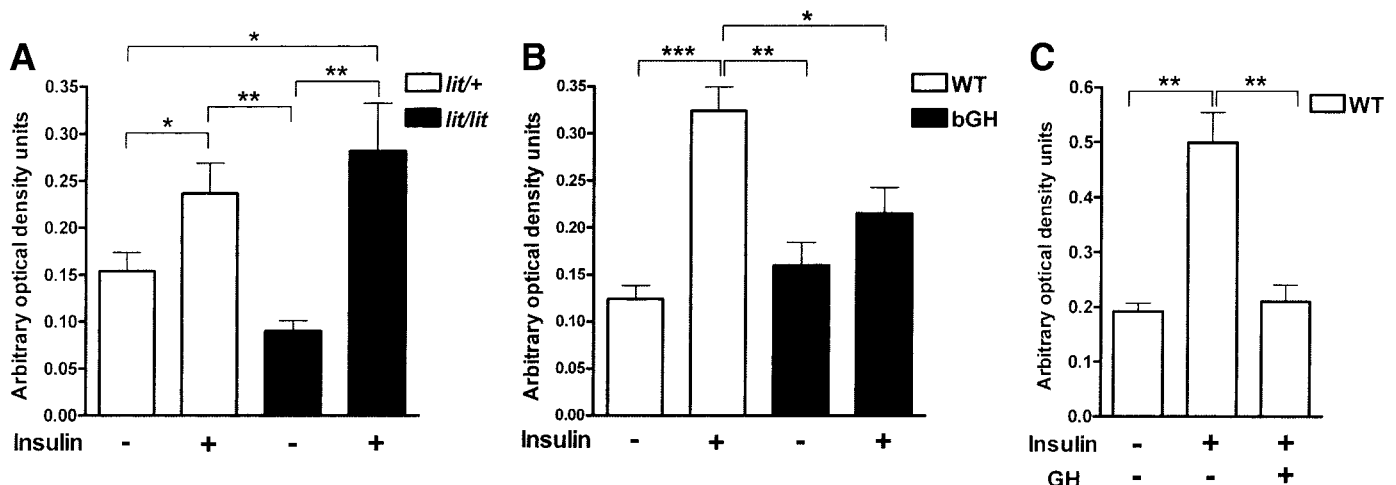


FIG. 5. Insulin-stimulated IRS-1-associated PI 3-kinase activity in epididymal fat from lit/lit and lit/+ mice (A), bGH mice and their wild-type littermates (B), and wild-type mice treated with rat recombinant GH (1 μ g/g twice daily) or vehicle for 3 days (C) was determined as described in RESEARCH DESIGN AND METHODS, analyzing individually (unpooled samples) four to seven mice per group. Densitometry values are the means \pm SE in arbitrary units. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. WT, wild type.

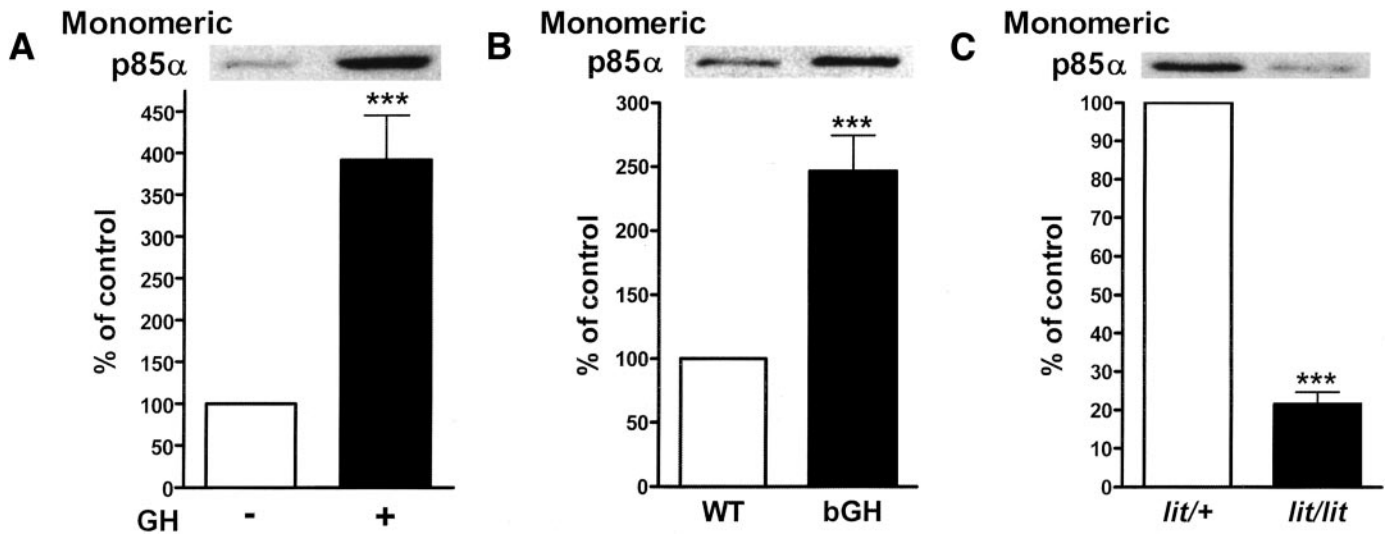


FIG. 6. Regulation of free p85 α protein by GH action in vitro and in vivo. p110-p85 α dimers were immunoprecipitated with anti-p110 α and anti-p110 β antibodies and the remaining p85 α in the supernatant was analyzed by Western blot. **A:** 3T3-F442A adipocytes treated with GH. Cells differentiated as described in RESEARCH DESIGN AND METHODS and Fig. 4 were incubated for 24 h with or without rat recombinant GH (500 ng/ml, $n = 4-5$ culture dishes per group). **B:** Perirenal fat from bGH mice and their wild-type littermates ($n = 6-9$ mice/group). **C:** Epididymal fat from lit/+ and lit/lit mice ($n = 5-6$ mice per group). Values in **A**, **B**, and **C** are the means \pm SE expressed in percent from three independent experiments performed with protein pools. Representative pictures are shown in the upper panels. *** $P \leq 0.001$. WT, wild type.

in p85 α in WAT (with a resultant decrease in PI 3-kinase responsiveness to insulin) might contribute to the reduced adiponectinemia. Consistent with our results in the lit/lit

Pik3r1 (p85 α ^{-/-}) (16) placed on a high-fat diet developed greater adipocyte size than their littermates but yet showed higher adiponectin in WAT (and greater insulin sensitivity) compared with their wild-type littermates on

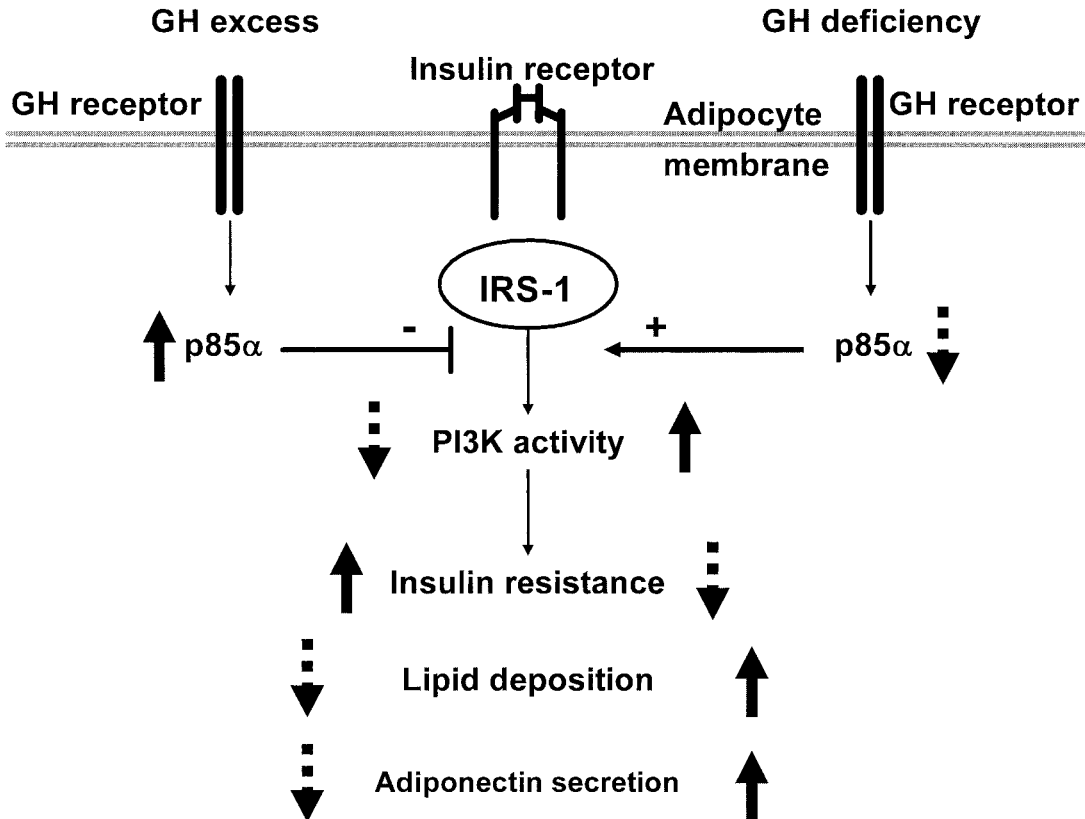


FIG. 7. Schematic representation of one proposed model illustrating modulatory effects of GH on insulin action through regulation of p85 α expression in WAT. p85 α has been extensively studied and shown to reciprocally regulate PI 3-kinase signaling (16,18,19,27,50). Increased levels of GH lead to increased expression of p85 α , decreasing insulin-stimulated IRS-1-associated PI 3-kinase activity. The latter is a sine qua non of insulin resistance and may also lead to decreased lipid deposition and adiponectin secretion. GH deficiency leads to a decline in p85 α expression, strong insulin-stimulated IRS-1-associated PI 3-kinase activity, and increased insulin sensitivity (decreased insulin resistance), and it may also lead to increased lipid deposition and enhanced adiponectin secretion. Thick arrows indicate either an increase (continuous arrow) or decrease (dashed arrow).

the same diet (27). We acknowledge that other factors beside adiponectin secretion, including adiponectin turnover, determine serum adiponectin concentrations and may be playing a role in these mouse models (46).

Besides changes in adipocytokines (e.g., adiponectin) (47), other mechanisms have been proposed to explain the modulatory effects of GH on insulin (5,48), including upregulation of suppressor of cytokine signaling (SOCS) proteins (e.g., SOCS-3). However, a role for SOCS is still undefined. It was suggested that SOCS-3 may be involved in the termination of insulin-like effects in 3T3-F442A cells incubated with GH, but because of its transient expression and rapid turnover, it is unlikely to play a relevant role in GH-mediated insulin resistance (24).

Because it has been reported that insulin per se can induce expression of p85 α (49), we analyzed the effect of GH in 3T3-F442A adipocytes in the absence of insulin; the resulting rapid and sustained increase in expression of the p85 α gene supports a direct effect of GH in adipocytes. Additional experiments to measure the abundance of the p85 α monomer further confirmed that GH per se induces p85 α protein expression. Monomeric or "free" p85 α protein (p85 α not bound to the p110 catalytic subunit of PI 3-kinase) is the p85 fraction more specifically linked with insulin resistance (37). In experiments performed to gain further insight into the direct effects of GH on p85 α in adipose cells, we exposed 3T3-F442A adipocytes to rat recombinant GH for 24 h and analyzed the amount of p85 α in lysate supernatants after three rounds of immunoprecipitation with anti-p110 α and anti-p110 β antibodies. The amount of p85 α monomer was markedly increased in GH-treated compared with control cells, thus demonstrating direct upregulation by GH of this critical negative regulator of insulin action.

Taken together, our results demonstrate that GH regulates the expression of p85 α , a critical modulator of insulin action. GH-deficient mice showed decreased p85 α expression and a concomitant increase in insulin-induced IRS-1-associated PI 3-kinase activity, whereas mice with GH excess showed increased p85 α levels and a concomitant suppression of the IRS-1-associated PI 3-kinase activity response to insulin. The GH-induced upregulation of p85 α expression may explain the GH-induced insulin resistance and reduced fat mass found in mice with GH excess. A schematic representation of a proposed model explaining GH effects on insulin action in adipose tissue is shown in Fig. 7. In the context of previous studies showing that genetic manipulations of Pik3r1 result in profound effects on insulin action (16–18,27,50), the results of the current study strongly support a role for regulation of p85 α expression in WAT as a mechanism involved in the insulin hypersensitivity of GH deficiency and in GH-mediated insulin resistance.

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