Induction of G\(_{\alpha_q}\)-specific Antisense RNA in vivo Causes Increased Body Mass and Hyperadiposity*

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Transgenic BDF-1 mice harboring an inducible, tissue-specific transgene for RNA antisense to G\(_{\alpha_q}\), provide a model in which to study a loss-of-function mutant of G\(_{\alpha_q}\) in vivo. G\(_{\alpha_q}\)-deficiency induced in liver and white adipose tissue at birth produced increased body mass and hyperadiposity within 5 weeks of birth that persisted throughout adult life. G\(_{\alpha_q}\)-deficient adipocytes display reduced lipolytic responses, shown to reflect a newly discovered, \(\alpha_q\)-adrenergic regulation of lipolysis. This \(\alpha_q\)-adrenergic response via phosphoinositide hydrolysis and activation of protein kinase C is lacking in the G\(_{\alpha_q}\) loss-of-function mutants in vivo and provides a basis for the increased fat accumulation.

Heterotrimeric G-proteins (G-proteins)\(^1\) mediate transmembrane signaling from a populous group of cell-surface receptors to a lesser group of effector molecules that includes adenyl cyclase, phospholipase C, and various ion channels (1). G-proteins have been shown to regulate complex biological processes, including cellular differentiation (2, 3), neonatal development (4–6), and oncogenesis (7). The expression of G\(_{\alpha_q}\), for example, is highly localized to the growth cones of developing neurites (2). Suppression of G\(_{\alpha_q}\) expression provokes the collapse of developing growth cones (8), whereas expression of constitutively active mutants of G\(_{\alpha_q}\) promote increased expression of neurites (9). In adiogenesis of 3T3 L1 embryonic fibroblasts, G\(_{\alpha_q}\) acts as a suppressor (2). Inducers of differentiation stimulate a sharp decline in G\(_{\alpha_q}\) levels, and constitutive expression of G\(_{\alpha_q}\) blocks induction of differentiation (2). G\(_{\alpha_q}\) has been shown to regulate the progression of embryonic stem cells to primitive endoderm (4), acting via phospholipase C (PLC) and protein kinase C to suppress progression (10). The morphogen retinoic acid induces primitive endoderm by stimulating a sharp decline in G\(_{\alpha_q}\) (4). Mimicking the decline with oligodeoxynucleotides antisense to G\(_{\alpha_q}\) provokes progression in the absence of retinoic acid (4, 10). Study of G-proteins in vivo is a formidable task. The role of G\(_{\alpha_q}\) in vivo has been studied through inducible, tissue-specific ablation by antisense RNA (5, 6) and gene inactivation by homologous recombination (11). Deficiency in G\(_{\alpha_q}\) leads to a runted phenotype (5, 6, 11), insulin resistance (12), and for the transgenic mice with the inactivated G\(_{\alpha_q}\) gene, ulcerative colitis and adenocarcinoma of the colon (11).

Little is known about the role of G-proteins of the G\(_{\alpha}\) family in vivo. Two highly homologous members of the G\(_{\alpha}\) subfamily of G-proteins, G\(_{\alpha_q}\) and G\(_{\alpha_i}\), can stimulate PLC and are insensitive to pertussis toxin (13–17). G\(_{\alpha_i}\) have been shown to mediate growth in fibroblasts in response to bradykinin and thrombin (18), hypertrophy in cultured neonatal ventricular myocytes (19), and transformation in NIH 3T3 cells (20). In the current work, we employ conditional, tissue-specific expression of RNA antisense to G\(_{\alpha_i}\) in transgenic mice to explore the role of this G-protein in vivo and more specifically in white adipocytes made deficient of G\(_{\alpha_i}\).

EXPERIMENTAL PROCEDURES

Reagents and Supplies—[\(^3\)H]cyclic AMP, [\(\gamma\)-\(^32\)P]dCTP, [\(^3\)H]inositol 1,4,5-trisphosphate (IP3), Gene Screen Plus, and anti-G\(_{\alpha_i}\) antibodies (EC2) were purchased from Sigma or standard suppliers (5). Mice—The B6D2F1 (BDF1) strain of mice was purchased from Tac tonic Farms Inc. and handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee at the State University of New York at Stony Brook.

Experimental Design of the Antisense RNA Strategy—The pPKC-ASG\(_{\alpha_q}\) expression vector was constructed as described below using standard techniques. In order to insert the antisense sequence at the BgII site within the first exon of the phosphoethanolamine corticosteroidase (PEPCK) gene, the 7.0-kb PEPCK gene was subcloned as a 1.0-kb EcoRI/HindIII and a 5.8-kb HindIII/BamHI fragment into the vector pGEMZ/f (Promega). The vector harboring the 1.0-kb gene fragment was digested with BgII, and the restriction ends were made flush using the Klenow fragment. The 39-bp antisense sequence was obtained within a 235-bp Nhel/SstI fragment excised from the vector pNC-AAG\(_{\alpha_q}\) (4). The restriction ends of this fragment were filled-in, ligated with the BgII-digested gene fragment, and used to transform XL-1 Blue strain of Escherichia coli (Stratagene) under selection with ampicillin. Plasmids with the 1.2-kb fragment and an insert oriented to produce antisense RNA were identified by direct DNA sequencing. The 1.2-kb fragment containing the antisense sequence was digested with SacII and CiaI (sites present in the 235-bp sequence harboring the antisense sequence), and a 59-bp oligomer containing the sense sequence to G\(_{\alpha_q}\) (\(\sim\)33 to +3) flanked by restriction enzyme sites for BcII (5) and SacI (3) was ligated via force cloning into the SacII and CiaI sites. Insertion of the oligomer was confirmed by restriction digest analysis with BcII and SacI. The presence of unique restriction sites within the 235-bp fragment facilitates the removal and insertion of different antisense sequences in a cassette-like fashion. The 1.2-kb fragment containing the antisense sequence was excised and ligated into the plasmid harboring the 5.8-kb gene fragment to produce the 7.9-kb pPKC-ASG\(_{\alpha_q}\) construct. In addition, the synthesis of primers complimentary to the flanking ends of the 235-bp insert allows for discrimination between the pPKC-ASG\(_{\alpha_q}\) RNA and the endogenous PEPCK RNA in subsequent reverse transcription-PCR amplification reactions (see below).

In choosing the antisense RNA target sequence, we had to consider...
the large degree of nucleotide identity among the G-protein α-subunits within their respective open reading frame regions. This prompted us to look for unique target sequences within the 5′- and 3′-untranslated regions of the Goq mRNA. The 36 nucleotides immediately upstream of and including the translation initiation codon were chosen to target the sequence. The 5′-untranslated sequence (5′-CGCGC- CGGCCGGGTGCAGCAGGACACTTCCGAAAGATG-3′) did not show any significant homology with sequences present in the GenBank™ data base, including Goq1 (33% homology) and Goq14 (27% homology).

Cell Culture and Transfection—FTO-2B cells were cultured in a 5% CO₂, 85% O₂ chamber and maintained in Ham's F-12/Dulbecco's modified Eagle's medium (1:1) supplemented with 10% fetal bovine serum. Cells were transfected with DNA in a 5-fold excess taken relative to the plasmid carrying the selectable marker. Transfection was carried out using the Lipofectin reagent (Life Technologies, Inc.) according to the manufacturer's protocol.

Detection of Antisense RNA Expression—Total RNA was extracted as described previously (4). One microgram of total RNA was reverse transcribed using a pCCK-ASGoq-specific downstream primer and then PCR-amplified in both the upstream and downstream primer set according to the manufacturer's protocol (Perkin-Elmer). The sequences for the upstream and downstream primers were dCGCTTATGTAACCCCTAGA and dAGGTTGGGTCTTATCCCC, respectively.

Production of Transgenic Mice—Transgenic lines of mice were produced at the Transgenic Mouse Facility at SUNY Stony Brook using standard techniques (5, 6). Briefly, the pCCK-ASGoq construct was excised free of vector sequences and purified prior to microinjection into single-cell preimplantation embryos. Microinjected embryos were then transferred to pseudopregnant females. Offspring carrying the transgene were identified by PCR amplification and subsequent Southern analysis using a pCCK-ASGoq-specific probe uniformly labeled with [32P]dCTP (5). Five separate founder lines were identified by Southern analysis and bred over 10 generations (5).

White Adipocyte Isolation—White adipocytes were isolated from epididymal and perirenal pads by collagenase digestion, as described previously (5). Briefly, 0.5–1.0 g of adipose tissue was excised from male and female mice, weighed, and added to an equal volume of Krebs-Ringer phosphate buffer (KRP) containing 3% bovine serum albumin (KRP/BSA), prewarmed to 37 °C. The tissue was digested for 1 h using collagenase (1 mg/ml) at 37 °C in an orbital, shaking water bath. The isolated adipocytes were washed twice with the KRP/BSA buffer and resuspended to a final volume to achieve 62.5 mg of total wet weight of packed adipocytes/ml in the same buffer. The KRP/BSA buffer was supplemented with adenosine deaminase at a concentration of 0.5 unit/ml.

Cyclic AMP Accumulation and Lipolysis—Briefly, collagenase-digested white fat cells from epididymal and epoophoronal pads were incubated at 37 °C in KRP buffer supplemented with 3% bovine serum albumin and DNAse (5 units/ml) for 30 min at room temperature. After the 30 min incubation period, the adipocytes were harvested by centrifugation and resuspended in fresh KRP buffer (50 μl/ml) and incubated for 20 min at 37 °C. Lipolysis was determined by measuring the release of glycerol into the medium as described (4, 5).

RESULTS AND DISCUSSION

Defining the role of a specific G-protein subunit, like Goq in vivo is a formidable task. We adopted the strategy of conditional, antisense RNA to ablate Goq in vivo in a tissue-specific manner, creating loss-of-function mutants in adipose and liver, prominent sites of Goq expression. The degree of nucleotide identity among the G-protein α-subunits within the open reading frames dictated selection of the 5′-untranslated region immediately upstream and including the ATG initiator codon (33–3 to +3) as the antisense RNA sequence targeting Goq (Fig. 1). This region is unique with respect to sequences with the GenBank™ data base and does not share significant homology with other G-protein α-subunits (Fig. 1A), including other members of the Gq family, Goq1 (33% homology with respect to Goq) and Goq14 (27% homology with respect to Goq). A double-stranded oligodeoxynucleotide fragment antisense to Goq was inserted into BclI and SalI sites of the pCCK-AS vector (Fig. 1B), an inducible expression vector driven by the promoter of the PEPCk gene (5, 6). Screening of FTO-2B hepatoma cell lines transfected with pCCK-ASGoq was performed from day 0 to day 12 following induction of the promoter with the chlorophenothio analogue of cyclic AMP (CPT-cyclic AMP, 25 μM). Immunoblots reveal that levels of Goq12, Goq14, and Gβδ2 were unaffected by induction of pCCK-ASGoq, while staining with an antibody to Goq displayed a 54% loss by day 6, and 86% loss of Goq by day 9 following induction (Fig. 1C). By day 12 of the induction with CPT-cyclic AMP, Goq was not detectable in the immunoblots of FTO-2B cell membranes. Induction of the pCCK-SGoq vector, harboring the sense as compared to antisense sequence for Goq, resulted in a null phenotype, i.e. Goq expression was normal. Goq activates PLC-β in the liver (12–16) and suppression of Goq in FTO-2B hepatoma cells reduced basal PLC activity from 2.7 ± 0.6 to 1.0 ± 0.3 (p ≤ 0.05 for difference) and abolished PLC stimulation in response to either 10 nM angiotensin II (0.9 ± 0.3) or 10 μM norepinephrine (0.95 ± 0.2), as determined by mass assay of intracellular IP3 accumulation at 30 s following hormonal stimulation (n = 5, pmol of IP3 accumulation/μg of cellular protein).

The pCCK-ASGoq construct was excised as a 7.0-kb EcoRI-BamHI fragment, microinjected into single cell, preimplantation embryos, and the microinjected embryos were transferred into pseudopregnant recipients. BDF1 mice harboring the transgene were identified by PCR of tail DNA. Five independent founders were identified from two rounds of microinjection and implantation. Five separate founder lines have been propagated for more than 10 generations. Immunoblots of crude membranes from fat, liver, brain, and lung subjected to SDS-PAGE and stained with a Goq-specific antisem reveal the near absence of Goq in the tissues, fat and liver, targeted by the transgene (Fig. 1D). Immunoblots of brain and lung, tissues not targeted by the pPEPCk vector, displayed normal levels of Goq (Fig. 1E). Expression of Goq12, Gβδ2, and Goq14 (not shown) were not significantly altered in the transgenic mice.

Necropsy and histology of the transgenic mice were performed. Prominent was the increase in body weight observed in the mice harboring the pCCK-ASGoq transgene (Fig. 2A). By 5 weeks of age, the transgenic mice were >135% of the body weight of their control littermates, for both male and female
**Fig. 1. Ga<sub>q</sub> expression is suppressed in hepatoma cell stably transfected with pPCK-ASG<sub>α</sub> and in mice harboring the pPCK-ASG<sub>α</sub> transgene.**

Comparison of the 5'-untranslated region from Ga<sub>q</sub> (nucleotides -33 to +3) with Ga<sub>11</sub> and Ga<sub>12</sub>, additional members of the Ga family (panel A). The pPCK-ASG<sub>α</sub> construct for inducible expression of RNA antisense to Ga<sub>q</sub> (panel B). The 36-nucleotide sequence upstream of and including the translation initiation codon was inserted into the first exon of the rat phosphoenolpyruvate carboxykinase gene (PCK) to provide a 2.9-kb hybrid pPCK-ASG<sub>α</sub> antisense RNA, driven by a promoter which is silent in utero and activated at birth. Crude membranes (0.2 mg of protein/SDS-polyacrylamide gel electrophoresis lane) were prepared from rat hepatoma FTO-2B cells that were stably transfected with the pPCK-ASG<sub>α</sub> construct and induced with CPT-cyclic AMP for 0, 6, 9, and 12 days, subjected to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose blots, and probed with rabbit polyclonal antisera specific for the G-protein subunits tested between control and transgenic mouse tissues, with the exception of the loss of Ga<sub>q</sub> in liver and fat tissues. Expression of Ga<sub>q</sub> was normal in liver and fat, although reduced (<15%) occasionally in fat, but not liver, of some transgenic mice (not shown). Scanning densitometry values for immunoblots from brain and lung revealed no significant differences in the values obtained with tissues from transgenic mice compared to control mice (not shown). The antibodies employed for staining of immunoblots for specific G-protein subunits were as follows: E973 for Ga<sub>q</sub>, CM112 for Ga<sub>11</sub>, CM129 for Ga<sub>12</sub>, E976 for Ga<sub>α</sub>; and CM162 for G<sub>β</sub>.

mice alike (Fig. 2B). Progeny of the five founder lines harboring the transgene all display the increased body weight (not shown). The fat mass at 4 weeks after birth increased by 50% in the transgenic mice (Fig. 2C). At 8 weeks of age, the white epididymal and epophoronal fat mass of the transgenic mice was 1.75-fold greater than that of the control mice. Segregated by gender for males, white fat mass (mg) was 215 ± 5 and 333 ± 8 (n = 5, p ≤ 0.05) for 12-week-old control and transgenic mice, respectively. For females, white fat mass was 160 ± 10 mg and 303 ± 8 mg (n = 6, p ≤ 0.05) for 12-week-old control and transgenic mice, respectively. By 24 weeks, the transgenic mice displayed a 1.4-fold increase in fat mass, and the percentage of whole body weight as fat mass was 2.3 ± 0.2 as compared to 1.1 ± 0.3 (n = 6) for control mice (Fig. 2C). Total body protein and nasal-anal length were unaffected by the presence of the transgene over this same range in age (not shown). Equally notable was the dramatic increase in adiposity, i.e. fat cell number, that occurred in the transgenic mice lacking Ga<sub>q</sub> expression in adipose tissue (Fig. 2D).

To assess the effects of Ga<sub>q</sub> deficiency on cell signaling, we investigated the adipocytes isolated from transgenic mice and their control littermates. PLC-β signaling by loss-of-function Ga<sub>q</sub>-deficient white adipocytes was virtually abolished, i.e. IP<sub>3</sub> and DAG accumulation in response to norepinephrine, vasopressin, phenylephrine, or bradykinin (all hormones that activate PLC) was markedly attenuated (Fig. 3, A and B, respectively). Suppression of Ga<sub>q</sub> in adipocytes of the pPCK-ASG<sub>α</sub> mice and the stably transfected hepatoma cells abolished PLC activation by a variety of hormones (Figs. 1 and 3). This loss of signaling in Ga<sub>q</sub> deficiency occurs, although expression of the Ga<sub>11</sub> subunit was found to be normal (not shown). Both Ga<sub>q</sub> and Ga<sub>11</sub> are expressed in a number of tissues (12–14), including fat and liver. The observations from the present study suggest that Ga<sub>q</sub> and Ga<sub>11</sub> may not be redundant with respect to PLC activation in vivo.

Since PLC activation and accumulation of either IP<sub>3</sub> or DAG have not been implicated in controlling lipolysis, the pharmacology of the lipolytic response observed in the Ga<sub>q</sub>-deficient adipocytes came as a great surprise (Fig. 3C). The lipolytic response to a mixed α- and β-adrenergic agonist norepinephrine for 0, 6, 9, and 12 days, subjected to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose blots, and probed with rabbit polyclonal antisera specific for the G-protein subunits indicated (5, 6). Immunoblots were made visible with goat anti-rabbit IgG coupled to calf alkaline phosphatase and colorimetric development (5, 6). Immunocomplexes were made visible with goat anti-rabbit IgG coupled to calf alkaline phosphatase and colorimetric development.
rine was blunted in the \(G_{\alpha q}\)-deficient adipocytes. Lipolysis in response to the \(\beta\)-adrenergic agonist isoprenaline was impaired, whereas the response to the \(\alpha_1\)-adrenergic agonist phenylephrine was abolished in the loss-of-function mutant cells. These results were unexpected, since neither a direct role of \(G_{\alpha q}\) in activating adenylyl cyclase nor the existence of a prominent \(\alpha_1\)-adrenergic stimulation of lipolysis have been reported. Analysis of cyclic AMP accumulation provided direct proof linking loss of \(G_{\alpha q}\) to impaired lipolysis in response to \(\beta\)-adrenergic stimulation (acting via adenylyl cyclase) as well as to \(\alpha_1\)-adrenergic stimulation (acting via PLC). Forskolin (10 \(\mu M\))-stimulated cyclic AMP accumulation, in contrast, was actually elevated in the \(G_{\alpha q}\)-deficient as compared to control adipocytes (125 \pm 15 and 160 \pm 5 pmol/10^6 cells, respectively). Forskolin (10 \(\mu M\))-stimulated lipolysis was equivalent in transgenic and control mice (14.1 \pm 2.9 and 13.9 \pm 0.8 \(\mu M\) of glycerol release/10^6 cells, respectively), as were the abundance of \(\beta\)-adrenergic receptors (140 \pm 4 and 133 \pm 9 fmol/mg of protein, respectively) in crude adipocyte membranes and the amounts of cyclic AMP phosphodiesterase activity (1.33 \pm 0.02 and 1.32 \pm 0.09 pmol/min/mg of protein, respectively) in extracts of whole fat pads.

In the \(G_{\alpha q}\)-deficient cells, the impaired lipolytic response stimulated by norepinephrine was sensitive to the \(\beta\)-adrenergic antagonist propranolol, reflecting a residual \(\beta\)-adrenergic, cyclic AMP-mediated response (Fig. 4A). Adipocytes from control mice display sensitivity to both propranolol and the \(\alpha_1\)-adrenergic antagonist prazosin. The former reflects the \(\beta\)-adrenergic response acting via cyclic AMP, while the latter reflects this newly discovered \(\alpha_1\)-adrenergic response first detected through its loss in the \(G_{\alpha q}\)-deficient cells. Vasopressin (1 \(\mu M\)), which activates PLC, also stimulated lipolysis in adipocytes from control mice 1.8-fold over basal. The \(\alpha_1\)-adrenergic stimulation of lipolysis was abolished by prazosin, but not by...
propranolol (Fig. 4B). The loss-of-function $G_{q}$-deficient cells, in contrast, have essentially lost the lipolytic response to phenylephrine stimulation.

Although $G_{q}$ is not known to regulate adenyl cyclase directly, the loss-of-function $G_{q}$ mutants displayed impaired $\beta$-adrenergic stimulation of cyclic AMP accumulation and lipol-
Gaq, acting via PLC to promote IP3 and DAG accumulation, may augment the cyclic AMP response indirectly, perhaps via effects on calcium- or protein kinase C-sensitive forms of adenylylcyclase (22–25). We tested the role of protein kinase C using bis-indolylmaleimide and calphostin C, selective inhibitors of protein kinase C (Fig. 4C). Both calphostin C (100 nM) and bis-indolylmaleimide (1 μM) abolished the α1-adrenergic stimulation of lipolysis, whereas the protein kinase A inhibitor KT-5720 (1 μM) was without effect. At 100 nM, bis-indolylmaleimide effectively blocked phenylephrine (10 μM)-stimulated lipolysis in adipocytes from control mice; glycerol release (μmol/10⁶ cells), in response to this α1-adrenergic agonist, declined from 4.7 to 1.2 in the absence versus presence of this protein kinase C inhibitor. The KI for nonselective inhibition of protein kinase A by bis-indolylmaleimide is 2 μM (26). Since the protein kinase A inhibitor KT-5720 itself was without effect, nonselective effects of protein kinase C inhibitors, if they indeed occurred at these lower concentrations, would be irrelevant.

**Fig. 4.** The pharmacology of the adrenergic lipolytic response reveals the existence of an α1-adrenergic stimulatory pathway, absent in the Gaq-deficient loss-of-function mutants. White adipocytes were isolated from transgenic mice and their control littermates for study of the lipolytic response to adrenergic agonists. The lipolytic response was measured as described in the legend to Fig. 3. Stimulation of lipolysis by either 10 μM norepinephrine (NOR, panel A) or 10 μM phenylephrine (PHEN, panels B and C) was observed in the absence and presence of either the β-adrenergic antagonist propranolol (PROP, 10 μM) or the α1-adrenergic antagonist prazosin (PRAZ, 1 μM). Inhibitors of protein kinase A (KT, KT7502, 1 μM) and protein kinase C (BIS, bis-indolylmaleimide, 1 μM; CAL, calphostin C, 100 nM) were examined for their ability to block the α1-adrenergic stimulation of lipolysis in adipocytes from transgenic mice and their control littermates (panel C). The results are mean values ± S.E. from three to five separate experiments for each. An asterisk denotes statistical significance with p ≤ 0.05 for the differences from mean basal values obtained with adipocytes isolated from both transgenic (Gaq-deficient) and control mice.

**Fig. 5.** Adipocytes from pPCK-ASGaq transgenic mice display loss of function with respect to activation of protein kinase C following challenge with norepinephrine. White adipocytes were isolated from epididymal and epoophoronal fat of transgenic mice and control littermates by collagenase digestion. The activity of protein kinase C was measured in cells from mice 18–24 weeks of age. The cells were challenged for 5 min without (Basal) and with 10 μM of the mixed α- and β-adrenergic agonist norepinephrine (NOR), in the absence or presence of 0.1 mM bis-indolylmaleimide (NOR + BIS), a potent protein kinase inhibitor. Protein kinase C activity was measured in DEAE-cellulose-purified cell homogenates, as described elsewhere (30). The data presented are mean values ± S.E. from at least three separate experiments, each performed on separate occasions. An asterisk denotes statistical significance with p ≤ 0.05 for the difference between the mean values for transgenic (Gaq-deficient) as compared to control mice.
Measurement of protein kinase C activity in \( \alpha\)-cellulose-purified homogenates of cells challenged with and without norepinephrine was performed using antibodies from the control and transgenic mice (Fig. 5). In adipocytes from control mice, norepinephrine stimulates protein kinase C activity, an action blocked by the addition of bis-indolylmaleimide. Suppression of \( \alpha\)-adrenergic receptors in adipocytes of the pPCK-ASG \( \alpha\) transgen results in a frank reduction in protein kinase C activity in the basal state and a loss of norepinephrine-induced activation of protein kinase C. Total protein kinase activities for adipocytes from control and transgenic mice are equivalent, 310 ± 20 and 315 ± 18 pmol/min/million cells, respectively. Thus, \( \alpha\)-adrenergic control of lipolysis is shown to be mediated via protein kinase C, a pathway revealed by its absence in the \( \alpha\)-deficient state.

The absence of \( \alpha\) resulted in increased fat accumulation and hyperadiposity, observed within 5 weeks of age and sustained through adult life. Obesity has been reported in transgenic mice after genetic ablation of brown adipose tissue (27), supporting the role of this specialized tissue in preventing obesity (28). The pPCK-ASG \( \alpha\) transgene was not expressed in brown adipose tissue (not shown). Expression of \( \alpha\), the uncoupling protein UCP, and the mRNAs for both were equivalent in brown adipose tissue from transgenic and control mice (not shown), suggesting no involvement of brown adipose tissue in enhanced fat accumulation by the pPCK-ASG \( \alpha\)-expressing mice.

The absence of \( \alpha\) abolished an important stimulatory control of lipolysis, apparently predisposing the mice to accumulation of fat. Recently, G-proteins have been shown to play prominent roles in differentiation (2, 3) and neonatal growth (4–6). For progression of F9 teratocarcinoma cells to primitive endoderm (4) and for development of nerve growth cones (3), G-proteins appear to be acting directly or indirectly via protein kinase C (10, 29). In the present study we demonstrate the key role of PLC and protein kinase C in adipocyte signaling in the mature cells. The basis for the hyperadiposity in the cells deficient in \( \alpha\), however, remains to be established, but may reflect a critical role of \( \alpha\) in controlling adipogenic conversion in vivo.

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