Endocrine regulation of G-protein subunit production in an animal model of type 2 diabetes mellitus

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Abstract

Adipocyte β -adrenergic sensitivity is compromised in animal models of obesity and type 2 diabetes. Although changes in the membrane concentrations of G-protein alpha subunits ($G\alpha$) have been implicated, it remains to be determined how these changes are affected by insulin resistance in the different animal models. Because previous studies used young animals, we measured the concentrations of $G\alpha$ and $G\beta$ subunits in epididymal fat from aged (48 weeks old) *db/db* mice and from their lean littermates to more closely reproduce the model of type 2 diabetes mellitus. Levels of immunoreactive $G\alpha$ s, $G\alpha_{i1/2}$, $G\alpha_o$ and $G\alpha_q/11$ were all significantly greater in adipocyte membranes from the *db/db* mice than in membranes from their lean non-diabetic littermate controls. Levels of $G\alpha_i$ and $G\alpha_i$ were also individually determined and although they appeared to be slightly higher in *db/db* membranes, these differences were not significant. Although the levels of both $G\alpha$ s isoforms were elevated, levels of the 42 and 46 kDa proteins rose by approximately 42% and 20% respectively, indicating differential protein processing of $G\alpha$ s. By contrast, levels of $G\alpha_i3$ were similar in the two groups. The levels of common $G\beta$ and $G\beta_2$ were also

elevated in *db/db* mice, whereas $G\beta_1$ and $G\beta_4$ levels were not different. To determine whether these changes were due to insulin resistance *per se* or to elevated glucocorticoid production, G-protein subunit levels were quantified in whole cell lysates from 3T3-L1 adipocytes that were stimulated with different concentrations of either insulin or corticosterone. Although none of the subunit levels was affected by insulin, the levels of both $G\alpha$ s isoforms were increased equally by corticosterone in a concentration-dependent manner. Since glucocorticoids are known regulators of $G\alpha$ s gene expression in many cell types and in adipocytes from diabetic rodents, the results presented herein appear to more accurately reflect diabetic pathophysiology than do those of previous studies which report a decrease in $G\alpha$ s levels. Taken together, these results indicate that most of the selective changes in G-protein subunit production in adipocytes from this animal model of type 2 diabetes may not be due to diminished insulin sensitivity, but may be due to other endocrine or metabolic abnormalities associated with the diabetic phenotype.

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Introduction

The signal transduction process of many ligand-activated transmembrane receptors begins with the activation of heterotrimeric guanine nucleotide-binding proteins (G-proteins) which ultimately help to mediate several physiological phenomena including photoreception, olfaction, synaptic transmission, membrane depolarization, muscle contraction and metabolism (Emala *et al.* 1994, Neer 1995). Each G-protein is composed of a single α subunit monomer ($G\alpha$) and a $\beta\gamma$ heterodimer ($G\beta\gamma$). At least 16 mammalian $G\alpha$ proteins have been identified and are divided into four distinct families based on sequence homology and biochemical characteristics: $G\alpha_s$, $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12}$ (Hamm & Gilchrist 1996). Although $G\alpha$ genes

are differentially expressed in different tissues, individual cell types express $G\alpha$ subunits from multiple families. By contrast, only five mammalian $G\beta$ subunits have been identified to date (Fong *et al.* 1986, 1987, Levine *et al.* 1990, von Weizsacker *et al.* 1992, Watson *et al.* 1994). Unlike the numerous and structurally distinct $G\alpha$ subunits, $G\beta_1$ –5 are relatively well conserved and belong to a single $G\beta$ family. Whereas $G\beta_3$ and $G\beta_5$ are expressed predominantly in the brain and retina, the remaining $G\beta$ homologs are ubiquitously expressed.

Ligand activation of G-protein-coupled receptors (GPCRs) facilitates $G\alpha$ binding to the receptor and the exchange of prebound GDP for cytosolic GTP. Dissociation of the trimeric complex results from a reduced affinity of the GTP-bound $G\alpha$ for the $G\beta\gamma$ dimer and

allows both to freely interact with their appropriate intracellular effectors. Regulator of G-protein signaling (RGS) proteins activate the intrinsic GTPase activity of $G\alpha$ which, in turn, hydrolyzes GTP to GDP and ultimately restores the G-protein to its inactive heterotrimeric state (Emala *et al.* 1994, Neer 1995). Although G-proteins typically associate with seven-transmembrane domain GPCRs, recent studies suggest that they also participate in transducing signals from receptor tyrosine kinases (RTKs), including receptors for insulin, insulin-like growth factor-I and epidermal growth factor (Ramirez *et al.* 1995, Butler *et al.* 1996, Krieger-Brauer *et al.* 1997, Imamura *et al.* 1999).

The lipolytic actions of catecholamines are mediated by β -adrenergic receptors that activate $G\alpha$ s and consequently increase adenylyl cyclase activity. However, adipose tissue becomes insensitive to catecholamines in obese humans with type 2 diabetes mellitus and in animal models of these disorders. In most instances, this occurs despite normal receptor concentrations and binding kinetics and in the presence of a functionally active adenylyl cyclase (Begin-Heick & McFarlane-Anderson 1992). Although previous studies have implicated changes in membrane concentrations of specific $G\alpha$ subunits as the basis for this reduced catecholamine sensitivity, it remains to be determined how the production of different G-protein subunits are affected by insulin resistance and whether these changes contribute to the pathogenesis of type 2 diabetes. Strassheim *et al.* (1991a) showed that levels of $G\alpha_i$ in adipocyte membranes from *db/db* mice were elevated twofold over those from the control mice whereas the levels of $G\alpha_i$, $G\alpha_o$, $G\alpha_s$ and of common (c) $G\beta$ were unchanged. The same group later reported a decrease in protein levels of $G\alpha_s$, $G\alpha_i$ and $G\alpha_o$ in obese (fa/fa) Zucker rat adipocytes (Strassheim *et al.* 1991b). These conflicting results may be due to inherent differences between the animal models. In fact, a comparison between diabetic *db/db* and *ob/ob* mice indicated that the adipocyte levels of $G\alpha_s$, $G\alpha_i$ and $G\alpha_o$ were differentially regulated in these two animal models (Begin-Heick 1992). In another study, levels of the same $G\alpha$ subunits were reduced in most tissues from *ob/ob* mice, but were either unchanged or elevated in a diet-induced model of diabetes (Gettys *et al.* 1995). Moreover, Begin-Heick (1992) previously reported changes in $G\alpha_s$, $G\alpha_i$ and $G\alpha_o$ in fat pad membranes of *db/db* mice by two different methods: adenosine diphosphate (ADP) ribosylation and western immunoblotting. The former technique detected an increase in the fat pad levels of both $G\alpha_s$ isoforms in *db/db* mice while those of the other subunits were not different from the non-diabetic controls. By contrast, the immunoblot results indicated that the levels of $G\alpha_i$, $G\alpha_o$ and the 46 kDa $G\alpha_s$ isoform were all reduced while that of the 42 kDa $G\alpha_s$ isoform was unaffected. Therefore, much of the discrepancy regarding changes in G-protein concentrations associated with obesity-induced diabetes mellitus

may be due to differential physiological responses of the animal models to insulin resistance, and possibly to the methods used to detect the changes.

In order to determine how different G-protein subunits change in response to obesity and type 2 diabetes, we measured their concentrations in epididymal fat pad membranes from a more physiologically relevant model than those previously used – the aged (48 weeks old) insulin-resistant *db/db* mouse. In addition to hyperinsulinemia, obese human and animal models of obesity and type 2 diabetes display increased activity of their hypothalamic–pituitary–adrenal (HPA) axis (Peeke & Chrousos 1995, Bjorntorp 1997, Bjorntorp & Rosmond 1999). Therefore, we additionally measured the levels of different G-protein subunits in fully differentiated 3T3-L1 adipocytes that were stimulated with varying concentrations of insulin or corticosterone.

Materials and Methods

Animals

Eight-week-old male homozygous C57BL/KsJ *db/db* and heterozygous C57BL/KsJ *db/+* mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed for 40 additional weeks at 23 °C, with 12 h light/day and food was available *ad libitum*. All experiments were described in a detailed protocol that was previously approved by the National Institute on Aging's Animal Care and Use Committee and were in compliance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Mice were killed by cervical dislocation followed by decapitation. Epididymal fat pads were subsequently harvested, frozen in liquid nitrogen and stored at –80 °C. Serum glucose and triglycerides were measured by the glucose oxidase and Abbott methods respectively, with a Spectrum CCX spectrophotometer (Abbott Laboratories, Abbott Park, IL, USA). Serum insulin concentrations were determined with a standard double antibody equilibrium radioimmunoassay for rat insulin (Peninsula Labs, San Carlos, CA, USA) in conjunction with [¹²⁵I]-rat insulin tracer (Amersham Pharmacia Biotech Inc., Piscataway, NY, USA).

Microsomal membrane isolation and western blotting

Microsomal membrane fractions were isolated by sucrose gradient ultracentrifugation. Frozen epididymal fat pads were submersed in buffer A (10 mM Tris-HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, 5 µg/ml leupeptin and 10% sucrose) and homogenized with a Polytron homogenizer (Brinkman, Westbury, NY, USA). The lysate was then layered on top of a 44.5% sucrose cushion and centrifuged for 30 min

Table 1 Non-fasting serum glucose, triglycerides and insulin levels in 48-week-old control non-diabetic *db/+* and diabetic *db/db* mice (*n*=15/group)

Genotype	Glucose (mg/dl)	Triglycerides (mg/dl)	Insulin (ng/ml)
Non-diabetic <i>db/+</i>	185 ± 8	101 ± 4	2.5 ± 0.2
Diabetic <i>db/db</i>	640 ± 18***	123 ± 9*	4.8 ± 0.9**

P*=0.03, *P*=0.02, ****P*<0.0001 compared with non-diabetic mice.

at $100\,000 \times g$ at 4 °C. The microsomal fraction was collected from the interface, concentrated by centrifugation and washed twice with buffer A lacking sucrose and was finally resuspended in the same buffer. The protein concentration was determined with the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA) on microsomes that were previously dissolved in 0.1 M NaOH. An equal amount of protein from each sample was then solubilized in SDS-reducing sample buffer and heated at 70 °C for 10 min.

Soluble membrane proteins (15 µg/lane) were electrophoresed on 4–12% gradient polyacrylamide gels under reducing conditions and transferred onto 0.2 µm polyvinylidene difluoride (PVDF) membranes (all from Novex, San Diego, CA, USA). The membranes were first incubated in a blocking buffer containing 5% non-fat milk (Bio-Rad, Hercules, CA, USA) prepared in 20 mM Tris-HCl (pH 7.5), 137 mM NaCl and 0.1% Tween-20 (TBST). They were then washed 3 times for 10 min in TBST and were probed individually with rabbit antisera (1:1000) against *G α s*, *G α i₁*, *G α i₂*, *G α i_{1,2}*, *G α i₃*, *G α o*, *G α q/11*, common (c) *G β* , *G β 1*, *G β 2*, *G β 3* and *G β 4* (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 1% non-fat milk/TBST. Antibody binding was detected using horseradish peroxidase-conjugated goat anti-rabbit antiserum (1:5000; Amersham Life Science, Arlington Heights, IL, USA) in TBST, and positive immunogenic reactions were visualized with the enhanced chemiluminescence reagents (ECL, Amersham Life Science) in combination with Hyperfilm-ECL (Amersham Life Science). Band intensities were densitometrically quantified with the ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). Primary and secondary antibodies were removed with a stripping buffer (Chemicon, Temecula, CA, USA) and each PVDF membrane was again blocked and reprobed with a different primary antiserum. Individual membranes were probed no less than two times and no more than three.

3T3-L1 adipocyte cultures

3T3-L1 preadipocytes were cultured in Dulbecco's minimal essential medium (DMEM) supplemented with 10% calf serum until two days postconfluency. Differentiation was induced by the addition of 0.5 mM

isobutylmethylxanthine, 1 mM dexamethasone and 167 nM insulin to DMEM that was supplemented with 10% fetal bovine serum (FBS). Cells were then incubated for two days in this differentiation medium, two days in DMEM/10% FBS with insulin and two days in DMEM/10% FBS. Fully differentiated cells were then pre-incubated for 48 h in serum-free DMEM before the addition of insulin (0, 0.01, 0.1, 1, 10 and 100 nM) or corticosterone (0, 0.1, 0.5, 1, 5, and 10 µM). After incubating for 16 h, cells were washed twice in PBS, rapidly frozen in liquid nitrogen, scraped and solubilized in 1.5 × Laemmli sample buffer. The levels of different G-protein subunits were determined by western blotting as described above. Membranes were also probed with G β -like antiserum as a positive control for insulin action.

Statistical analysis

Differences between the mean levels of serum glucose, triglycerides and insulin and of different G-protein subunits in control versus *db/db* mice were determined by a Student's *t*-test using Statview for the Macintosh.

Results

Serum glucose, triglyceride and insulin concentrations

The diabetic and insulin-resistant status of the mice was determined by measuring the non-fasting serum concentrations of glucose, triglycerides and insulin. All three indices were significantly elevated in the 48-week-old *db/db* mice as compared with those of control mice (Table 1). The insulin concentration of the *db/db* mice was approximately twice that of the control mice. This is in contrast to substantially younger insulin-resistant *db/db* mice, in which insulin concentrations can be 10-fold greater than those in the non-diabetic heterozygotes (Hunt *et al.* 1976, Leiter *et al.* 1983).

G-protein subunit levels in epididymal fat pads of non-diabetic control and diabetic *db/db* mice

Differences in individual G-protein subunit levels in epididymal fat pads from control and *db/db* mice were

Figure 1 Gα subunit levels in non-diabetic control and diabetic *db/db* mice. An equal amount of membrane protein from epididymal fat pads of non-diabetic control *db/+* ($n=5$) and diabetic *db/db* ($n=5-6$) mice was analyzed by western blotting with antisera against the indicated proteins as described in the Materials and Methods section. Band intensities were quantified by scanning densitometry. Mean differences between specific Gα subunit levels in control and *db/db* fat pad membranes were determined by a Student's *t*-test and are indicated by the presence of a probability (*P*) value. An absence of a *P* value indicates no difference between control and *db/db* band intensities for a given Gα subunit. Levels of Gα_{i1} and Gα_{i2} were also individually determined and although they appeared to be slightly higher in *db/db* membranes, these differences were not significant.

determined by western blotting of equal amounts of plasma membrane protein. The concentrations of Gα_{i1,2}, Gαo and Gαq/11 were all significantly elevated in *db/db* mice by approximately 50%, 70% and 120% respectively, as compared with those in control mice (Fig. 1). By contrast, the levels of Gα_{i3} did not differ between groups, indicating that the aforementioned differences were not due to loading error. Levels of Gα_{i1} and Gα_{i2} were also individually determined (data not shown) and although they appeared to be slightly higher in *db/db* membranes, these differences were not significant. Both isoforms of Gαs were increased in *db/db* membranes; the 42 kDa isoform increased by approximately 40% and the 46 kDa

Figure 2 Gαs levels in non-diabetic control and diabetic *db/db* mice. An equal amount of membrane protein from epididymal fat pads of non-diabetic control *db/+* ($n=5$) and diabetic *db/db* ($n=5-6$) mice was analyzed by western blotting with antisera against the indicated proteins as described in the Materials and Methods section. Band intensities were quantified by scanning densitometry (left histogram) and the 46 kDa/42 kDa Gαs isoform ratio was calculated (right histogram). Mean differences were determined by a Student's *t*-test and are indicated by the presence of a probability (*P*) value. An absence of a *P* value indicates no difference.

isoform by 20% (Fig. 2). This resulted in a 28% decrease in the ratio of the 46 kDa to 42 kDa isoforms.

The membrane concentrations of different Gβ subunits were also determined by stripping and reprobing the same membranes used to evaluate changes in Gα concentrations. Fat pad levels of cGβ and Gβ2 were elevated in *db/db* mice by approximately 350% and 140% respectively, while those of Gβ1 and Gβ4 were equal in both groups (Fig. 3). Although the cGβ antiserum crossreacts with Gβ2, the substantial increase in cGβ cannot be attributed to an increase in Gβ2 alone as the magnitude of change in cGβ levels was considerably greater than that of Gβ2 (approximately 350% versus 140% respectively).

Insulin and glucocorticoid regulation of G-protein subunit production in fully differentiated 3T3-L1 adipocytes

To determine whether the alterations in G-protein subunit levels in *db/db* fat pads were due to insulin insensitivity, glucocorticoids or to other physiological factors, the G-protein subunit profile in fully differentiated 3T3-L1 adipocytes stimulated with varying concentrations of either hormone was determined. Although insulin levels are elevated in *db/db* mice, adipocytes from these animals and from other animal models of obesity and type 2

Figure 3 Gβ subunit levels in non-diabetic control and diabetic *db/db* mice. An equal amount of membrane protein from epididymal fat pads of non-diabetic control *db/+* ($n=5$) and diabetic *db/db* ($n=5-6$) mice was analyzed by western blotting with antisera against the indicated proteins. Band intensities were quantified by scanning densitometry. Mean differences between specific Gβ subunit levels in control and *db/db* fat pad membranes were determined by a Student's *t*-test and are indicated by the presence of a probability (*P*) value. An absence of a *P* value indicates no difference between control and *db/db* band intensities for a given Gβ subunit.

diabetes are resistant to insulin stimulation. If insulin resistance was responsible for increased G-protein subunit production, then the intracellular concentrations of these proteins would be inversely related to insulin concentrations *in vitro*. However, none of the different *Gα* or *Gβ* subunit levels were affected by insulin. By contrast, the level of Gβ-like, a novel WD-40 protein whose expression is upregulated by insulin, increased in a dose-dependent manner (data not shown). These data indicate that the increase in *Gαs*, *Gαi1,2*, *Gαo*, *Gαq/11*, *cGβ* and *Gβ2* in fat pad membranes of *db/db* mice may not be due to insulin resistance *per se* as the levels of these G-protein subunits in insulin-stimulated cells were not different from those in cells cultured in the absence of insulin. Corticosterone failed to alter the concentrations of any G-protein

Figure 4 Corticosterone regulation of *Gαs* and *cGβ* levels in 3T3-L1 adipocytes. Fully differentiated 3T3-L1 adipocytes were serum starved for 48 h before the addition of corticosterone (*B*) at the indicated concentrations. After 16 h, cells were washed three times in PBS, frozen in liquid nitrogen and equal amounts of the whole cell lysates were analyzed by western blotting with antisera against several different G-protein subunits. Levels of *Gαi1*, *Gαi2*, *Gαi3*, *Gαo*, *Gαq/11*, *Gβ1*, *Gβ2*, *Gβ3* and *Gβ4* were unaffected by corticosterone and are not shown. Levels of *cGβ* are shown as a loading control. Similar experiments were conducted with varying concentrations of insulin, although the levels of each subunit discussed were unaffected and are thus not shown. Each corticosterone concentration was repeated within each experiment and each experiment was repeated twice. Representative blots are shown.

subunit measured, except for *Gαs* whose two isoforms increased equally and in a dose-dependent manner that spans physiologically relevant concentrations of the steroid (Fig. 4).

Discussion

The epididymal fat pads from *db/db* mice contained elevated levels of *Gαs*, *Gαi1,2*, *Gαo*, *Gαq/11*, *cGβ* and *Gβ2* by comparison to those in fat pads from the non-diabetic control mice. These differences do not appear to be due to adipocyte insulin resistance as insulin failed to alter the production of any of these subunits in cultured adipocytes. The progression of obesity and ultimately type 2 diabetes in animal models of these disorders is accompanied by changes in the endocrine milieu and include an elevation in the production and circulating levels of glucocorticoid hormones (Peeke & Chrousos 1995, Bjorntorp 1997, Bjorntorp & Rosmond 1999). In non-diabetic rats, adrenalectomy reduces the steady state adipocyte levels of both *cGβ* and *Gαs* while glucocorticoid supplementation restores the levels of both subunits (Ros *et al.* 1989a,b). This suggests that the rise in *Gαs*, *cGβ* and *Gβ2* reported herein may be due to glucocorticoids. Indeed, the levels of *Gαs* were increased by corticosterone in 3T3-L1 adipocytes suggesting that glucocorticoids are capable of modulating *Gαs* levels in these and possibly other adipocytes (Fig. 4). If these effects are conserved among all adipocytes (cell lines and primary cells), corticosterone may not be responsible for the preferential

increase in the 42 kDa isoform that occurs in fat pads of aged *db/db* mice (Fig. 2) since it elevated levels of both *Gαs* isoforms equally *in vitro*. Similarly, the rise in cGβ and Gβ2 may not be attributed to corticosterone as the 3T3-L1 adipocyte levels of these subunits were not affected by this steroid.

The physiological consequences of the changes observed cannot be determined at the moment. Nevertheless, one can presume that changes in the relative concentrations and ratios of many different G-protein subunits may influence the signaling capacity of a multitude of hormones and cytokines. Although the functional capacity of adenylyl cyclase and the binding capacity of catecholamines have been shown to be unaltered in adipocytes from several genetic and diet-induced models of obesity and type 2 diabetes (Begin-Heick & McFarlane-Anderson 1992), concentrations of the β3-adrenergic receptor have been shown to be reduced in adipose tissue from *ob/ob* mice (Begin-Heick 1996). This alone could account for reduced cholinergic tone in this animal model, but not in *db/db* mice where perturbations in catecholamine binding have yet to be described. Notwithstanding, alterations in the intracellular composition of different *Gα* and *Gβ* subunits could probably contribute as well.

In humans, type 2 diabetes characteristically develops later in adulthood and is exacerbated by age. By contrast, hyperphagia develops shortly after birth in *db/db* mice, which rapidly become obese and insulin resistant. The diabetic phenotype worsens with age as animals eventually become ketotic and the insulin content within β-cells and in the circulation drops (Hunt *et al.* 1976, Leiter *et al.* 1983). The aged animals (48 weeks old) used in our study were considerably older than those used in previous studies (Begin-Heick 1992, 1994, Strassheim *et al.* 1991a, 1992) and were displaying typical signs of type 2 diabetes which include hyperglycemia, insulin insensitivity and pancreatic β-cell decompensation. Although hyperinsulinemic and hyperglycemic, the mice used in these other studies were young, only 8–14 weeks old. Furthermore, the reduction in *Gαs* levels previously reported (Begin-Heick 1992) is inconsistent with the glucocorticoid regulation of *Gαs* gene expression in these animals, which display heightened HPA activity (Saito & Bray 1983, Shimomura *et al.* 1987). Thus, the changes in the G-protein subunit profile and concentrations presented herein may be more reflective of the pathophysiology associated with obesity and type 2 diabetes than the results presented in former studies.

Potential changes in *Gβ* subunit levels in animal models of obesity and diabetes, regardless of tissue, have not been well characterized (Begin-Heick & McFarlane-Anderson 1992). To our knowledge, this is the first report of alterations of any kind in the adipocyte membrane concentrations of any *Gβ* subunit. The cGβ antiserum used in our study crossreacts with Gβ1–4. Since the levels of Gβ1 and Gβ4 were not different between diabetic and non-

diabetic mice, the differential increase in cGβ versus Gβ2 may be due to the crossreaction of anti-cGβ with additional and possibly novel *Gβ* subunits.

Insulin-stimulated glucose transport, glycogen synthesis and cell growth are believed to occur primarily in response to the ligand-activated autophosphorylation of the insulin receptor beta subunits and the subsequent phosphorylation and activation of cytosolic kinases and adapter proteins (Cheatham & Kahn 1995). However, early studies on the mechanisms of insulin receptor signaling indicated that some actions of insulin were sensitive to pertussis toxin, suggesting that Gi or Go heterotrimers participated in transducing insulin signals (Heyworth *et al.* 1986, Gawler *et al.* 1987, Pyne *et al.* 1989, Jo *et al.* 1992, 1993a, Butler *et al.* 1996, Krieger-Brauer *et al.* 1997). Two unidentified *Gα* subunits were later shown to interact with specific regions of the insulin receptor beta subunits (Jo *et al.* 1992, 1993a,b) while *Gαi2* was shown more recently to copurify with the insulin receptor (Krieger-Brauer *et al.* 1997). Deficiency of *Gαi2* induces insulin resistance while, conversely, mice overexpressing a constitutively active mutant *Gαi2* do not develop diabetic symptoms when made insulin-deficient by streptozotocin injections (Moxham & Malbon 1996, Zheng *et al.* 1998). More recently, Imamura *et al.* (1999) determined that insulin-induced phosphatidylinositol 3-kinase activation, GLUT4 translocation and consequently, glucose transport into 3T3-L1 adipocytes is dependent on *Gαq/11*. Thus, both structural and functional links have been established between *Gα* subunits and the insulin receptor. If Gi and Gq/11 heterotrimers truly contribute to insulin receptor signaling *in vivo*, their adipocyte membrane concentrations would be expected to fall if such changes were responsible for insulin resistance. Therefore, the increase in their concentrations reported herein are likely symptomatic of the diabetic state, and not causative. Nevertheless, all of the reported changes probably contribute to altered adipocyte physiology and possibly to hormone insensitivity in this animal model of type 2 diabetes.

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