Tissue-Specific Alterations in G Protein Expression in Genetic Versus Diet-Induced Models of Non–Insulin-Dependent Diabetes Mellitus in the Mouse

Thomas W. Gettys, Vickram Ramkumar, Richard S. Surwit, and Ian L. Taylor

Various tissues were obtained from the well-characterized genetic model (C57BL/6J-ob/ob) of non–insulin-dependent diabetes mellitus (NIDDM) and from a diet-induced model of NIDDM produced in the same genetic background (C57BL/6J). The objectives were to determine whether the previously observed changes in guanine nucleotide-binding regulatory protein (G protein) expression in adipose tissue from ob/ob mice were mirrored by concomitant changes in other tissues, and whether NIDDM of a different etiology would share similar alterations in G protein expression. Plasma membranes from adipocytes, brain, heart, liver, and testes were probed with α-subunit–specific antisera, and the level of G protein expression in each model was compared with that in its lean littermate control. Adipose, heart, and liver cell membranes from ob/ob mice contained significantly less α-subunit of stimulatory G protein ($G_s\alpha$) than those from their lean littermates. As compared with the lean littermates, heart α-subunit-2 of inhibitory G protein ($G_i\alpha$-2), liver $G_i\alpha$-3, and adipocyte $G_i\alpha$-1 and $G_i\alpha$-3 were also reduced in ob/ob mice. In contrast, $G_i\alpha$-2 and $G_o\alpha$ were increased over lean-control levels in brain tissue from ob/ob mice, whereas $G_s\alpha$ was unchanged. G protein expression in the testes did not differ between lean and ob/ob mice. In the diet-induced model of NIDDM, $G_s\alpha$ expression in the liver was twofold greater in obese/diabetic mice as compared with lean controls. However, G protein expression in all other tissues examined did not differ between obese/diabetic animals and lean littermates. Although the severity of overt symptoms of NIDDM is comparable between the two models, the diet-induced model does not suffer from the documented endocrine abnormalities of hypothyroidism and hyperadrenocorticism noted in the ob/ob mouse. Thus, it seems likely that the G protein changes noted in the ob/ob mouse are not the direct result of the obese or diabetic condition, but may be secondary to endocrine differences between the two models.

THE MOUSE STRAIN C57BL/6J-ob/ob has been widely used as a model of non–insulin-dependent diabetes mellitus (NIDDM) and obesity.[1] However, its usefulness as an analog of the human disease is compromised somewhat by the fact that animals develop the syndrome on a diet of animal chow that is essentially devoid of fat (typically 4%), even if food intake is controlled.[1,2] Understanding the sequence of events culminating in obesity and diabetes is further confounded by the presence of several endocrine abnormalities in the C57BL/6J-ob/ob mouse.[1,2] Thus, it has been difficult to determine whether documented alterations in hormonal signaling in the ob/ob mouse are a product of its diabetes or its altered endocrine environment.[3-7]

Guanine nucleotide-binding regulatory proteins (G proteins) are membrane proteins that relay information from occupied receptors to intracellular effector systems using the binding and hydrolysis of guanosine triphosphate as the regulatory mechanism.[8-12] As such, they are essential to the integrity of signaling mechanisms in all tissues. Changes in G protein expression have been associated with changes in glucocorticoid levels,[13,14] testosterone levels[15] and thyroid status,[16-18] and with pathologic states such as NIDDM[19-22] and hereditary osteodystrophy.[23] Studies of disease-associated changes in G protein expression have been guided by the belief that altered hormonal signaling may subserve several of the associated metabolic disturbances. However, what has remained unclear is whether changes in G protein expression play a significant role in the etiology of disease. In the case of the obese mouse (C57BL/6J-ob/ob), the development of the syndrome after weaning is associated with a series of pathologic changes in adipose tissue, including decreased G protein expression.[7,19,22,24-26] Studies addressing the mechanism of these changes have been guided by reports that the ob/ob mouse suffers from hyperadrenocorticism and hypothyroidism, and by the knowledge that alterations in adrenal[13,14] and thyroid[16-18] function have profound effects on G protein expression in a number of tissues. In the present study, we have attempted to evaluate the role of the altered endocrine status of the ob/ob mouse by determining whether diminished G protein expression is a consistent effect across tissues. These studies have been complemented by examining the same questions in a diet-induced model of NIDDM created in the background strain of the C57BL/6J-ob/ob mouse that does not display altered adrenal or thyroid function. Using tissues from both models, it is shown that altered G protein expression is a characteristic of the ob/ob mouse, and is not necessarily a product of its obesity or diabetes.

MATERIALS AND METHODS

Materials

N-tris(hydroxylmethyl)methyl-2-aminoethanesulfonic acid buffer (TES),[2] sucrose, mercaptoethanol, EDTA, *N,N'*-diallyltartardiamide, dithiothreitol, bovine serum albumin, leupeptin, phenylmethylsulfonyl fluoride, adenosine triphosphate, adenosine, soybean trypsin inhibitor, and other common chemicals were obtained from Sigma Chemical (St Louis, MO). Diethylaminoethyl Sephacel and Sephadex G-25 resin were from Pharmacia (Piscataway, NJ).

From the Department of Medicine, Medical University of South Carolina, Charleston, SC; the Department of Pharmacology, Southern Illinois University, Springfield, IL; and the Department of Psychiatry, Duke University Medical Center, Durham, NC.

Submitted May 17, 1994; accepted July 31, 1994.

Supported by grants from the National Institutes of Health to T.W.G. (DK 42486), R.R.S. (DK 42923 and K05MH 00303), and I.L.T. (DK 44072).

Address reprint requests to Thomas W. Gettys, PhD, 751 Basic Science Bldg, Medical University of South Carolina, 171 Ashley Ave, Charleston, SC 29425.

0026-0495/95-4406-0015$03.00/0

Immobilon-P polyvinylidene difluoride (PVDF) membranes and 10,000–molecular-weight cutoff low–protein-binding membranes were from Millipore (Bedford, MA). The α-subunit-2 of inhibitory G protein ($G_i\alpha$-2)-specific antiserum, J883, was a gift from Dr Susanne Mumby of the Department of Pharmacology, University of Texas Southwestern Medical Center (Dallas, TX). Bacteria expressing the different G protein α-subunits were a gift from Dr Pat Casey of Duke Medical Center (Durham, NC). [^{125}I]Na was purchased from Dupont NEN Radiochemicals (Boston, MA).

Animals

Obese mice (C57BL/6J-ob/ob) and their lean siblings were obtained from Jackson Laboratories (Bar Harbor, ME) and used at approximately 14 weeks of age. Body weights averaged 24.6 ± 0.6 g for lean and 49.4 ± 0.9 for ob/ob mice. Additional C57BL/6J mice were obtained from Jackson Laboratories at 4 weeks of age. Half the animals were fed Purina Rodent Chow (St Louis, MO), and the other half were given ad libitum access to a high-fat, high–simple-carbohydrate, low-fiber diet as described by Surwit et al.[27] The two groups were maintained on this diet until they were 17 weeks of age, at which time the group receiving the high-fat, high–simple-carbohydrate diet was documented to be hyperinsulinemic (125 ± 16 *v* 13.2 ± 2.6 μU/mL for control), hyperglycemic (252 ± 6.7 *v* 161 ± 4.7 mg/dL for control), and diabetic in a manner consistent with previous reports.[27,28] Mice receiving the diabetogenic diet were also obese, and their body weights (53.5 ± 0.7 g) were nearly twice those of their control littermates (26.4 ± 0.2 g). At the indicated ages, tissues were rapidly removed under Nembutal (Abbott Laboratories, North Chicago, IL) anesthesia and snap-frozen in liquid nitrogen. Adipocytes were isolated according to the following description.

Isolation of Adipocytes

Adipocytes were prepared from epididymal fat pads of male mice of each phenotype as previously described by Rodbell,[29] with slight modification.[22,30] The cells were washed and resuspended in Krebs-Ringer-HEPES buffer containing 1 mmol/L $CaCl_2$.

Preparation of Plasma Membranes

Tissue samples (2 to 4 g) were homogenized in 10 mmol/L TES buffer (pH 7.0) containing 0.25 mol/L sucrose, 1 mmol/L phenylmethylsulfonyl fluoride, 40 μmol/L leupeptin, and 1 mmol/L EDTA in a polytron homogenizer, and the homogenate was subjected to low-speed centrifugation (3,000 × *g* for 5 minutes) to remove unbroken cells. The supernatant was homogenized additionally with a Dounce glass homogenizer, and a crude membrane preparation was obtained by centrifugation at 48,000 × *g* for 20 minutes at 4°C. After removal of the supernatant, the crude membrane pellet was resuspended in the same TES buffer (pH 7.5) and plasma membranes were prepared according to the following description.

For isolated adipocytes, cells were lysed in a Dounce homogenizer in hypotonic buffer containing 10 mmol/L TES (pH 7.0) and 1 mmol/L EDTA. Unbroken cells and nuclei were removed by an initial centrifugation at 12,000 × *g* for 2 minutes. After this step, unbroken cells and the fat cake floated and were removed by aspiration, while the nuclei and mitochondria were pelleted by this initial centrifugation. The supernatants were transferred to separate tubes, and crude membranes were collected by a 20-minute centrifugation at 48,000 × *g*. The pelleted membranes were resuspended in 10 mmol/L TES (pH 7.5) containing 0.25 mol/L sucrose. Plasma membranes were prepared from crude membrane pellets of each tissue type by aqueous two-phase partition with dextran and polyethylene glycol as described previously.[22,31] The purified plasma membranes were resuspended at 1 mg/mL in 25 mmol/L HEPES (pH 7.4) containing 140 mmol/L NaCl, 40 μmol/L leupeptin, 1 μg/mL soybean trypsin inhibitor, and 1 mmol/L EDTA, and stored at −80°C.

Antisera for Western Blotting

Antisera were raised against the C-terminal decapeptide (amino acids 345 to 354) of $G_i\alpha$-3,[32,33] against the C-terminal decapeptide (amino acids 345 to 354) shared by both $G_i\alpha$-1 and $G_i\alpha$-2,[32] and against an internal sequence (amino acids 159 to 168) of $G_i\alpha$-1.[32] The peptides were conjugated to keyhole limpet hemocyanin via a cysteine placed on the *N*-terminal end of each peptide,[34] and rabbits were immunized with each conjugate according to the method reported by Green et al.[35] The anti–$G_i\alpha$-1, anti–$G_i\alpha$-3, and anti–$G_i\alpha$-(1-2) antisera were desalted using Sephadex G-25, and IgGs from each sera were purified by high-performance liquid chromatography using a Protein-A affinity column (Rainin Instrument, Woburn, MA). Purified IgGs were standardized to 6 mg/mL and characterized with respect to titer, specificity, and cross-reactivity using lysates from bacteria transformed with the cDNA for each of the G proteins.[34,36] Anti–$G_i\alpha$-1 and anti–$G_i\alpha$-(1-2) IgGs were used at a final concentration of 1.5 μg/mL, and anti–$G_i\alpha$-3 IgG was used at 0.75 μg/mL. The anti–$G_i\alpha$-2 antiserum J883 was used at a final dilution of 1:500.

Solubilization of Membranes and Immunologic Detection of G Proteins

Purified plasma membranes from each experimental replicate and tissue were solubilized on ice for 1 hour in 20 mmol/L Tris, 1 mmol/L EDTA, 1 mmol/L dithiothreitol, 100 mmol/L NaCl, and 0.9% Na cholate (pH 8.0). The supernatants were collected after centrifugation at 13,000 × *g* for 5 minutes at 4°C, and protein was carefully assayed using a detergent-compatible protein assay from Biorad (Melville, NY). Bovine serum albumin diluted in the solubilization buffer was used as the standard. Preliminary experiments were conducted to determine the appropriate amount of solubilized membrane protein to use for each tissue type. Thereafter, experimental replicates from each tissue type and phenotype were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis ([SDS-PAGE] 12.5% acrylamide, 0.051% *N,N'*-diallytartardiamide), and transferred by electrophoresis to Immobilon-P (Millipore, Bedford, MA) PVDF membranes.[22,37] Using the procedure reported by Mumby et al,[38] PVDF membranes were probed with specific G protein antisera described earlier and the bands were detected with ^{125}I-labeled goat anti-rabbit IgG (1×10^6 cpm/ml). The membranes were washed, blotted dry, and exposed to Kodak XAR film (Eastman Kodak, Rochester, NY) with intensifying screens overnight. The autoradiograms were scanned on a Molecular Dynamics (Sunnyvale, CA) densitometer at 50-μm scanning resolution, and densitometric intensities for replicates of each tissue type and phenotype were compared by one-way ANOVA as described previously.[39]

RESULTS

G Protein Expression in Tissues From C57BL/6J-ob/ob Mice

Initial experiments were conducted with each tissue source to establish that equivalent amounts of protein were loaded on the gels for each experimental replicate and across phenotypes. These studies were necessary to guarantee that denominators for phenotypic comparisons were the same, and initial gels for each tissue were evaluated by Coomassie staining before subjecting the replicate extracts

for each tissue and phenotype to Western blotting. For example, the gel presented in Fig 1A illustrates that equivalent amounts and types of solubilized heart cell plasma membrane protein were loaded in each lane for the experimental replicates within and across phenotypes. Similar patterns were observed in the initial gels evaluated with other tissues from lean and ob/ob mice (data not shown). The overall protein composition was also similar in experimental replicates of liver plasma membrane preparations from control and diet-induced diabetic animals (Fig 1B).

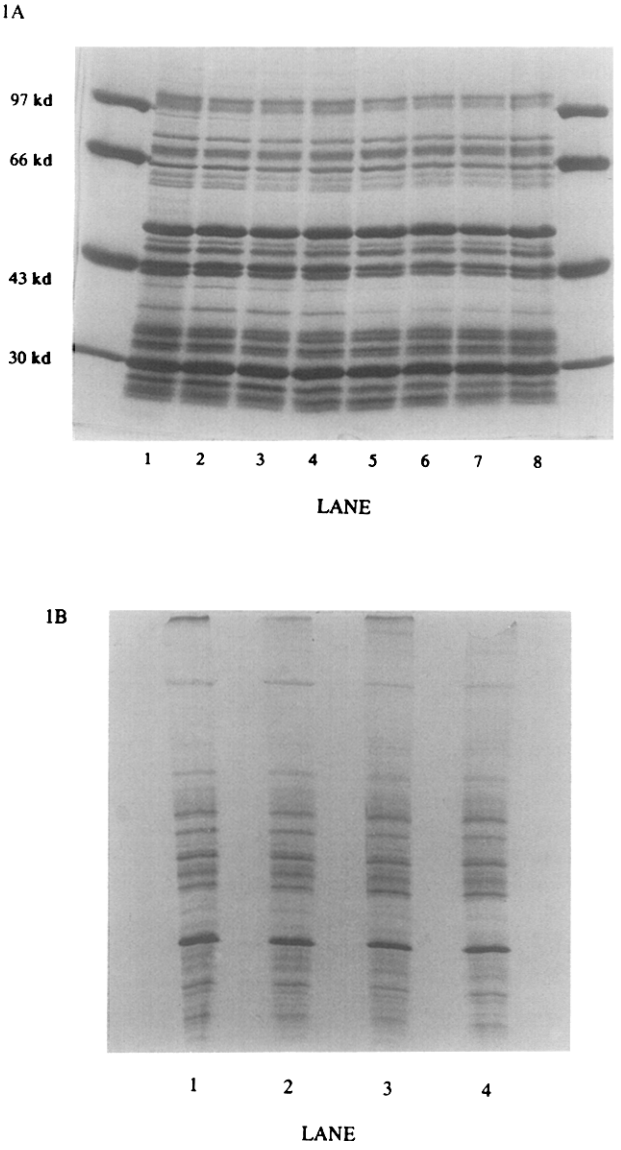

Fig 1. Coomassie blue–stained gels of solubilized plasma membranes from hearts of lean and ob/ob mice (A) and livers of lean and diet-induced obese mice (B). Equal amounts of protein were loaded onto the gel for the replicates within each tissue type (heart, 25 μg per lane; liver, 25 μg per lane), and after SDS-PAGE, resolved proteins were visualized by Coomassie staining. Lanes 1 to 4 and 5 to 8 represent heart cell membranes from lean and ob/ob mice, respectively (A), and lanes 1 to 2 and 3 to 4 represent liver cell membranes from lean and diet-induced obese mice, respectively (B).

Table 1. Comparison of $G_s\alpha$ Expression in Tissues From Lean and ob/ob Mice

Source of Membranes (kd)	Densitometric Intensity (mean ± SE)	
	Lean	ob/ob
Brain		
48	1,050 ± 85	848 ± 85
43	140 ± 36	158 ± 36
Adipocyte		
48	2,202 ± 82	1,238 ± 82*
43	2,122 ± 94	990 ± 94*
Testes		
48	2,006 ± 148	1,789 ± 148
43	78 ± 32	69 ± 32
Liver		
48	1,060 ± 73	160 ± 73*
43	2,233 ± 97	408 ± 97*
Heart		
48	1,453 ± 80	819 ± 80*
43	1,887 ± 85	851 ± 85*

NOTE. The 43- and 48-kd forms of $G_s\alpha$ are products of alternative splicing of $G_s\alpha$ mRNA and are expressed in varying ratios from tissue to tissue. Autoradiograms were scanned by laser densitometry, and the estimates of densitometric intensity were compared by 1-way ANOVA. The means were calculated from 2 blots for each tissue, and pooled standard errors were obtained from ANOVA. The level of protection against type I errors was set at 1%, and the result of all hypothesis tests is indicated in the ob/ob column.

*$P < .01$ for hypothesis: mean1 = mean2.

These experiments indicated that solubilization of purified plasma membranes, followed by removal of insoluble material via centrifugation, and assay of protein in the extracts using a detergent-compatible Lowry assay from Biorad achieved the stated objectives and provided a sound basis for subsequent comparisons.

In the next series of experiments, $G_s\alpha$ expression was compared in tissues from lean and genetically obese (ob/ob) mice by Western blotting using an antipeptide antiserum of defined specificity for $G_s\alpha$.[34] Representative blots for various tissues are presented in Fig 2, and the ANOVAs of the densitometric data from each tissue are presented in Table 1. The 43- and 48-kd forms of $G_s\alpha$ are produced by alternative splicing of $G_s\alpha$ mRNA, but vary in their relative expression among various tissues. In the brain (blot not shown) and testes (Fig 2), the 48-kd form is the predominant splice variant expressed, whereas the smaller 43-kd form is present in much smaller amounts (Table 1). ANOVA of densitometric data from the blots indicates that $G_s\alpha$ expression did not differ between phenotypes in these two tissues (Table 1). However, it is clear from both Fig 2 and Table 1 that both forms of $G_s\alpha$ are significantly reduced in heart, liver, and adipose tissue from ob/ob mice as compared with their lean littermates. The decreased expression in adipose and heart cell membranes was on the order of 40% to 60%, whereas $G_s\alpha$ expression in liver cell membranes of ob/ob mice was reduced 70% to 80% as compared with lean mice (Table 1). It is interesting that in the three tissues where reduced $G_s\alpha$ expression was detected, fairly equivalent expression of both G protein isoforms was also observed (Fig 2).

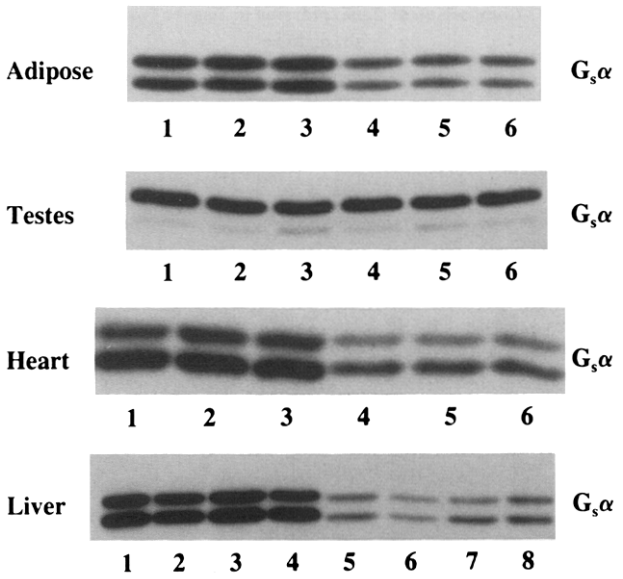

Fig 2. Immunoblots of plasma membranes from adipocytes, testes, heart, and liver of lean and ob/ob mice. Equal amounts of protein were loaded onto the gel for the replicates within each tissue type (adipose, 10 μg per lane; testes, 25 μg per lane; heart, 25 μg per lane; liver, 25 μg per lane), and after SDS-PAGE, resolved proteins were transferred to PVDF membranes in a semi-dry blotter. Blots were probed with an antiserum directed against the C-terminal decapeptide of $G_s\alpha$,[34] and the detected proteins were visualized using ^{125}I-goat anti-rabbit IgG. Autoradiograms were developed after overnight exposure with intensifying screens, and bands were quantified using scanning laser densitometry at 50-μm resolution. Blots are representative of two similar blots for each tissue, and the summary analysis of pooled data is presented in Table 1. Lanes 1 to 3 were loaded with membranes from separate animals within the group of lean littermates, and lanes 4 to 6 were loaded with membranes from separate ob/ob mice in the first 3 blots. In the blot of liver membranes, lanes 1 to 4 and 5 to 8 represent experimental replicates of lean and ob/ob mice, respectively.

Expression of the pertussis-toxin–sensitive G proteins $G_i\alpha$-1, $G_i\alpha$-2, $G_i\alpha$-3, and $G_o\alpha$ was also compared in the same tissues using subtype-specific antisera.[34] $G_i\alpha$-1 was only detected in the brain and adipose tissue, and in the brain expression did not differ between the phenotypes (Table 2). However, $G_i\alpha$-1 expression was significantly lower in adipocyte membranes from ob/ob mice (Table 2). $G_i\alpha$-2 was detected in all tissues studied, and with the exception of the heart and brain, its expression did not differ between phenotypes (Table 2 and Fig 3). In the brain, $G_i\alpha$-2 expression was increased slightly in ob/ob mice (Fig 3 and Table 2), whereas in the heart, $G_i\alpha$-2 expression was reduced by approximately 60% in ob/ob mice as compared with their lean littermates (Table 2). This decrease in $G_i\alpha$-2 expression in the heart of ob/ob mice is comparable to the decrease in $G_s\alpha$ noted in the same tissue, but the almost identical levels of expression of $G_i\alpha$-3 in the two phenotypes argue against a systematic source for the differences (Fig 3 and Table 2). However, it is interesting that $G_i\alpha$-2 expression did not differ between phenotypes with respect to the adipocyte and liver, whereas $G_s\alpha$ expression was reduced in these tissues from ob/ob mice (Tables 1 and 2). Compared with tissues from lean mice, $G_i\alpha$-3 expression was also significantly reduced in adipocytes and livers from ob/ob mice. In the testes, $G_i\alpha$-3 expression did not differ between phenotypes (Table 2). $G_i\alpha$-3 was weakly detected in the brain as a faint band above a more strongly detected band at 39-kd, which was $G_o\alpha$. The anti–$G_i\alpha$-3 used in these experiments was raised against the C-terminal decapeptide sequence of $G_i\alpha$-3, which is similar to the C-terminus of $G_o\alpha$. Therefore, it was not unexpected that some of the epitopes represented in this polyclonal antiserum should recognize $G_o\alpha$. Thus, the combination of high-level expression of $G_o\alpha$ coupled with modest expression of $G_i\alpha$-3 in the brain allowed us to detect both G proteins. Due to low levels of expression and incomplete resolution from $G_o\alpha$, it was not possible to determine whether $G_i\alpha$-3 expression differed between phenotypes. However, the level of $G_o\alpha$ per unit of protein was increased significantly in brain membranes from ob/ob animals (lean, 1,240 ± 170 densitometric units; ob/ob, 2,121 ± 170 densitometric units).

G Protein Expression in Tissues From Diet-Induced Model of NIDDM

Consumption of the diabetogenic diet (high-fat, high–simple-carbohydrate, and low-fiber) described by Surwit et al[27] for 13 weeks after weaning produced animals that were comparable to genetically obese mice (ob/ob) in terms of the severity of obesity and diabetes. At the time of study, diet-induced obese animals weighed twice as much as their lean littermates (54 *v* 26 g), had fasting hyperglycemia

Table 2. Comparison of $G_i\alpha$ Expression in Tissues From Lean and ob/ob Mice

Source of Membranes	Densitometric Intensity (mean ± SE)		
	$G_i\alpha$-1	$G_i\alpha$-2	$G_i\alpha$-3
Brain			
Lean	1,277 ± 186	544 ± 38	†
ob/ob	1,602 ± 186	788 ± 38*	†
Adipocyte			
Lean	1,446 ± 51	705 ± 87	1,140 ± 13
ob/ob	1,083 ± 51*	764 ± 87	773 ± 13*
Testes			
Lean	ND	628 ± 48	1,784 ± 251
ob/ob	ND	533 ± 48	1,266 ± 251
Heart			
Lean	ND	2,141 ± 140	1,411 ± 100
ob/ob	ND	761 ± 140*	1,371 ± 100
Liver			
Lean	ND	2,106 ± 283	1,094 ± 45
ob/ob	ND	2,199 ± 283	819 ± 45*

NOTE. The autoradiograms were scanned by laser densitometry, and the estimates of densitometric intensity were compared by 1-way ANOVA. The means were calculated from 2 blots for each tissue, and pooled standard errors were obtained from ANOVA. The level of protection against type I errors was set at 1%, and the result of all hypothesis tests is indicated in the ob/ob column.

Abbreviation: ND, none detected.

*$P < .01$ for hypothesis: mean1 = mean2.

†Low levels of $G_i\alpha$-3 were detected in brain plasma membranes that were not resolved from the substantially more prominent band of $G_o\alpha$ migrating at 39 kd. Means for the 39-kd band were 1,240 ± 170 U (lean) and 2,121 ± 170 (ob/ob; $P < .01$).

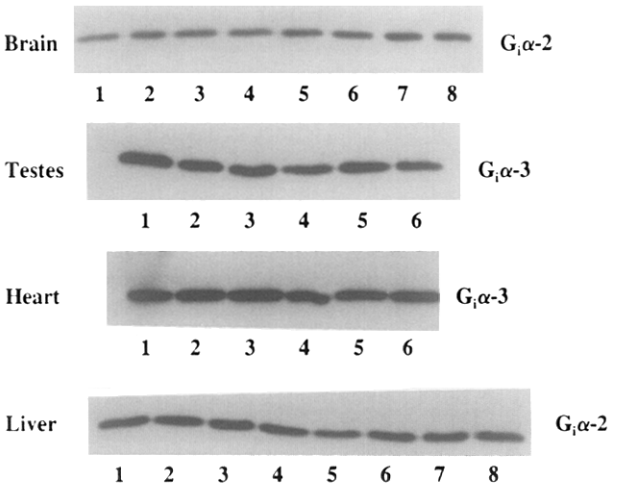

Fig 3. Immunoblots of plasma membranes from adipocytes, testes, heart, and liver of lean and ob/ob mice. Equal amounts of protein were loaded onto the gel for the replicates within each tissue type (brain, 25 μg per lane; testes, 25 μg per lane; heart, 25 μg per lane; liver, 25 μg per lane), and after SDS-PAGE, resolved proteins were transferred to PVDF membranes in a semi-dry blotter. Blots were probed with antisera directed against the C-terminal decapeptides of $G_i\alpha$-3 or $G_i\alpha$-2,[34] and the detected proteins were visualized using ^{125}I-goat anti-rabbit IgG. Autoradiograms were developed after overnight exposure with intensifying screens, and bands were quantified using scanning laser densitometry at 50-μm resolution. Blots are representative of two similar blots for each tissue, and the summary analysis of pooled data is presented in Table 2. Lanes 1 to 3 were loaded with membranes from 3 lean littermates and lanes 4 to 6 were loaded with membranes from 3 ob/ob mice in the first 3 blots. In the blot of liver membranes, lanes 1 to 4 and 5 to 8 represent experimental replicates of lean and ob/ob mice, respectively.

greater than 240 mg/dL, and were hyperinsulinemic as reported previously.[27] But in contrast to C57BL/6J-ob/ob mice, G protein expression was essentially unaffected in the diet-induced model of NIDDM. This was particularly evident for expression of pertussis-toxin–sensitive G proteins, where no phenotypic differences were observed in any of the five tissues surveyed. $G_s\alpha$ expression was also unaffected in all tissues from the diet-induced model of NIDDM except the liver, where a significant increase in expression of both splice variants was observed in obese animals ($P < .01$; Fig 4). This finding is particularly interesting in light of the exaggerated glycemic responses to epinephrine and the more pronounced fasting hyperglycemia in the diet-induced model of NIDDM.[27] The increase in liver $G_s\alpha$ expression in the diet-induced model contrasts with results from the genetic model of NIDDM, where $G_s\alpha$ expression in livers of ob/ob animals is decreased by 60% to 80% (Fig 2 and Table 1).

DISCUSSION

The α-subunits are the most structurally diverse of the G protein subunits, with at least 16 genes identified to date.[40-43] The list of effector systems regulated by G proteins also continues to grow,[10,44-46] and recent reports have indicated the involvement of βγ-subunits in receptor desensitization mechanisms.[47,48] Alterations in G protein expression have been associated with changes in glucocorticoid levels,[13,14] testosterone levels,[15] and thyroid status,[16-18] and with pathologic states such as hereditary osteodystrophy[23] and NIDDM.[19-22] Since G proteins are involved in signaling cascades in essentially every cell and tissue, early reports of diabetes-associated loss of G protein expression raised the question of whether this might be a fundamental defect in the disease that gave rise to the plethora of associated metabolic disturbances.[49] Many subsequent reports have documented altered G protein expression in genetic models of NIDDM,[19,21,22,50,51] but in no case has expression or function been lost completely. It has also been shown that the genes responsible for genetic models of obesity in rodents reside on different chromosomes than the genes that encode G proteins.[52,53] Together, these results suggest that decreased G protein expression is not a cause of NIDDM, but instead occurs in tandem with development of the syndrome.

The present studies were designed to test whether the development of obesity and/or diabetes, regardless of cause, results in altered G protein expression or whether other characteristics of the syndrome play a causative role. Thus, availability of the diet-induced model of NIDDM in the same genetic background as the C57BL/6J-ob/ob mouse[27,28] made it possible to determine whether changes in G protein expression occur in animals with obesity/diabetes of different etiology but comparable severity. The

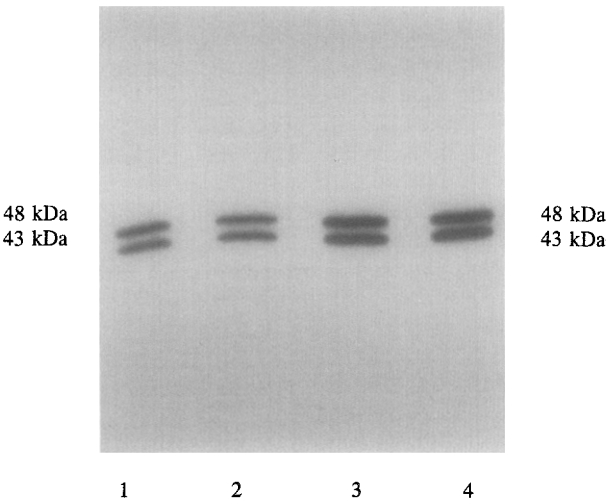

Fig 4. Immunoblot of plasma membranes from liver of lean mice and mice that have consumed a diabetogenic diet. Equal amounts of protein were loaded onto the gel for the replicates of each phenotype (liver, 25 μg per lane), and after SDS-PAGE, resolved proteins were transferred to PVDF membranes in a semi-dry blotter. Blots were probed with an antiserum directed against the C-terminal decapeptide of $G_s\alpha$,[34] and the detected proteins were visualized using ^{125}I-goat anti-rabbit IgG. Autoradiograms were developed after overnight exposure with intensifying screens, and the bands were quantified using scanning laser densitometry at 50-μm resolution. Blots are representative of two similar blots, and pooled data were compared by ANOVA (lean: 43 kd = 963 ± 88 U, 48 kd = 941 ± 90; obese: 43 kd = 2,335 ± 88 U, 48 kd = 2,358 ± 90; $P < .01$ for hypothesis: means1 = means2). Lanes 1 to 2 were loaded with membranes from 2 lean littermates, and lanes 3 to 4 were loaded with membranes from 2 diet-induced obese mice.

significant feature of the diet-induced model of NIDDM is the absence of endocrine abnormalities in thyroid and adrenal function that are characteristic of the ob/ob mouse.[2,27,54] Perhaps the most important finding of the present study was that G protein expression in tissues from the diet-induced model of NIDDM was essentially unchanged between lean and obese animals. The one documented change occurred in the liver, where $G_s\alpha$ expression was increased twofold in obese mice. This finding is interesting in that it may be relevant to the exaggerated glycemic responses to epinephrine reported in the diet-induced model of NIDDM.[27] Fasting hyperglycemia was also more severe in the diet-induced compared with the genetic model of NIDDM,[27] and may be related to the altered sympathetic outflow noted in the diet-induced model.[54a] The increase in hepatic $G_s\alpha$ is completely opposite to what was observed in genetically obese animals, where a 70% to 80% decrease in liver $G_s\alpha$ expression was noted. Similar decreases in $G_s\alpha$ and $G_i\alpha$ expression were noted in three of five tissues surveyed in the ob/ob mouse. However, it was interesting that in the three tissues where decreased expression was noted (adipose, liver, and heart), not all $G_i\alpha$ subtypes were affected. In adipocyte and liver membranes, $G_i\alpha$-2 expression was identical in lean and ob/ob mice, whereas in the heart, $G_i\alpha$-3 did not differ between phenotypes. The brain and testes were unique in that G protein subtypes were either unchanged (testes) or increased (brain: $G_i\alpha$-2 and $G_o\alpha$) in ob/ob mice. These results suggest that there are tissue-specific effects of the ob/ob syndrome on G protein expression, and that obesity and/or diabetes by itself is probably not the cause of altered G protein expression in the ob/ob mouse.

As noted earlier, several genetic models of NIDDM including the ob/ob mouse share the characteristics of hypothyroidism and hyperadrenocorticism.[1,2,54] The connection to genetically transmitted obesity lies in the fact that the syndrome will not develop if the homozygous individuals receive glucocorticoid antagonists[55] or are adrenalectomized at weaning.[56,57] Moreover, the syndrome can be re-created in adrenalectomized animals given steroid replacement.[58,59] The significance of hyperadrenocorticism to the development of insulin resistance and hyperglycemia in rodent models of NIDDM is that it has been linked to a reduction in expression of the high-K_m glucose transporter in pancreatic β cells.[60-62] Glucocorticoids have also been shown to decrease expression of β_3-adrenergic receptors in 3T3-F442A adipocytes.[63] In addition, we[26] and others[64] have shown that expression of the β_3-adrenergic receptor subtype is specifically reduced in adipocytes from ob/ob mice and Zucker rats, respectively. Although negative promoters for the β_3-adrenergic receptor have not been identified in the 5′ untranslated region of the receptor gene, it is possible that negative effects result from interaction of the glucocorticoid receptor with transcriptional regulators.[26] Decreased expression of both the high-K_m glucose transporters and the β_3-adrenergic receptors is central to the development of characteristic features of the obese/diabetic syndrome in rodents, and evidence for the involvement of glucocorticoids is indirect but strong. Studies to evaluate directly the involvement of glucocorticoids in NIDDM-associated decreases in G protein expression have not been conducted, but the results presented herein are consistent with that possibility. It will be of interest to determine whether the tissue-specific changes in G protein expression noted in the present study are also involved in the development of specific features of the genetically transmitted obese/diabetic syndrome. Results from the present study suggest that the diet-induced model of NIDDM may be more similar to the human form of NIDDM and provides a better representation of the natural etiology of the disease.

ACKNOWLEDGMENT

We thank Lucy Seger for excellent technical assistance and Dr Pat Casey for the gift of bacteria expressing the different G protein α-subunits.

REFERENCES

1. Bray GA, York DA: Genetically transmitted obesity in rodents. Physiol Rev 51:598-646, 1979

2. Bray GA: Pathophysiology of obesity. Am J Clin Nutr 55:488S-494S, 1992 (suppl)

3. Bégin-Hieck N: Quantification of the α and β subunits of the transducing elements (G_s and G_i) of adenylate cyclase in adipocyte membranes from lean and obese (*ob/ob*) mice. Biochem J 268:83-89, 1990

4. Dehaye JP, Hebbelinck M, Winand J, et al: Indirect evidence against a contribution of the guanine nucleotide-binding inhibitory component of adenylate cyclase to impaired lipolysis in the epididymal adipose tissue of congenitally obese (ob/ob) mice. Horm Metab Res 17:333-336, 1985

5. Black MA, Heick HM, Begin-Heick N: Abnormal regulation of insulin secretion in the genetically obese (ob/ob) mouse. Biochem J 238:863-869, 1986

6. Begin-Heick N: Differential response of adenylate cyclase to beta-adrenergic agonists in white adipocyte membranes from lean and obese (ob/ob) mice. Can J Biochem 59:816-820, 1981

7. Marshall NB, Engel FL: The influence of epinephrine and fasting on adipose tissue content and release of free fatty acids in obese-hyperglycemic and lean mice. J Lipid Res 1:339-342, 1960

8. Gilman AG: G proteins: Transducers of receptor-generated signals. Annu Rev Biochem 56:615-649, 1987

9. Bourne HR, Sanders DA, McCormick F: The GTPase superfamily: A conserved switch for diverse cell functions. Nature 348:125-132, 1990

10. Birnbaumer L: Receptor-to-effector signaling through G proteins: Roles for βgamma dimers as well as α subunits. Cell 71:1069-1072, 1992

11. Birnbaumer L, Codina J, Mattera R, et al: G proteins and transmembrane signalling, in Cooke BA, King RJB, van der Molen HJ (eds): Hormones and Their Actions, Part II. New York, NY, Elsevier Science, 1988

12. Casey PJ, Gilman AG: G protein involvement in receptor-effector coupling. J Biol Chem 263:2577-2580, 1988

13. Ros M, Watkins DC, Rapiejko PJ, et al: Glucocorticoids modulate mRNA levels for G-protein beta-subunits. Biochem J 260:271-275, 1989

14. Ros M, Northup JK, Malbon CC: Adipocyte G-proteins and

adenylate cyclase. Effects of adrenalectomy. Biochem J 257:737-744, 1989

15. Dieudonne M-N, Pecquery R, Dausse J-P, et al: Regulation of white adipocyte guanine nucleotide binding proteins Gsα and $Gi\alpha_{1-2}$ by testosterone in vivo: Influence of regional fat distribution. Biochim Biophys Acta 1176:123-127, 1993

16. Levine MA, Feldman AM, Robishaw JD, et al: Influence of thyroid hormone status on expression of genes encoding G protein subunits in the rat heart. J Biol Chem 265:3553-3560, 1990

17. Rapiejko PJ, Watkins DC, Ros M, et al: Thyroid hormones regulate G-protein beta-subunit mRNA expression in vivo. J Biol Chem 264:16183-16189, 1989

18. Begin-Heick N, Heick HM: Increased response of adipose tissue of the ob/ob mouse to the action of adrenaline after treatment with thyroxin. Can J Physiol Pharmacol 55:1320-1329, 1977

19. Begin-Heick N: Quantification of the alpha and beta subunits of the transducing elements (Gs and Gi) of adenylate cyclase in adipocyte membranes from lean and obese (ob/ob) mice. Biochem J 268:83-89, 1990

20. Strassheim D, Palmer T, Milligan G, et al: Alterations in G-protein expression and the hormonal regulation of adenylate cyclase in the adipocytes of obese (fa/fa) Zucker rats. Biochem J 276:197-202, 1991

21. Bushfield M, Griffiths SL, Murphy GJ, et al: Diabetes-induced alterations in the expression, functioning and phosphorylation state of the inhibitory guanine nucleotide regulatory protein G_i-2 in hepatocytes. Biochem J 271:365-372, 1990

22. Gettys TW, Ramkumar V, Uhing RJ, et al: Alterations in mRNA levels, expression, and function of GTP-binding regulatory proteins in adipocytes from obese mice (C57BL/6J-ob/ob). J Biol Chem 266:15949-15955, 1991

23. Levine MA, Ahn TG, Klupt SF, et al: Genetic deficiency of the alpha subunit of the guanine nucleotide-binding protein Gs as the molecular basis for Albright hereditary osteodystrophy. Proc Natl Acad Sci USA 85:617-621, 1988

24. Begin-Heick N: Absence of the inhibitory effect of guanine nucleotides on adenylate cyclase activity in white adipocyte membranes of the ob/ob mouse. J Biol Chem 269:6187-6193, 1985

25. Dehaye JP, Winand J, Christophe J: Adenylate cyclase activity in the epididymal adipose tissue from obese-hyperglycaemic mice. Diabetologia 15:45-51, 1978

26. Collins S, Daniel KW, Rohlfs EM, et al: Impaired expression and functional activity of the beta-3 and beta-1 adrenergic receptor in adipose tissue of congenitally obese mice (C57BL/6J-ob/ob). Mol Endocrinol 8:518-527, 1994

27. Surwit RS, Kuhn CM, Cochrane C, et al: Diet-induced type II diabetes in C57BL/6J mice. Diabetes 37:1163-1167, 1988

28. Surwit RS, Seldin MF, Kuhn CM, et al: Control of expression of insulin resistance and hyperglycemia by different genetic factors in diabetic C57BL/6J mice. Diabetes 40:82-87, 1991

29. Rodbell M: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem 239:375-380, 1964

30. Gettys TW, Blackmore PF, Redmon JB, et al: Short-term feedback regulation of cAMP by accelerated degradation in rat tissues. J Biol Chem 262:333-339, 1987

31. Morre DJ, Morre DM: Preparation of mammalian plasma membranes by aqueous two-phase partition. Biotechniques 7:946-958, 1989

32. Goldsmith P, Gierschik P, Milligan G, et al: Antibodies directed against synthetic peptides distinguish between GTP-binding proteins in neutrophil and brain. J Biol Chem 262:14683-14688, 1987

33. Goldsmith P, Rossiter K, Carter A, et al: Identification of the GTP-binding protein encoded by Gi3 complementary DNA. J Biol Chem 263:6476-6479, 1988

34. Raymond JR, Olsen CL, Gettys TW: Cell-specific physical and functional coupling of human 5-HT1A receptors to inhibitory G protein alpha-subunits and lack of coupling to Gs-alpha. Biochemistry 32:11064-11073, 1993

35. Green N, Alexander H, Olson A, et al: Immunogenic structure of the influenza virus hemagglutin. Cell 28:477-487, 1982

36. Linder ME, Gilman AG: Purification of recombinant Gi-alpha and Go-alpha proteins from *Escherichia coli.* Methods Enzymol 195:202-215, 1991

37. Uhing RJ, Polakis PG, Snyderman R: Isolation of GTP-binding proteins from myeloid HL-60 cells. J Biol Chem 262:15575-15579, 1987

38. Mumby S, Pang I-K, Gilman AG, et al: Chromatographic resolution and immunologic identification of the alpha-40 and alpha-41 subunits of guanine nucleotide-binding regulatory proteins. J Biol Chem 263:2020-2026, 1988

39. Cochran WG, Cox GM: Experimental Designs. New York, NY, Wiley, 1957

40. Strathmann M, Wilkie TM, Simon MI: Diversity of the G-protein family: Sequences from five additional alpha subunits in the mouse. PNAS 86:7407-7409, 1989

41. Strathmann M, Simon MI: G protein diversity: A distinct class of alpha subunits is present in vertebrates and invertebrates. PNAS 87:9113-9117, 1990

42. Jones DT, Reed RR: Molecular cloning of five GTP-binding protein cDNA species from rat olfactory neuroepithelium. J Biol Chem 262:14241-14249, 1987

43. Casey PJ, Fong HK, Simon MI, et al: Gz, a guanine nucleotide-binding protein with unique biochemical properties. J Biol Chem 265:2383-2390, 1990

44. Hepler JR, Gilman AG: G proteins. Trends Biochem Sci 17:383-387, 1992

45. Exton JH: Role of G proteins in activation of phosphoinositide phospholipase C. Adv Second Messenger Phosphoprotein Res 28:65-72, 1993

46. Bourne HR, Stryer L: G proteins: The target sets the tempo. Nature 358:541-543, 1992

47. Pitcher JA, Inglese J, Higgins JB, et al: Role of βgamma subunits of G proteins in targeting the β-adrenergic receptor kinase to membrane-bound receptors. Science 257:1264-1267, 1992

48. Kim CM, Dion SB, Benovic JL: Mechanism of β-adrenergic receptor kinase activation by G proteins. J Biol Chem 268:15412-15418, 1993

49. Gawler D, Milligan G, Spiegel AM, et al: Abolition of the expression of inhibitory guanine nucleotide regulatory protein Gi activity in diabetes. Nature 327:229-232, 1987

50. Begin-Heick N: Alpha-subunits of Gs and Gi in adipocyte plasma membranes of genetically diabetic (db/db) mice. Am J Physiol 263:C121-C129, 1992

51. Strassheim D, Milligan G, Houslay MD: Diabetes abolishes the GTP-dependent, but not the receptor-dependent inhibitory function of the inhibitory guanine-nucleotide-binding regulatory protein (Gi) on adipocyte adenylate cyclase activity. Biochem J 266:521-526, 1990

52. Johnson PR, Greenwood MRC, Horwitz BA, et al: Animal models of obesity: Genetic aspects. Annu Rev Nutr 11:325-353, 1991

53. Ashley PL, Ellison J, Sullivan KA, et al: Chromosomal assignment of the murine Gi alpha and Gs alpha genes. Implications for the obese mouse. J Biol Chem 262:15299-15301, 1987

54. Bray GA: The Zucker-fatty rat: A review. Fed Proc 36:148-153, 1977

54a. Kuhn CM, Surwit RS, Feinglos MN: Glypizide stimulates

sympathetic outflow in diabetes-prone mice. Life Sci 56:661-666, 1994

55. Okada S, York DA, Bray GA: Mifepristone (RU 486), a blocker of type II glucocorticoid and progestin receptors, reverses a dietary form of obesity. Am J Physiol 262:R1106-R1110, 1992

56. Saito M, Bray GA: Adrenalectomy and food restriction in the genetically obese (ob/ob) mouse. Am J Physiol 248:E20-E25, 1984

57. Yukimura LM, Bray GA: Effects of adrenalectomy on body weight and the size and number of fat cells in the Zucker (fatty) rat. Endocr Res Commun 103:1924-1928, 1978

58. Freedman MR, Horwitz BA, Stern JS: Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am J Physiol 250:R595-R607, 1986

59. Castonguay TW, Dallman MF, Stern JS: Some metabolic and behavioral effects of adrenalectomy on obese Zucker rats. Am J Physiol 251:R923-R933, 1986

60. Ogawa A, Johnson JH, Ohneda M, et al: Roles of insulin resistance and β-cell dysfunction in dexamethasone-induced diabetes. J Clin Invest 90:497-504, 1992

61. Johnson JH, Ogawa A, Chen L, et al: Underexpression of β cell high K_m glucose transporters in noninsulin-dependent diabetes. Science 250:546-549, 1990

62. Orci L, Ravazzola M, Baetens D, et al: Evidence that down-regulation of β-cell glucose transporters in non–insulin-dependent diabetes may be the cause of diabetic hyperglycemia. Proc Natl Acad Sci USA 87:9953-9957, 1990

63. Feve B, Baude B, Krief S, et al: Inhibition by dexamethasone of β3-adrenergic receptor responsiveness in 3T3-F442A adipocytes. Evidence for a transcriptional mechanism. J Biol Chem 267:15909-15915, 1992

64. Muzzin P, Revelli JP, Kuhne F, et al: An adipose tissue–specific beta-adrenergic receptor. J Biol Chem 266:24053-24058, 1991