Published in final edited form as:

Int J Obes (Lond). 2010 January ; 34(1): 6–17. doi:10.1038/ijo.2009.222.

The Role of *GNAS* and Other Imprinted Genes in the Development of Obesity

Lee S. Weinstein, M.D., Tao Xie, Ph.D., Ahmed Qasem, Ph.D., Jie Wang, Ph.D., and Min Chen, M.D., Ph.D.

Signal Transduction Section, National Institute of Diabetes, Digestive, and Kidney Disease, National Institutes of Health, Bethesda, MD 20892 USA

Abstract

Genomic imprinting is an epigenetic phenomenon affecting a small number of genes which leads to differential expression from the two parental alleles. Imprinted genes are known to regulate fetal growth and a 'kinship' or 'parental conflict' model predicts that paternally- and maternally-expressed imprinted genes promote and inhibit fetal growth, respectively. In this review we examine the role of imprinted genes in postnatal growth and metabolism, with an emphasis on the *GNAS/Gnas* locus. *GNAS* is a complex imprinted locus with multiple oppositely imprinted gene products, including the G protein α -subunit $G_s\alpha$ which is expressed primarily from the maternal allele in some tissues and the $G_s\alpha$ isoform XL α s which is expressed only from the paternal allele. Maternal, but not paternal, $G_s\alpha$ mutations lead to obesity in Albright hereditary osteodystrophy. Mouse studies show that this phenomenon is due to $G_s\alpha$ imprinting in the central nervous system leading to a specific defect in the ability of central melanocortins to stimulate sympathetic nervous system activity and energy expenditure. In contrast mutation of paternally-expressed XL α s leads to opposite metabolic effects in mice. While these findings conform to the 'kinship' model, the effects of other imprinted genes on body weight regulation do not conform to this model.

Keywords

G protein; genomic imprinting; obesity; pseudohypoparathyroidism; Prader-Willi syndrome; Angelmans syndrome

Introduction

Genomic imprinting is an epigenetic phenomenon affecting a small number of genes which results in partial or complete suppression of gene expression from one parental allele¹. The primary biochemical imprint 'marks' (differences in DNA methylation and/or histone modification) which distinguish the two parental alleles are erased in primordial germ cells, reestablished during oogenesis or spermatogenesis, and maintained in all somatic tissues throughout development. All imprinted genes have one or more regions in which the maternal and paternal allele are differentially methylated. In many cases DNA methylation is associated with silencing of the affected allele as the methylation occurs within the gene's promoter region. In other cases DNA methylation may be present on the active allele, particularly when the methylation silences a *cis*-acting negative regulatory element for the gene promoter or when the methylation occurs on a promoter for an interfering RNA.

The most widely accepted hypothesis for why imprinting occurs is the 'kinship' or 'parental conflict' hypothesis which predicts that paternally and maternally transmitted alleles promote and inhibit fetal growth, respectively ^{2, 3}. This model assumes that father wants to allocate finite resources to his offspring while it is in the mother's interest to conserve resources over multiple litters that she will bear throughout life. In addition to their effects on fetal growth, it is clear that imprinted genes also have important roles in postnatal energy metabolism and in many cases obesity is associated with altered function of imprinted genes. Several human genetic disorders affecting imprinted genes lead to obesity and population studies have identified several chromosomal regions associated with parent-of-origin effects on body weight regulation in humans ⁴⁻⁷. In this review we will summarize the what is presently known about the role of imprinted genes in the development of obesity and regulation of energy balance, with a particular focus on the *GNAS/Gnas* locus.

Organization and Gene Products of *GNAS/Gnas*

GNAS and the mouse ortholog *Gnas* are located within syntenic regions at 20q13.2-13.3 and distal chromosome 2, respectively and have similar overall organization and imprinting patterns ⁸. *GNAS/Gnas* generates multiple gene products through the use of alternative promoters and first exons that splice onto a common exon (exon 2, Fig. 1). The most downstream alternative promoter generates transcripts encoding the ubiquitously expressed G protein α -subunit $G_s\alpha$ that couples many receptors for hormones, neurotransmitters and other extracellular signals to adenylyl cyclase ⁹. $G_s\alpha$ is required for receptor-stimulated cAMP production and most of its downstream effects are mediated via cAMP, which in turn activates signaling molecules such as protein kinase A (PKA) and cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFs). PKA is a serine/threonine kinase which acutely activates many metabolic processes such as lipolysis, gluconeogenesis, and glycogenolysis through direct phosphorylation of enzymes and other factors involved in intermediary metabolism. In addition PKA has more chronic effects on gene expression by phosphorylation of transcription factors such as the cAMP response element binding protein (CREB) ¹⁰. cAMP-GEFs are highly expressed in neuroendocrine cells and may lead to activation of the ras-like protein Rap1. There is some evidence that $G_s\alpha$ may also stimulate other effectors besides adenylyl cyclase and may also be activated by receptors outside of the seven-transmembrane G protein-coupled receptor family ⁸.

The $G_s\alpha$ coding region spans 13 exons (12 exons in mice) and generates two long and two short forms of $G_s\alpha$ protein by alternative splicing of exon 3 ^{9, 11}. The $G_s\alpha$ promoter resides within a unmethylated CpG island (GC-rich region with a high frequency of CpG dinucleotides) ^{9, 12-14}. Although its promoter is not methylated, $G_s\alpha$ is imprinted in a tissue-specific manner, being expressed primarily from the maternal allele in several tissues, including pituitary, thyroid, renal proximal tubules, gonads, and the paraventricular nucleus of the hypothalamus, but is biallelically expressed in most tissues ¹⁵⁻²³. $G_s\alpha$ also appears to be imprinted in neonatal brown adipose tissue (BAT) ²⁴, but is not imprinted in adult BAT or white adipose tissue (WAT) ²⁵⁻²⁷. Temporal changes in *Gnas* regulation appear to occur in adipose tissue in the early postnatal period, as the alternative $G_s\alpha$ isoform XL α s is expressed in neonatal BAT and WAT but this expression is lost within 1-2 weeks ²⁸. Splicing to an alternative terminal exon (N1) located between $G_s\alpha$ exons 3 and 4 generates a neural-specific truncated splice variant of unknown function ($G_s\alpha$ N1) ²⁹.

The most upstream alternative promoter (referred to as *Nesp* in the mouse; Fig. 1) generates transcripts for the neuroendocrine-specific protein of 55 kDa (NESP55) ^{13, 30, 31}. NESP55 is a chromogranin-like protein located within large dense core granules of secretory cells and is presumed to be a cosecretagogue ³² which is totally unrelated to the G protein α -subunit family. It is primarily expressed in neuroendocrine tissues, including the adrenal medulla, pituitary,

hypothalamus, and other midbrain and brainstem regions^{32–35}. The entire NESP55 coding sequence is within its specific upstream exon and *G_sα* exons 2–13 are within the 3' untranslated region of NESP55-specific transcripts^{30, 32}. NESP55 is only transcribed from the maternal allele and its promoter region is methylated only on the paternal allele^{13, 30, 36}. Imprinting of NESP55 in mice is not established until after implantation, suggesting that this region is not a primary imprinting control region for the *GNAS/Gnas* locus¹⁴ but is rather its imprinting is dependent on the promoter for the antisense transcript *Nespas*²⁴ (see Fig. 1 and below).

Downstream of the *Nesp* promoter is another alternative promoter (referred to as *Gnasxl* in the mouse, Fig. 1) that generates transcripts encoding the *G_sα* isoform XLas^{12, 13, 31, 37, 38}. *Gnasxl* is oppositely imprinted to *Nesp*, being transcriptionally active only on the paternal allele and methylated on the maternal allele^{12, 13, 36}. XLas has a long amino-terminal extension encoded by its specific first exon while the remainder of the protein encoded by exons 2–13 is identical to *G_sα*. XLas has been shown to be capable of mediating receptor signaling in a similar fashion as *G_sα* in cultured cells³⁹. XLas has a more restricted tissue distribution than *G_sα*, being primarily expressed in neuroendocrine tissues including the brain, adrenal medulla, pars intermedia of the pituitary, stomach, heart, kidney and pancreatic islets^{37, 40, 41, 42}. Mouse brain regions where XLas is expressed include the hypothalamus, locus coeruleus, and the laterodorsal tegmental, hypoglossal, trigeminal, ambiguous, medullary reticular, raphe obscurus and facial nuclei⁴². XLas is expressed in mouse adipose tissue in the early postnatal period but its expression is lost within two weeks after birth²⁸.

High levels of a neural-specific truncated form of XLas (XLN1) are generated by alternative splicing of XLas to alternative terminal exon N1⁴⁰ which has been proposed to be potential dominant negative regulator of *G_sα* function^{28, 42}. The XLas first exon has been shown to also generate the unrelated protein Alex from a second open reading frame, and this protein has been reported to directly interact with the XLas protein^{38, 43, 44}. Polymorphisms within the XLas exon 1 may generate alternative forms of Alex and XLas which fail to interact with each other⁴⁴.

Just upstream of the XLas promoter and within the same differentially methylated region is the *Nespas* promoter which generates paternally expressed antisense transcripts that traverse the NESP55 exon^{36, 45–47}. Within the *Nespas* promoter region is a primary DNA methylation imprint mark in which methylation is established during oogenesis and maintained throughout development⁴⁸. A mouse model in which the *Nespas* promoter region was deleted confirmed that *Nespas* is the imprint control center for *Nesp* and probably is important for normal imprinting throughout the *Gnas* locus²⁴.

The exon 1A promoter region (also referred to as exon A/B in humans) located just upstream of *G_sα* exon 1 (Fig. 1) is within a differentially methylated region which is a primary imprint control region as methylation on the maternal allele is established during oogenesis^{14, 49, 50}. Exon 1A generates untranslated transcripts from the paternal allele of unknown function.¹⁴ In pseudohypoparathyroidism type 1B, a form of renal parathyroid hormone resistance, maternal-specific methylation of the exon 1A region is absent due to either a failure to establish or maintain the methylation in this region^{50–53}. Based upon this observation it has been suggested that tissue-specific *G_sα* imprinting (suppression of the paternal *G_sα* promoter) is due to the presence of one or more negatively regulatory *cis*-acting elements within the exon 1A differentially methylated region that are both tissue-specific and methylation-sensitive (and therefore does not suppress *G_sα* expression on the methylated maternal allele). Evidence for this model comes from mice in which paternal deletion of the exon 1A region resulted in reversal of *G_sα* imprinting with biallelic expression of *G_sα* in all tissues that were examined^{54, 55}.

Evidence for a Role of *GNAS* in Weight Regulation in Humans

The most direct evidence for an important role of *GNAS* in the regulation of energy balance comes from patients with Albright hereditary osteodystrophy (AHO), a congenital disorder characterized by the presence of short stature, shortening of various long bones in the hands and feet (brachydactyly), subcutaneous ossifications, rounded face, and neurobehavioral abnormalities, which is caused by heterozygous *GNAS* mutations which disrupt $G_s\alpha$ expression or function⁵⁶. Patients in whom the mutation is present on the maternal *GNAS* allele also develop resistance to various hormones, including parathyroid hormone, thyrotropin, growth hormone-releasing hormone, and gonadotropins, a condition known as pseudohypoparathyroidism type 1A (PHP1A) while patients with mutations on the paternal allele develop the features of AHO without multihormone resistance, a condition referred to as pseudopseudohypoparathyroidism (PPHP)^{15, 56}. This is the result of $G_s\alpha$ imprinting in specific hormone target tissues: mutation of the active maternal $G_s\alpha$ allele leads to severe $G_s\alpha$ deficiency and hormone resistance while mutation of the inactive paternal allele has little effect on $G_s\alpha$ expression or hormone sensitivity^{17, 18, 20–22}.

Similar to the parent-of-origin effect on the development of hormone resistance, it has recently been shown that the development of obesity only occurs with maternal, but not paternal, $G_s\alpha$ mutations (PHP1A but not PPHP)⁵⁷. The obesity generally occurs very early (within the first year) and tends to be severe in early childhood, similar to other monogenic forms of obesity (e.g. those secondary to melanocortin 4 receptor [MC4R] mutations).

Few studies have been performed in humans examining the underlying mechanism for the obesity in PHP1A. Although one study showed evidence that adipocytes from PHP1A patients have reduced lipolytic responsiveness to epinephrine due to reduced levels of $G_s\alpha$ /cAMP signaling⁵⁸, the adipose tissue is unlikely to be involved in this parent-of-origin effect on energy balance as studies have shown no evidence for $G_s\alpha$ imprinting in adipose tissue^{25–27}. This same study⁵⁸ did show PHP1A patients to have extremely low circulating norepinephrines, even when compared to similarly obese children without PHP1A, suggesting that the defect may lie in central nervous system (CNS) regulation leading to low sympathetic nervous system activity and metabolic rates. Although food intake has not been systematically examined in these patients, one recent case was reported in which severe obesity developed in the first year of life in the absence of hyperphagia, suggesting that low energy expenditure rates may be the major contributor to obesity in this disorder⁵⁹. There is some evidence that growth hormone deficiency due to growth hormone releasing hormone resistance in the pituitary may contribute to obesity in some cases⁶⁰. Although the incidence of comorbid metabolic abnormalities such as insulin resistance and diabetes in PHP1A has not been systematically examined, recently a case of severe insulin resistance in a young PHP1A patient has been reported⁶¹.

In addition to the monogenic obesity resulting from clear loss-of-function mutations, two single nucleotide polymorphisms have been identified within the *GNAS* locus that have been associated with altered body weight or response to weight loss regimens. In one study in German women with polycystic ovarian syndrome, the common T393C polymorphism within the *GNAS* coding region (which does not alter the protein sequence) was associated with increased obesity and insulin resistance, although it should be noted that no such association was noted in their larger control population⁶². A functional polymorphism within the $G_s\alpha$ promoter region (G vs. A at nucleotide –1211 relative to the $G_s\alpha$ translational start site) was shown to be associated with differences in binding of the transcriptional factor upstream stimulatory factor 1 (USF-1), $G_s\alpha$ expression levels, lipolytic rates in adipocytes, and weight loss on short-term calorie restriction, but was not associated with adiposity at baseline^{63, 64}.

G_sα Imprinting Underlies the Parent-of-Origin Metabolic Effects of *GNAS* Mutations

The initial *GNAS* knockout line that was developed harbored a large insertion mutation within exon 2 ($E2^{-}$)¹⁷ (the metabolic phenotypes of various *Gnas* knockout are summarized in Table 1). Homozygotes were embryonically lethal. Interestingly heterozygous $E2^{-}$ mice showed opposite changes in energy metabolism depending on whether the mutation was present on the maternal ($E2^{m/+}$) or paternal ($E2^{p/+}$) allele⁶⁵. $E2^{m/+}$ mice developed obesity with increased lipid accumulation in BAT and WAT and proportionally increased serum leptin levels. This obesity was primarily associated with reduced resting and total energy expenditure levels, with no evidence for hyperphagia. These effects seemed to be due to reduced activation of the sympathetic nervous system, as these mice had reduced urine excretion of norepinephrine and its metabolites, normal metabolic and biochemical responsiveness to a β 3-adrenergic agonist which only activates adipose tissue, and normal energy expenditure at thermoneutral temperature (30°C) when sympathetic activity is minimized⁶⁵. Despite the presence of obesity, $E2^{m-}$ had very mild hypolipidemia and slightly improved glucose tolerance and insulin sensitivity with increased insulin-stimulated glucose uptake in isolated skeletal muscles⁶⁵. In contrast, $E2^{p/+}$ mice had a severely lean phenotype with markedly increased sympathetic nervous system activity, glucose tolerance, and insulin sensitivity that is the result of XL α s deficiency (see below).

Although NESP55 deficiency could potentially contribute to the $E2^{m/+}$ metabolic phenotype as it is a maternally-expressed *GNAS* gene product which includes exon 2 in its mRNA, this is not likely to be the case, as exon 2 is not within the NESP55 coding region and NESP55 transcripts are still present in $E2^{m/+}$ mice (T.X., L.S.W., unpublished results). Moreover mice with specific disruption of NESP55 expression do not develop metabolic abnormalities⁶⁷. NESP55 is unlikely to significantly contribute to regulation of energy balance in humans as PHP1B patients with NESP55 deficiency due to imprinting defects involving the NESP55 promoter leading to promoter methylation on both parental alleles do not appear to be prone to obesity⁵⁰.

Studies in *G_sα*-specific knockout mice in which *G_sα* exon 1 was deleted ($E1^{-}$) have confirmed that the obesity observed in $E2^{m/+}$ mice is due to loss of expression of *G_sα* from the maternal *Gnas* allele^{25, 26}. Like $E2^{m/+}$ mice, $E1^{m/+}$ mice develop obesity associated with normal food intake but reduced sympathetic nervous system activity and energy expenditure levels in the presence of normal thyroid function. Unlike $E2^{m/+}$ mice, $E1^{m/+}$ mice have a phenotype more reminiscent of metabolic syndrome, in that in addition to obesity $E1^{m/+}$ mice develop glucose intolerance, insulin resistance, and hyperlipidemia²⁶. Mice with a single base missense mutation (V159E) within *G_sα* exon 6 on the maternal allele (known as *Oed* mice) also developed obesity with reduced energy expenditure⁶⁸. As the *Oed* mutation disrupts *G_sα* function without affecting expression of the neural-specific truncated *G_sα* isoform *G_sαN1*, this is consistent with loss of *G_sα* rather than loss of *G_sαN1* as the cause of the obesity that occurs secondary to maternal *GNAS* mutations. Unlike $E1^{m/+}$ mice, *Oed* mice did not develop glucose intolerance or hypertriglyceridemia, although this difference between the two lines may be due to the fact that they were on different genetic backgrounds.

In contrast to $E1^{m/+}$ mice, $E1^{p/+}$ mice with the paternal *G_sα* mutation developed a very minimal increase in adiposity and minimal glucose intolerance and insulin resistance²⁶. Therefore the development of obesity in $E1^{-}$ mice mimics the pattern of inheritance observed in AHO patients, in whom obesity is much more prominent in PHP1A (who have maternal *G_sα* mutations) than in PPHP patients (who have paternal *G_sα* mutations)⁵⁷. These similar observations in both *G_sα* knockout models and AHO patients strongly implicates tissue-specific *G_sα* imprinting as being a required for the metabolic consequences of maternal *G_sα*

mutations, with obesity resulting from severe $G_{s\alpha}$ deficiency in one or more metabolically active tissues due to mutation of the maternal $G_{s\alpha}$ allele and suppressed $G_{s\alpha}$ expression from the paternal allele due to imprinting. To further test this model female $E1^{-}$ mice were mated with males harboring a deletion of the 1A imprinting control region for $G_{s\alpha}$ ⁶⁹. The metabolic phenotype in $E1^{m-/+}$ offspring was completely reversed in those mice which also harbored the paternal 1A⁻ deletion, and therefore had reversal of tissue-specific $G_{s\alpha}$ imprinting, thus confirming that the maternal $G_{s\alpha}$ metabolic phenotype is dependent on tissue-specific $G_{s\alpha}$ imprinting. In addition, mice with only the paternal 1A⁻ deletion had reduced body growth (both lean and fat mass) and hyperactivity, without any measurable changes in food intake or energy expenditure rates ⁶⁹.

The *GNAS* Metabolic Phenotype is Due to $G_{s\alpha}$ Imprinting in the Central Nervous System

$G_{s\alpha}$ is ubiquitously expressed and therefore any tissue involved in regulation of energy balance and glucose metabolism is a potential candidate as being the site where $G_{s\alpha}$ imprinting produces the parent-of-origin metabolic effects of $G_{s\alpha}$ mutations. Liver, adipose tissue, and muscle are not likely candidates as $G_{s\alpha}$ expression is not affected by imprinting in these tissues ^{8, 17, 25, 27, 65}. Moreover mice with loss of $G_{s\alpha}$ expression in liver, muscle, adipose tissue, or pancreatic β cells do not develop the metabolic phenotype observed in mice with maternal $G_{s\alpha}$ mutations in the germline ^{70–73}.

Recent work shows that $G_{s\alpha}$ imprinting in the CNS underlies the *GNAS* metabolic phenotype ²³. Mice with CNS-specific disruption of maternal $G_{s\alpha}$ allele (mBrGsKO mice) which were generated by mating female $G_{s\alpha}$ E1-floxed mice with male Nestin-cre mice developed severe obesity starting at age 5–6 weeks which was at least initially not associated with hyperphagia, but rather with lower sympathetic nervous system activity, energy expenditure rates, and activity levels and greater metabolic efficiency (weight gain/calorie intake). In contrast mice with CNS-specific disruption of the paternal $G_{s\alpha}$ allele (pBrGsKO mice) maintained a normal phenotype. The effects in mBrGsKO mice were not due to maternal gestational effects as the mothers were Nestin-cre⁻ and therefore had normal $G_{s\alpha}$ expression levels and metabolic phenotype. Thyroid hormone levels were also normal in mBrGsKO mice.

In addition to abnormal energy balance, mBrGsKO mice also developed hyperglycemia, glucose intolerance, and insulin resistance, while glucose metabolism and insulin sensitivity remained normal in pBrGsKO mice. The abnormalities in glucose metabolism and insulin sensitivity in mBrGsKO mice began prior to the development of obesity and therefore CNS-specific $G_{s\alpha}$ deficiency has a primary effect on peripheral glucose metabolism independent of obesity.

Overall, these findings in BrGsKO mice show that the metabolic phenotype generated by germline maternal $G_{s\alpha}$ mutations can be reproduced by loss of maternal $G_{s\alpha}$ expression in the CNS alone and therefore $G_{s\alpha}$ imprinting in the CNS underlies this parent-of-origin effect. Initial studies have shown that $G_{s\alpha}$ undergoes imprinting in the paraventricular nucleus of the hypothalamus, an area known to be important for central regulation of metabolism ²³. In contrast, there was no evidence for $G_{s\alpha}$ imprinting in the nucleus of the solitary tract, another site that is important for metabolic regulation.

$G_{s\alpha}$ Mutation Leads to Loss of Stimulation of Energy Expenditure by Central Melanocortins

The central melanocortin pathways work downstream of leptin to promote negative energy balance by inhibiting food intake and stimulating sympathetic nervous system activity and

energy expenditure^{74–76}. Proopiomelanortin neurons which synthesize and release melanocortins are located in the arcuate nucleus of the hypothalamus where they are stimulated by leptin. These neurons have axonal projections to the paraventricular nucleus and other CNS sites where they synapse with downstream neurons which express the melanocortin receptors MC3R and MC4R. These receptors are classic seven-transmembrane G protein-coupled receptors which activate $G_s\alpha$ /cAMP pathways. MC4R deficiency leads to obesity in both mice and humans^{75, 77, 78} and MC4R receptors mediate the acute effects of melanocortins on food intake and energy expenditure^{79, 80}. In addition, MC4R also directly regulates glucose metabolism and peripheral insulin sensitivity^{74, 81, 82}. MC3R deficiency leads to only minor changes in adiposity⁷⁵.

In many respects mBrGsKO mice have a similar metabolic phenotype to MC4R knockout mice and therefore the primary defect leading to the metabolic consequences of maternal $G_s\alpha$ mutation may be resistance to central melanocortin action through loss of MC4R/ $G_s\alpha$ signalling. In fact the ability of a central melanocortin agonist to acutely stimulate energy expenditure was significantly impaired in mBrGsKO mice²³. This finding is consistent with the reduction in sympathetic nervous activity and energy expenditure measured at baseline in mBrGsKO, $E1^{m-/+}$, $E2^{m-/+}$, and *Oed* mice^{23, 26, 65, 68, 69}, the absence of diet-induced thermogenesis in mBrGsKO mice (which is known to be dependent on MC4R signalling)^{23, 83, 84}, and the reduction of heart rate and diastolic blood pressure in mBrGsKO mice in the setting of severe obesity²³. Reduced heart rate and blood pressure was also observed in obese patients and mice with MC4R mutations^{85, 86}. Loss of MC4R-mediated stimulation of sympathetic nervous system activity and energy expenditure rates may also underlie the obesity observed in PHP1A patients, as these patients were also shown to have low circulating levels of norepinephrine⁵⁸.

Although MC4R-mediated effects on sympathetic nervous system activity and energy expenditure were impaired in mBrGsKO mice, the ability of the same melanocortin agonist to acutely inhibit food intake was maintained in these same mice (as well as in $E1^{m-/+}$ mice)²³. This finding is consistent with fact that the onset of obesity in mBrGsKO mice is not associated with hyperphagia and in at least one child it was documented that early weight gain occurred in the absence of hyperphagia⁵⁹.

Mice with maternal $G_s\alpha$ mutation had some of the same features as those of MC4R mutations (obesity, reduced sympathetic nervous system activity and energy expenditure, and impaired glucose metabolism), while several features associated with MC4R mutation were absent (hyperphagia, increased linear growth). MC4R signalling stimulates the expression of *Sim1*, a transcription factor expressed in the paraventricular nucleus which mediates some of the physiological actions of central melanocortins⁸⁷. *Sim1* mutations in humans and in mice leads to effects that are reciprocal to those of $G_s\alpha$ mutations, namely obesity due primarily to hyperphagia and increased linear growth, with no effects on energy expenditure or glucose metabolism^{88–92}. Moreover, *Sim1* haploinsufficient mice had an impaired anorexic response to administered melanocortins while the ability of melanocortins to stimulate energy expenditure in these mice were normal⁸⁸, a pattern opposite to what is observed in mBrGsKO mice. It is therefore possible that melanocortins mediate their physiological effects through multiple pathways downstream of MC4R: a $G_s\alpha$ -dependent pathway which mediates the effects on sympathetic nervous system activity, energy expenditure, and glucose metabolism, and a $G_s\alpha$ -independent pathway through *Sim1* that mediates the effects on food intake and linear growth. Further studies are required to determine whether this model is correct, and if so, what are the signals between MC4R and *Sim1* (another G protein such as $G_q\alpha$ or a G protein-independent signaling mechanism).

XLas Has Metabolic Effects Opposite to Those of $G_{s\alpha}$

$E2^{+/P-}$ mice with the $E2^{-}$ mutation on the paternal allele showed a metabolic phenotype opposite to that of $E2^{m-/+}$ mice⁶⁵. Neonatal $E2^{+/P-}$ mice had poor suckling and most died within hours of birth. Adult $E2^{+/P-}$ mice were severely lean with markedly reduced lipid accumulation in WAT and BAT. This change in energy balance was primarily due to a marked increase in sympathetic nervous system activity, energy expenditure, and activity levels. In addition $E2^{+/P-}$ mice had severe hypolipidemia and increased triglyceride clearance with markedly improved glucose tolerance and insulin sensitivity in liver, muscle and adipose tissue^{66, 93}. A similar overall phenotype was also observed in mice with the V159E *Oed* mutation on the paternal allele (referred to as the *Sml* mutation when on the paternal allele)⁶⁸. These mutations disrupt the coding sequences of both $G_{s\alpha}$ and the paternally-expressed $G_{s\alpha}$ isoform XLas. In contrast to $E2^{+/P-}$ and *Sml* mice, $E1^{+/P-}$ mice in which only $G_{s\alpha}$ expression from the paternal allele is disrupted developed a marginal increase in adiposity and very mild insulin resistance²⁶. It therefore appeared likely that the lean, hypermetabolic phenotype observed in $E2^{+/P-}$ and *Sml* mice were the direct consequence of XLas deficiency.

An important role for XLas in metabolic regulation in mice was confirmed in mice with specific deficiency of XLas due to a deletion in the upstream XLas first exon (*Gnasxl*⁻)⁴². The *Gnasxl*⁻ deletion had no effect on *Gnas* imprinting and only produced an abnormal phenotype when it was present on the paternal allele (*Gnasxl*^{+P-}) due to fact that XLas is only expressed from this parental allele. Overall *Gnasxl*^{+P-} mice had a phenotype very similar to that of $E2^{+/P-}$ and *Sml* mice. The initial prominent feature was a failure to suckle which was associated with hypoglycemia and significant neonatal lethality. Reduced suckling in *Gnasxl*^{+P-} mice may be a direct effect on orofacial muscle activity as XLas is expressed in CNS nuclei involved in orofacial motor activity, including the facial, hypoglossal, and trigeminal nuclei⁴².

Gnasxl^{+P-} mice mice, like $E2^{+/P-}$ mice^{65, 66, 93}, had markedly reduced lipid accumulation in BAT and WAT^{28, 42}. Studies of adult *Gnasxl*^{+P-} mice²⁸ showed them to continue to maintain a markedly lean phenotype associated with increased energy expenditure, hypolipidemia with increased triglyceride clearance, and markedly improved glucose tolerance and insulin sensitivity. Based upon gene expression studies, it appears that the *Gnasxl*^{+P-} phenotype is due to increased lipid oxidation in adipose tissue, particularly in BAT. However these changes were not due to cell-autonomous effects on BAT function as *Gnasxl*^{+P-} mice did not have increased metabolic responsiveness to a β_3 adrenergic agonist which specifically activates adipose tissue. Moreover XLas is not expressed in adipose tissue after two weeks of age²⁸. Rather BAT was overstimulated due to the mice having markedly increased sympathetic nervous system activity as they had increased urinary norepinephrine excretion²⁸, similar to $E2^{+/P-}$ mice⁶⁵.

Based upon the similar findings in *Gnasxl*^{+P-}, $E2^{+/P-}$, and *Sml* mice, it is clear that the severe metabolic phenotype found in all of these mice is due to XLas deficiency, which appears to have a dominant effect. It appears that XLas is involved in regulation of sympathetic output from the central nervous system in a manner opposite to that of $G_{s\alpha}$. Whether this occurs by acting at CNS sites that are distinct from $G_{s\alpha}$ or whether it directly inhibits $G_{s\alpha}$ signaling remains to be determined. It is unlikely that XLN1 deficiency plays a significant role in producing the *Gnasxl*^{+P-} phenotype as the *Sml* exon 6 mutation, which produces a similar phenotype, does not alter the coding sequence XLN1⁶⁸. Paternal deletion of 20q13 including *GNAS* has also been associated with feeding difficulties, poor growth, and abnormal adipose tissue distribution in humans⁹⁴. However the significant role that XLas plays in metabolic regulation may be species-specific, as PPHP patients with paternal *GNAS* mutations that disrupt XLas expression or function do not produce a similar phenotype.

Tissue-Specific $G_s\alpha$ Knockout Models

Important insights into the effects of $G_s\alpha$ signaling in specific tissues on metabolic regulation have been derived from various mouse models with tissue-specific $G_s\alpha$ deficiency. In these models both $G_s\alpha$ alleles were disrupted and therefore the effects of imprinting are not relevant. Mice with liver-specific $G_s\alpha$ deficiency (LGsKO) showed effects consistent with loss of glucagon action in the liver, with reduced gluconeogenesis and increased hepatic glycogen synthesis, along with increased insulin sensitivity in other tissues⁷². LGsKO mice were hypoglycemic and hypoinsulinemic in the fed state but maintained normal glucose and insulin levels after prolonged fasting, probably due to prolonged breakdown of increased hepatic glycogen stores and increased extrahepatic gluconeogenesis. LGsKO mice also developed pancreatic islet cell hyperplasia with markedly increased circulating glucagon and glucagon-like peptide 1 (GLP1) levels and inappropriately high levels of insulin release in response to administered glucose. In addition LGsKO mice had significantly reduced adiposity although the mechanisms underlying this effect on energy balance were not apparent from food intake and energy expenditure studies.

Muscle-specific $G_s\alpha$ knockout mice (MGsKO) showed no differences in body weight or composition but did show evidence of glucose intolerance despite the lack of an apparent defect in insulin sensitivity or glucose-stimulated insulin secretion⁷³. This may be due to the fact that these mice had a low muscle mass, confirming an important role for β -adrenergic signaling in maintenance of muscle mass. Interestingly the skeletal muscle fibers in MGsKO mice appeared to shift towards type 1 slow twitch (aerobic) fibers based upon myosin heavy chain subtype and kinetic properties but had lower expression of peroxisomal proliferator-activated receptor γ coactivator 1 α (PGC1 α) and lower oxidative metabolic capacity, which is characteristic of type 2 fast twitch (glycolytic) fibers. Therefore characteristics of muscle fiber type based upon kinetic and other properties can be dissociated from their metabolic characteristics. Finally mice with loss of $G_s\alpha$ expression in pancreatic β cells (β GsKO) developed early postnatal insulin-deficient diabetes with a severe defect in β cell proliferation⁷⁰. Although it has been proposed that $G_s\alpha$ signaling promotes β cell growth through activation of the Irs2-Pdx1 pathway⁹⁵, expression of both of these signaling proteins was unaffected in β cells in β GsKO mice. These mice also had a severe defect in linear growth and reduced adiposity.

Prader-Willi and Angelman's Syndromes

Prader-Willi syndrome (PWS) is a common congenital disorder that in addition to severe hyperphagia and obesity, is also associated with short stature, muscular hypotonia, neurobehavior abnormalities and dysmorphic features⁹⁶. Severe hyperphagia is presumed to be secondary to a primary hypothalamic defect. PWS is also associated with abnormally high levels of the orexigenic peptide ghrelin, although in one study reducing ghrelin levels in PWS patients with somatostatin did not correct the hyperphagia⁹⁷. PWS patients also have increased lipoprotein lipase activity levels suggesting that in addition to hyperphagia, there may be abnormalities in lipid uptake in adipose tissue⁹⁸. PWS is associated with uniparental disomies, deletions, or abnormal imprinting of 15q11-13 leading to loss of expression of a series of contiguous paternally-expressed imprinted genes in this region, including *NDN*, *MAGEL2*, *MKRN3*, *SNURF-SNRPN*, and several sno-RNAs⁹⁹. As no specific gene mutation has been found to cause PWS, this disorder is believed to be a contiguous gene syndrome which results from the combined effects of loss of several genes. However there is some evidence that two of these genes (*MAGEL2*, *NDN* [Necdin]) may be involved in abnormal hypothalamic development underlying the hyperphagia and obesity in PWS¹⁰⁰.

Angelman's syndrome (AS) is characterized by severe mental retardation, ataxia, seizures, a happy disposition, and obesity. AS is associated with reciprocal uniparental disomies or

deletions of the same 15q11-13 region as that affected in PWS. AS is believed to be primarily due to loss of expression of the ubiquitin protein ligase E3A, the product of the *UBE3A* gene which is located just telomeric to the PWS region, as mutations affecting this gene have been associated with AS¹⁰¹. *UBE3A* undergoes tissue-specific imprinting in the CNS. However *Ube3a* knockout mice did not show clear evidence of obesity^{102, 103}. More recent studies have identified another gene *ATP10C* (encoding an ATPase believed to be phospholipid translocase) located nearby *UBE3A* which also undergoes brain-specific imprinting and which leads to obesity, glucose intolerance, and insulin resistance when mutated in mice^{104, 105}.

Other Imprinted Genes Associated with Body Weight Dysregulation

Initial evidence for an important role for imprinted genes, particularly those that are paternally-expressed, in hypothalamic development came from the observation in chimeric mice that androgenetic cells (those disomic for the paternal genome) are prevalent in hypothalamus while parthenogenetic cells (those disomic for the maternal genome) are excluded from the hypothalamus¹⁰⁶. In addition to the potential role of the PWS-associated paternally-expressed genes *NDN* and *MAGEL2* in hypothalamic function discussed above, the paternally-expressed imprinted gene *Peg3* (paternally-expressed gene 3) which encodes a zinc-finger protein is expressed in the hypothalamus and *Peg3* knockout mice develop obesity in adulthood primarily due to reduced sympathetic nervous system activity and metabolic rate¹⁰⁷. While *Igf2* (insulin-like growth factor) is a paternally-expressed imprinted gene which promotes prenatal growth, one mouse model in which *Igf2* expression was reduced in brain developed increased adiposity, suggesting that it may have an opposite effect on postnatal growth¹⁰⁸.

Two imprinted genes have been linked to obesity primarily via effects on adipocyte differentiation and function. *Pref1/Dlk1* (preadipocyte factor 1/Delta, Drosophila Homolog-like 1) is a paternally-expressed gene that is expressed in preadipocytes and which encodes a transmembrane protein which regulates adipocyte differentiation. *Pref1/Dlk1* inhibits the differentiation of preadipocytes into mature adipocytes¹⁰⁹ and *Pref1/Dlk1*-null mice develop adult-onset obesity¹¹⁰ while mice overexpressing *Pref1/Dlk1* in adipose tissue develop a lean phenotype with glucose intolerance¹¹¹. Another imprinted gene which affects adiposity through a primary effect on adipocytes is *Peg1/Mest* (paternally-expressed gene 1/mesoderm-specific transcript) which promotes adipogenesis and obesity when overexpressed and which is induced in WAT in the setting of diet-induced or genetic obesity due to loss of its imprinting^{112–115}.

Conclusion

The *GNAS* locus is a unique gene with oppositely imprinted gene products ($G_s\alpha$ and XLas) which have opposite effects on energy and glucose metabolism. These reciprocal effects occur primarily through opposite CNS regulation of sympathetic nervous system activity and energy expenditure. The effects of maternal and paternal mutations of the *GNAS* locus on postnatal growth are consistent with pattern predicted by that predicted by the 'kinship' theory of imprinting, in that paternal *Gnas* mutation leads to XLas deficiency and a severely lean phenotype in mice while maternal *GNAS/Gnas* mutations in mice and humans lead to $G_s\alpha$ deficiency in specific-tissues results and obesity. However when one examines the effects of other imprinted genes on adult energy balance, this same pattern does not hold up. For example, obesity is a feature of both PWS and AS even though these two syndromes result from loss of paternally- and maternally-expressed imprinted genes from the same chromosomal region, respectively. In addition, paternally-expressed imprinted genes can have opposite effects on sympathetic nervous system activity and energy expenditure (e.g. *Gnasxl* and *Peg3*) as well as opposite effects on adipocyte differentiation and function (e.g. *Peg1/Mest* and *Pref1/Dlk1*). In addition, both a paternally-expressed (*Peg3*) and maternally-expressed imprinted gene

(*Gnas*) have similar effects on hypothalamic regulation of sympathetic nervous system activity and energy expenditure.

Acknowledgments

Support: This work was supported by the Intramural Research Program of the National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, U.S. Department of Health and Human Services.

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Fig. 1.

Organization and imprinting of the *GNAS/Gnas* locus. The maternal (top) and paternal (bottom) alleles of *GNAS/Gnas* are depicted with regions of methylation shown above and splicing patterns shown below for each allele. The regions designated *Nesp*, *Nespas-Gnasxl*, and *Gnas* in mice are indicated at the top. Active promoters are indicated with horizontal arrows while inactive promoters are indicated with shaded boxes. First exons for transcripts encoding NESP55 (NESP), *Nespas*, *XL α s*, and *G α* (exon 1) are shown along with downstream exons 3–13 and the alternative neural-specific terminal exon N1 (exons 4–13 are shown as a single box). The dashed horizontal line at the paternal *G α* promoter indicates that transcription is suppressed due to imprinting in a tissue-specific manner. For clarity only the first exon for *Nespas* is shown. The figure is not drawn to scale.

Table 1Metabolic Phenotypes of *Gnas* Knockout Models

Model	Gene Defect	Transcripts Disrupted	Metabolic Phenotype	References
E2 ^{m-/+}	G _s α exon 2 insertion (maternal)	<ul style="list-style-type: none"> G_sα (maternal); ? other maternal <i>Gnas</i> transcripts 	<ul style="list-style-type: none"> ↑ BAT mass Obesity ↓ Energy expenditure ↓ Sympathetic activity ↓ Activity levels ↑ Glucose tolerance ↑ Insulin sensitivity 	17, 65, 66
E1 ^{m-/+}	G _s α exon 1 deletion (maternal)	<ul style="list-style-type: none"> G_sα (maternal) 	<ul style="list-style-type: none"> Severe obesity Severe glucose intolerance Severe insulin resistance ↓ Energy expenditure ↓ Sympathetic activity Phenotype reversed with reversal of G_sα imprinting secondary to paternal 1A⁻ deletion 	25, 26, 55, 69
<i>Oed</i>	G _s α exon 6 Val ¹⁵⁹ Glu point mutation (maternal)	<ul style="list-style-type: none"> G_sα (maternal) ? other maternal <i>Gnas</i> transcripts not G_sαN1 	Similar phenotype to E2 ^{m/+}	68
E2 ^{+/-p-}	G _s α exon 2 insertion (paternal)	<ul style="list-style-type: none"> G_sα (paternal); XLas, XLN1 	<ul style="list-style-type: none"> Poor suckling at birth Perinatal hypoglycemia Small body size ↓ BAT mass Reduced adiposity ↑ Energy expenditure ↑ Sympathetic activity ↑ Activity levels ↑ Glucose tolerance Markedly ↑ insulin sensitivity 	17, 65, 66, 93
E1 ^{+/-p-}	G _s α exon 1 deletion (paternal)	<ul style="list-style-type: none"> G_sα (paternal) 	<ul style="list-style-type: none"> Very mild obesity Very mild insulin resistance 	25, 26
<i>Sml</i>	G _s α exon 6 Val ¹⁵⁹ Glu point mutation (maternal)	<ul style="list-style-type: none"> G_sα (paternal) XLas Not XLN1 	Similar to E1 ^{+/-p-}	68
<i>Gnasxl^{+/-p-}</i>	XLas exon 1 deletion	<ul style="list-style-type: none"> XLas, XLN1 	<ul style="list-style-type: none"> Similar to E1^{+/-p-} Normal responsiveness to β3 agonist 	28, 42

Model	Gene Defect	Transcripts Disrupted	Metabolic Phenotype	References
NESP55 KO	<i>Nesp</i> deletion	<ul style="list-style-type: none"> NESP55 	<ul style="list-style-type: none"> No metabolic phenotype 	67
mBrGsKO	CNS-specific $G_s\alpha$ exon 1 deletion (maternal)	<ul style="list-style-type: none"> $G_s\alpha$ (maternal in CNS) 	<ul style="list-style-type: none"> Severe obesity Severe glucose intolerance Severe insulin resistance ↓ Energy expenditure ↓ Sympathetic activity Normal food intake Impaired stimulation of energy expenditure by melanocortin agonist Normal inhibition of food intake by melanocortin agonist ↓ Diet-induced obesity 	23
pBrGsKO	CNS-specific $G_s\alpha$ exon 1 deletion (both alleles)	<ul style="list-style-type: none"> $G_s\alpha$ (paternal in CNS) 	Normal phenotype	23
LGsKO	Liver-specific $G_s\alpha$ exon 1 deletion (both alleles)	<ul style="list-style-type: none"> $G_s\alpha$ (both alleles in liver) 	<ul style="list-style-type: none"> ↓ Adiposity ↓ Gluconeogenesis ↑ Glycogen synthesis ↑ Glucagon levels ↑ GLP1 levels Islet cell hyperplasia Increased insulin sensitivity Inappropriate insulin secretion 	72
MGsKO	Skeletal muscle-specific $G_s\alpha$ exon 1 deletion (both alleles)	<ul style="list-style-type: none"> $G_s\alpha$ (both alleles in skeletal muscle) 	<ul style="list-style-type: none"> Normal body weight Glucose intolerance Normal insulin sensitivity Normal insulin secretion Reduced muscle mass Switch to type 1 muscle fibers Reduced oxidative capacity in skeletal muscles 	73
β GsKO	β cell-specific $G_s\alpha$ exon 1 deletion (both alleles)	<ul style="list-style-type: none"> $G_s\alpha$ (both alleles in β cells) 	<ul style="list-style-type: none"> Severe insulin-deficient diabetes ↓ β cell proliferation ↓ insulin secretion Lean, poor growth 	70