Annu. Rev. Nutr. 1999. 19:437–61

TRANSGENIC MODELS OF GROWTH HORMONE ACTION

John J. Kopchick,[1,2] *Linda L. Bellush,*[1] *and Karen T. Coschigano*[1]
[1]Edison Biotechnology Institute, Konneker Research Laboratories, and [2]Department of Biomedical Sciences, College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701; e-mail: jkopchick1@ohiou.edu, lbellush1@ohiou.edu, kcoschiga1@ohiou.edu

KEY WORDS: microinjection, metabolism, anti-insulin activity, insulin-like growth factor I, nutrition

ABSTRACT

A growth-promoting principle of the pituitary gland was discovered in 1921, and bovine growth hormone (GH) was isolated in 1944. Since then, the structure of GH as it relates to its biological activities has been an exciting research topic. Equally fascinating is the relationship between GH structure and its metabolic activities. In attempts to define some of these activities, several investigators have used GH transgenic mice as models. In this review we summarize what is known about the molecular mechanisms of GH action. We then describe some of the GH transgenic models and point out potential targets for nutrition research.

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0199-9885/99/0715-0437$08.00

INTRODUCTION

Growth hormone (GH) belongs to a family of hormones that probably evolved from a common precursor (98, 104); the family includes prolactin (Prl) and chorionic somatomammotropin (CS), also known as placental lactogen (PL). GHs are encoded by genes that span approximately 2.0 kb each and contain five exons and four intervening sequences. The translation start and stop codons are located in exons 1 and 4, respectively (98). The genes for Prl and CS also possess this general arrangement.

Members of the GH family are proteins that (*a*) contain approximately 200 amino acids with two (GH) or three (Prl) disulfide bonds (152), (*b*) have molecular masses of approximately 22,000, and (*c*) share similar sedimentation and diffusion coefficients (86, 152). GH molecules from various species are approximately 60–90% identical with respect to amino acid sequence (125).

In a 1987 study, the crystal structure of porcine GH (pGH) reveals an elongated molecule with approximate dimensions of 55 Å by 35 Å by 35 Å (1). The molecule contains four alpha helices tightly packed as antiparallel bundles aligned in up-up-down-down orientations. Of the 191 amino acids of pGH, 54% are contained in these four alpha helices. The molecule also contains a large loop between residues 33 and 75, a smaller loop between residues 129 and 154, and a small loop located at the carboxyl terminus (1).

During the late 1950s and early 1960s, a great deal of work was performed on GH isoforms. The results revealed several heterogeneous types of GH (76, 84, 85, 87, 120, 124, 152). The heterogeneity of GH genes, isohormones, and their variants has been reviewed and is not described here (12).

As the name implies, one of the major functions of GH is in the promotion of growth. In young vertebrates, hyposecretion of GH during development leads to dwarfism and hypersecretion before puberty leads to gigantism. In adults, hypersecretion of GH from selected pituitary adenomas results in the clinical condition known as acromegaly, characterized by enlarged bones of the face, hands, and feet and by fatigue and weight gain. As many as 25% of individuals with acromegaly develop type 2 diabetes, a condition that results from chronically elevated circulating insulin levels and subsequent insulin resistance (136). In healthy adults, GH exerts effects on protein, fat, and carbohydrate metabolism. These effects, as they relate to GH activities, are discussed in this review.

Among the many metabolic activities of GH, two contradictory actions have been described: acute, or early insulin-like activities; and late anti-insulin, or diabetogenic activities. Acute insulin-like activities include hypoglycemia (99, 140), increased glucose and amino acid transport and metabolism (10, 56, 71, 82), increased protein synthesis (82), increased glycogenesis (103, 113), and increased lipogenesis (60, 112). These insulin-like activities are seen

primarily in vitro, or under special circumstances, for example following hypophysectomy. The delayed anti-insulin activities of GH include hyperglycemia (43), hyperinsulinemia (3), increased lipolysis (49, 58), decreased glucose transport (59), increased serum levels of nonesterified fatty acids (72), decreased glucose metabolism (2), and insulin resistance (51, 88, 89). These anti-insulin activities require relatively long periods of GH treatment in cultured cells or in vivo, and are thought to represent a major physiological effect of GH.

In addition to the insulin- and anti-insulin–like activities of the molecule, other metabolic effects of GH include increased rat tibia size (62), altered fat, protein, and carbohydrate metabolism in hypophysectomized animals (42), and enhanced growth rates in transgenic mice (30–36). Additionally, in vitro or in cultured cells GH stimulates transcription of cotransfected reporter genes and stimulates cell division, provided that the GH receptor (GHR) gene is endogenously expressed or expressed via transfection of the cells with recombinant GHRs.

Many of the functional effects of GH are mediated by insulin-like growth factors (IGF-I and IGF-II) produced in liver, bone, and other tissues in response to GH (139). One important experimental task is to determine which GH-associated effects are caused indirectly through IGF activities and which are directly due to GH.

The relationship of the structure of the GH molecule to its biological activity has been the subject of several excellent reviews (11, 12, 81, 98, 105, 112, 125, 152). This article first describes GH-induced signal transduction systems and protein intermediates. We then present data concerning mice that express heterologous GH genes, mutated GH genes or cDNAs which encode GH analogs—including GH antagonists—and mice that lack the gene for GH receptor and GH-binding protein (GHR/BP knock out mice). The possibility of using these animal models for nutritional studies is then discussed.

GH-DEPENDENT SIGNAL TRANSDUCTION

The major site of GH production is in somatotropic cells of the anterior pituitary. Production is regulated by the opposing action of two hypothalamic neuropeptides: GH-releasing hormone (GHRH), which stimulates synthesis and secretion of GH; and somatostatin (SST), which inhibits secretion of GH (for recent reviews, see 80, 139). The essential role of GH in growth promotion is achieved primarily by the GH-induced action of IGF-I. In contrast, regulation of energy metabolism by GH is thought to be mediated by direct interaction of GH with GHR on target cells (57). Thus, GHRs are found in many tissues throughout the body, including liver, muscle, adipose, and kidney (122). The intracellular molecular mechanisms known to date by which the effects of

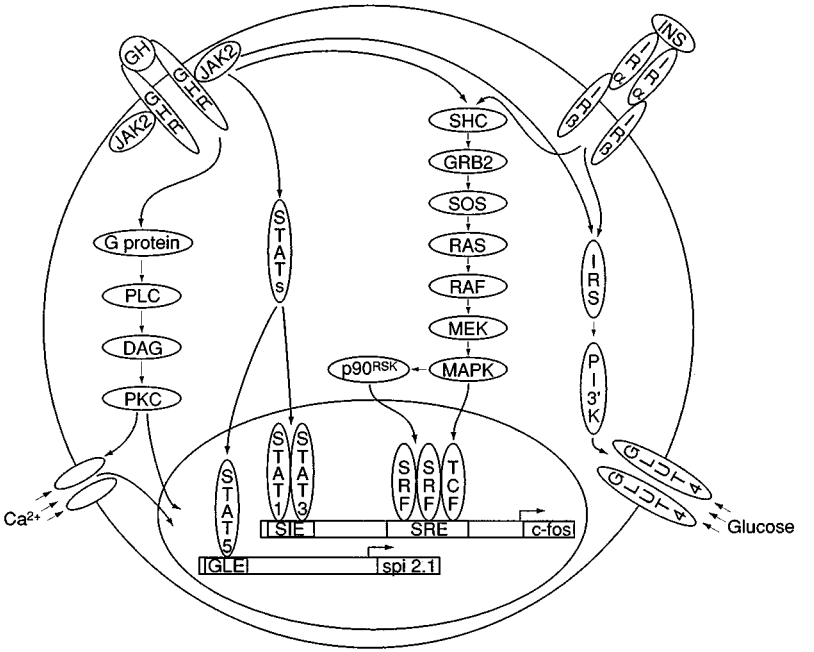

Figure 1 Growth hormone (GH) signaling pathways. Signal transduction pathways purported to be activated by GH in adipocytes. Pathways activated by insulin are also indicated. All abbreviations are defined in the text with the following exceptions: insulin (INS); insulin receptor (IR); and PI 3′-kinase (PI3′K) [Modified from Argetsinger & Carter Su (7)].

GH are mediated have been elucidated primarily by experiments in cultured cells or hypophysectomized rats (for a recent in-depth review, see 7). Signal transduction systems that mediate GH actions in vivo are largely unknown and are the subject of continued research in our own laboratory (21) as well as in those of others. Below, and in Figure 1, we summarize the current state of GH-mediated intracellular signal transduction systems and indicate pathways that may overlap with signal transduction pathways induced by insulin, another nutritionally important hormone.

GH Binding and Receptor Dimerization

A single GH molecule has at least two separate sites through which it interacts with GHR. Experiments utilizing the soluble extracellular domain of GHR indicate that these two GH sites interact sequentially with different receptor molecules, resulting in receptor dimerization (41). Mutational and crystallographic analyses indicate that contact points of site 1, the stronger binding site,

are comprised of amino acids found primarily in alpha helices I and IV as well as in a large loop between alpha helices I and II (40, 45). The weaker binding site—site 2—involves residues at the amino terminus as well as in alpha helices I and III (41, 45). Analysis of transgenic mice over-expressing GH genes mutated in helix III demonstrates that glycine 119 of bovine GH (or glycine 120 of human GH), located in a cleft region of the third alpha helix, is crucial for growth (30, 32, 34, 35). Substitution of this crucial amino acid with another amino acid, with the exception of alanine, results in a GH antagonist (30, 35). Overexpression of GH or a GH agonist in transgenic mice usually results in gigantism; overexpression of GH antagonists results in dwarfism (Figure 2) (30, 32, 34, 35).

Dimerization of the GHR appears to be essential for transduction of the GH signal (52, 157). However, dimerization and subsequent internalization of the GH/GHR complex are not sufficient for GH-induced intracellular signaling because GH antagonists form dimers with the GHR and are internalized (68).

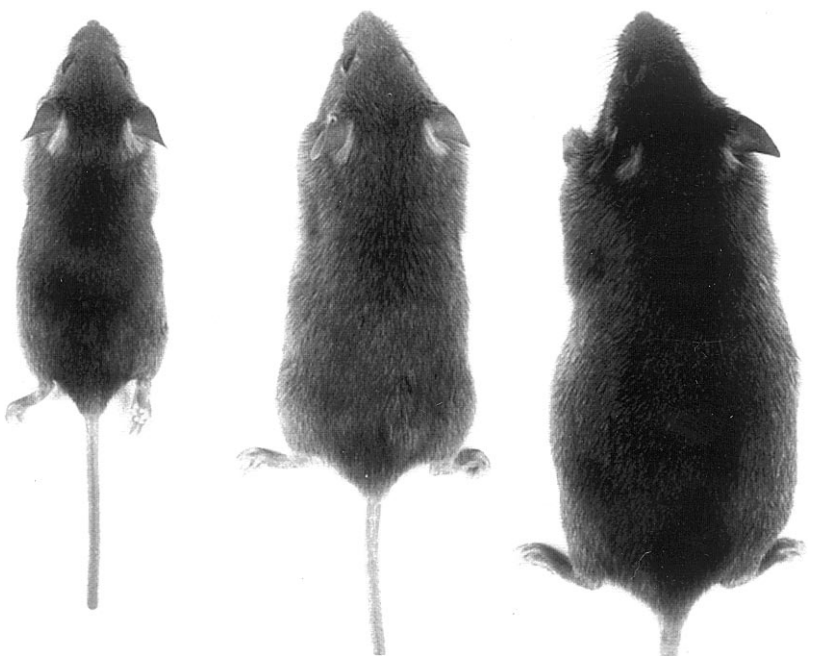

Figure 2 A bovine growth hormone (bGH)-transgenic giant mouse (*right*) and a bGH-G119K transgenic dwarf mouse (*left*) compared with a nontransgenic control mouse of the same age (*center*).

Experiments with monoclonal antibodies seem to indicate that binding of GH, but not of GH antagonists, leads to a conformational change in the extracellular domain of GHR and gives rise to an active dimer configuration that is necessary for signal transduction (93).

JAK Activation

Activation of Janus kinases (JAKs) appears to be an initial step in most signal transduction cascades initiated by GH binding to the GHR (Figure 1). Of the three JAK molecules activated by GH, JAK2 exhibits the greatest degree of activation (6, 75, 133). GH-dependent JAK2 activation requires interaction between JAK2 and the membrane-proximal, proline-rich motif, box 1, located in the intracellular region of GHR (46, 50, 148, 150). Because GHR itself has no intrinsic kinase activity, the bringing together of two JAK2 molecules by the dimerized GHR may result in transphosphorylation of one JAK2 by the other, and thus JAK2 activation (74). Activated JAK2, in turn, phosphorylates GHR on multiple tyrosyl residues, possibly providing docking sites for signal transducer and activator of transcription (STAT)5 (149).

STAT Signaling Pathway

Many of the physiological effects of GH result from transcriptional regulation of a variety of genes. Several different signaling pathways contribute to this regulation (Figure 1), but the most direct pathway involves the STAT proteins. On phosphorylation, cytoplasmic STAT proteins form dimers, translocate into the nucleus, bind DNA, and activate transcription (74, 126).

GH-dependent tyrosyl phosphorylation of STAT1, STAT3, and STAT5 requires JAK2 activation (18, 61, 63, 64, 96, 133, 150, 158). In addition, activation of STAT5 requires regions of GHR not involved in JAK2 activation, which suggests that STAT5 also interacts directly with GHR (133, 137, 149, 150). STAT1 and STAT3 do not exhibit this additional GHR requirement and thus probably interact directly with JAK2 but not with GHR (133, 137, 150).

STAT1, STAT3, STAT5, and possibly STAT4 are found in GH-induced DNA binding complexes of several genes (Figure 1). For example, STAT1, STAT3, and in some cells STAT4 bind to the *sis*-inducible element (SIE) in the c-*fos* promoter (18, 64, 96). Similarly, STAT5 binds to the interferon-γ–activated sequence (GAS)-like response element (GLE) in the serine protease inhibitor (*spi*) 2.1 gene (156). The presence of these proteins is required for maximum transcriptional activation of the genes (14, 26).

MAPK Signaling Pathway

Another GH-inducible pathway involves an extremely complex sequence of protein intermediates. This pathway culminates in transcriptional regulation

of a number of genes and involves activation of two mitogen-activated protein kinases (MAPKs), termed extracellular signal-regulated kinase (ERK) 1 and ERK2 (Figure 1) (5, 19, 100, 153). This pathway, initially described for insulin-mediated signal transduction, probably begins with GH-stimulated binding of SHC variants to phosphorylated residues in both GHR and JAK2, followed by phosphorylation of the SHCs by JAK2 (146). Subsequently, the tyrosyl-phosphorylated SHC proteins interact with growth factor receptor-bound 2 (GRB2), which in turn probably interacts with son of Sevenless (SOS) (146, 147). Finally, GH activates RAS, RAF, and MAP-ERK kinase (MEK; 147). These studies, as well as others (154), implicate the SHC-GRB2-SOS-RAS-RAF-MEK pathway in the activation of MAPK by GH.

GH also activates the S6 kinase, $p90^{RSK}$, most likely via MAPK (5). $p90^{RSK}$, in turn, can phosphorylate serum response factor (SRF), a transcription factor that binds to the GH-responsive serum response element (SRE) of the c-*fos* promoter (97, 118). Another protein, the ternary complex factor $p62^{TCF}$/ELK1, interacts with SRF to bind the SRE, but is directly activated by ERK1 and ERK2 (54, 70), possibly also in response to GH. Further evidence that MAPKs are involved in the GH-dependent transcriptional regulation of c-*fos* is that the same regions of GHR are required for activation of MAPK and for c-*fos* expression (55). As mentioned above, STAT proteins also are involved in regulation of the c-*fos* gene, demonstrating a convergence of two divergent GH signaling pathways in the regulation of a single gene. MAPK is activated by a number of growth factors and thus may represent a common signal transduction point for those growth factors. Activation of STAT proteins (in particular STAT5) may be more specific to GH (69).

The MAPK signaling pathway also may contribute to the ability of GH to stimulate glucose uptake (55). An inhibitor that blocks GH-induced tyrosyl phosphorylation of ERK1 and ERK2, but not of JAK2, partially inhibits GH-stimulated glucose uptake, implicating involvement of inhibitor-sensitive kinases downstream of JAK2 but upstream of ERK1 and ERK2 (55).

IRS Signaling Pathway

In addition to the MAPK pathway intermediates, GH also activates members of another insulin signaling pathway, insulin receptor substrate (IRS)-1 and IRS-2 (Figure 1) (9, 138). Although the nature of the interaction between the IRS molecules and the GHR/JAK2 complex is not clear, it does appear that JAK2 activation is involved in the insulin-like effects of GH and tyrosyl phosphorylation of IRS-1 and IRS-2 (8, 9, 48). Phosphatidylinositol (PI) 3′-kinase also appears to be involved in the insulin-like effects of GH; GH stimulates interaction between the regulatory subunit of PI 3′-kinase and tyrosyl phosphorylated IRS-1 and IRS-2 (8, 9, 116, 117). PI 3′-kinase, in turn, may regulate

translocation of the insulin-dependent glucose transporter (GLUT)4 from low-density microsomes to the plasma membrane, nuclear translocation of ERK1, and activation of protein kinase C (PKC), but whether each of these activities is GH responsive remains speculative (9, 25, 142, 144). Involvement of the IRS signaling pathway in the more physiologically relevant anti-insulin effects of GH has yet to be determined.

PKC Signaling Pathway

Experiments designed to inhibit or deplete PKC activity suggest the involvement of PKC in a number of physiological responses to GH stimulation (Figure 1). These responses include the insulin-like stimulation of lipogenesis (132), induction of c-*fos* gene expression (65, 131), and stimulation of Ca^{2+} uptake (53). In turn, GH-induced Ca^{2+} oscillations seem to be involved in GH-dependent *spi* 2.1 gene transcription (15) and in the refractoriness of certain cells to the insulin-like effects of GH (127).

One pathway for PKC activation involves GH-induced 1,2diacylglycerol (DAG) production by phospholipase C (PLC), possibly coupled to GHR via a G protein (Figure 1) (22, 119). Another proposed pathway for PKC activation involves the IRS/PI 3′-kinase pathway, but its dependency on GH is unclear (7, 142). We have shown that GH promotes activation and translocation of an IRS/PI 3′-kinase activated isoform, PKC-ε, which suggests that GH-dependent activation of PKC also may involve the IRS/PI 3′-kinase pathway (106).

As mentioned earlier, the GH-responsive pathways described have been elucidated mainly by experiments performed in cell culture or under special circumstances in vivo. One challenge researchers are just beginning to address is verification of these pathways in intact animals (37). The ultimate challenge, however, will be the correlation of physiological GH functions, some of which are described below, with each signal transduction pathway.

GH TRANSGENIC MICE

Given the complexities of signal transduction pathways involved in GH functions, the task of identifying molecular mechanisms for individual GH effects has been enormous. Human studies are necessarily limited in scope because of ethical constraints. Studies in animals have depended on repeated administration of GH, often following hypophysectomy, a fact that must be considered in interpreting experimental results. The development of technology for the expression of foreign genes in mammals has allowed the exposure of animals to high or low GH levels without the use of invasive procedures. Furthermore, expression of GH in transgenic animals can be targeted with respect to both developmental stage and specific tissues. As a result, the transgenic approach has led to significant advances in the understanding of GH functions.

GH Transgenic Mouse Models

The first GH transgenic mouse was described in 1982, when Palmiter and Brinster microinjected a fusion gene, consisting of the 5′ transcriptional regulatory element (TRE) (often termed promoter) of the mouse metallothionein-I (MT) gene linked to the rat GH (rGH) gene, into the male pronucleus of fertilized mouse eggs. The eggs were then implanted into pseudopregnant females (110). rGH was incorporated into the genome of a small number of the mice, which expressed the gene and transmitted the DNA to their descendants in a stable, Mendelian fashion. In addition, beginning at about 3 weeks of age, the transgenic mice started growing dramatically larger than their nontransgenic littermates, indicating that the transgene was functional. This initial achievement opened an exciting new approach to the study of gene function in general, and of GH in particular. Other techniques for the insertion of foreign DNA have been developed, but pronuclear microinjection remains the approach most widely used (151). Cloned GH genes from several other species, including human GH (hGH) (30, 111, 161), bovine (bGH) (32–34, 66), and ovine (oGH) (107, 108), have since been incorporated into the mouse genome (see Table 1). Early studies were important in establishing the validity of using GH transgenic mice for evaluation of long-term exposure to elevated levels of GH.

An important consideration when using the transgenic method is the use of heterologous GH genes (i.e. derived from species other than mouse). The similarity of the amino acid sequences of mouse GH and GH from other mammals varies anywhere from 98 (rGH) to 66.3% (hGH). These differences in amino acid sequence could produce unique functional effects (151). This is certainly true with hGH transgenic mice, because hGH has both somatotropic and lactotropic effects, whereas mouse GH is strictly somatotropic. In addition, because the insertion site is thought to be random, the transgene could disrupt the transcription of an endogenous mouse gene, producing functional

Table 1 Transgenic mouse models of GH function[a]

TRE	Gene	Size[b]	Fertility	Kidney	References
MT	rGH	1.87	Decreased	Damaged	110, 155
MT	hGH	2.03	Decreased	Damaged	23, 24, 102, 111
MT	bGH	1.91	Decreased	Damaged	23, 24, 47, 66, 102
MT	oGH	2.0	Unaffected	—	107
MT	hGHRH	2.0	Unaffected	Damaged	47, 66, 67
MT	IGF-I	1.3	—	Enlarged	47, 90, 115
PEPCK	bGH	2.0	Decreased	Damaged	23, 24, 92, 102

[a]GH, growth hormone; TRE, transcriptional regulatory element; MT, metallothionein; PEPCK, phosphoenolpyruvate carboxykinase; rGH, rat growth hormone; hGH, human growth hormone; bGH, bovine growth hormone; oGH, ovine growth hormone; hGHRH, human growth hormone-releasing hormone; IGF-I, insulin-like growth factor.

[b]Fold increase relative to nontransgenic control mice.

changes unrelated to expression of the GH transgene. Similarly, a single copy or many copies of the gene may be incorporated, and copy number could affect expression levels. Finally, GH transgenes are not expressed under control of their own TREs. As a result, GH levels produced in GH transgenic mice are often supraphysiological, and they are expressed constitutively and ectopically in tissues that do not normally release GH. Changes in circulating concentrations of hormones, or in the patterns of their release, can have dramatic impacts on normal hormonal function. Many of these issues can be addressed experimentally. To deal with species differences in GH proteins, mice expressing different heterologous GH genes have been compared. To address the issues of random insertion and multiple copies, measurements have been made comparing several different founder animals in which both factors presumably differ.

The choice of TRE used to express the GH gene is another powerful tool in the experimental analysis of GH action in transgenic mice. Different TREs can result in distinct developmental timing of gene expression. For example, when the phosphoenolpyruvate carboxykinase (PEPCK) TRE is used to drive the bGH gene, expression of bGH first occurs just after birth (92). If the MT TRE is used, bGH expression begins around day 13 of fetal development, although growth enhancement does not occur until 3 weeks postnatally (92). Different TREs also have different response elements, which provide opportunities for experimental regulation of gene expression. For example, the MT TRE contains response elements for both glucocorticoids and heavy metals. The PEPCK TRE has stimulatory response elements for glucocorticoids and cAMP and an inhibitory one for insulin (92).

Tissue expression is also determined by the choice of TRE. For example, PEPCK-GH fusion genes are expressed almost exclusively in liver and kidney, whereas MT-GH fusion genes are expressed in a much wider group of tissues (92). GH was targeted selectively to the pancreas by fusing the hGH gene to the 5′-flanking region of the rat elastase-I gene (109).

The rGH TRE was fused to the diphtheria toxin A-chain gene, which targeted the pituitary somatotrophs, and caused their destruction (73). The resultant GH-deficient mice had a dwarf phenotype. Similarly, the somatotrophs were targeted with a gene that contained the herpesvirus 1 thymidine kinase (HSV1-TK) gene under the control of the rGH, or rat prolactin, TRE (16). Cells expressing the HSV1-TK gene were sensitive to destruction by synthetic nucleotides, making dwarfism inducible in this model. GH-deficient dwarf mice also were produced by using the tyrosine hydroxylase TRE fused to the hGH gene. This targeted hypothalamic cells containing GHRH and SST (77, 83, 141), resulting in feedback suppression of GH release from the pituitary.

Genes for other components of the GH axis, including hGHRH and hIGF-I, also have been introduced into the mouse genome. Instead of introducing supraphysiological levels of heterologous GH, as with other models, expression

of MT-hGHRH caused an increase in circulating levels of endogenous mouse GH (67). The large phenotype of these mice (up to twice the size of nontransgenic littermates) (Table 1) confirms that elevated circulating levels of GH per se are likely responsible for enhanced growth in GH transgenic mice. These mice have very high levels of IGF-I but low levels of endogenous GHRH, as a result of negative feedback inhibition on somatotrophs and on hypothalamic GHRH. IGF-I transgenic mice show some increase in adult weight (Table 1), but unlike other GH models, there is no increase in linear skeletal growth (90, 115).

We have undertaken another approach to investigate the functional roles of GH. Specifically, we carried out oligonucleotide-directed mutagenesis of the GH gene, producing GH analogs that have subsequently been studied in cultured cells as well as in transgenic mice (Table 2) (31, 32, 34, 36, 79). The purpose of this work has been to identify the specific regions of the GH protein involved in various aspects of its functioning, including binding to the GHR, initiation of signal transduction, promotion of growth, and metabolic activities. Much of this work has focused on alpha helix III of the GH molecule, which encompasses amino acids 96–126 (31). A number of transgenic mouse lines have been generated that express individual GH analogs, and the study of these mice has produced important insights. Recently, we also generated a model complementary to the transgenics: specifically, a mouse in which the GHR/BP gene was disrupted or knocked out (GHR-KO) (162). In this mouse, endogenous GH levels are high, but unlike GH transgenic mice, IGF-I levels are very low. In the following section, characteristics of GH transgenic mice are described in detail.

Functional Effects of GH, GHRH, and IGF-I Transgenes

ALTERED GROWTH Expression of any of the GH genes (bGH, hGH, oGH, rGH) in mice, except when targeted selectively to the somatotrophs, leads to enhanced overall growth, with some mice growing to nearly twice normal size (Figure 2; Table 1; 151). The degree of growth enhancement is not always correlated with gene copy number or with circulating levels of the heterologous GH, nor does gene copy number always correlate with circulating levels of the heterologous GH (107, 111, 151). Even offspring of a single founder may vary in the degree of growth enhancement (92, 108). Both hGHRH and IGF-I transgenic mice also weigh significantly more than nontransgenic littermates, but as noted above, IGF-I transgenic mice do not show an increase in linear skeletal growth (115). In our mutation studies, we found that bGH amino acid 119 [glycine (G); amino acid 120 in hGH] is essential for growth-enhancing properties (30, 32, 34, 35). When G119 was changed to any of several amino acids (arginine, G119R; proline, G119P; leucine, G119L; tryptophan, G119W; or lysine, G119K), expression of these bGH analogs resulted in transgenic mice with a dwarf phenotype (Figure 2; Table 2). These analogs of bGH and hGH

Table 2 Characteristics of transgenic mice expressing bGH analogs and of GHR-KO mice[a]

Name	Substitutions	Size[b]	bGH (μg/ml)	IGF-I (ng/ml)	Insulin (ng/ml)	Kidney	References
MT-bGH	None	1.7	0.5–6.0	501 ± 89	3.0–4.0	Damaged	31, 32, 35, 92, 159
M8	E117L, G119R, A122D	0.80	0.6–5.0	119 ± 38	—	Unaffected	32, 35, 159
M10	K114P, E118P	1.0	2.0–3.0	280 ± 53	—	—	32
M11	L121P, E126G	1.0	0.2–10.0	537 ± 29	—	Damaged	33, 78, 159
E117L	E117L	1.7	0.4–5.0	793 ± 83	2.2 ± 0.54	Damaged	27, 29, 31, 34
G119K	G119K	0.65	0.4–10.0	111 ± 26	0.3 ± 0.06	Unaffected	27, 29, 31, 34
GHR-KO	None	0.4	0.13[c]	33 ± 1	—	—	39, 162
NT	None	1.0	0.01[c]	286 ± 44	0.56 ± 0.08	Unaffected	29, 31

[a]bGH, bovine growth hormone; GHR-KO, knocked-out, or disrupted GH receptor gene; IGF-I, insulin-like growth factor; NT, nontransgenic.
[b]Fold increase or decrease relative to nontransgenic control mice.
[c]Endogenous mouse GH.

bound to the liver GHR with affinities equal to that of bGH, and subsequently we showed that they act as functional GH antagonists (30, 32, 34, 35).

We generated other mutations in codons of GH helix III (Table 2). Changing bGH amino acid 117 from glutamic acid to leucine (E117L) had no effect on bGH-induced growth enhancement (34). However, a bGH analog (bGH-M8) with both E117L and G119R plus a third mutation at amino acid 122 (alanine to aspartic acid, A122D) caused a dwarf phenotype (32, 35). Two other bGH analogs, one called bGH-M10 (K114 and E118 both changed to P) and the other called bGH-M11 (L121P and E126G), produced mice of normal size (32, 159). The GHR-KO mouse, like the mice with mutations at bGH amino acid 119 (hGH 120), had dramatically reduced growth (162).

In addition to alterations in general linear growth, GH transgenes affected the size of various organs in a nonallometric fashion. In rGH transgenic mice, body weight nearly doubled, liver and spleen weights were increased threefold, but brain size and tail and hind foot lengths were barely changed (129). In hGH transgenic mice, the liver and kidney showed the greatest increase in growth; the spleen was much less affected (151). Not all organs were enlarged by excess GH. Female mice transgenic for rGH had smaller ovaries than control mice had (66).

To our knowledge, the only examination of the body composition of mice expressing various GH genes was carried out in 1- to 10-week-old male mice expressing either bGH-E117L (giant phenotype), bGH-G119K (dwarf phenotype), or bGH-M11 (L121P-E126G: near-normal size) (78, 143). Both the dwarf and the giant mice displayed increased percentages of fat and decreased percentages of protein relative to M11 and nontransgenic control mice (78). However, during the first 68 days of development, which was the limit of their study, fat represented >72% of total weight gained by G119K mice but only 53%, 35%, and 41% of total weight gained by nontransgenic controls, M11, and E117L mice, respectively. Consistent with this latter finding, G119K also had greater feed efficiency than all other groups. Additionally, in control mice and G119K dwarf mice, protein accretion was decreasing and fat accretion was increasing by 68 days of age, indicating that physiological maturity was achieved. In contrast, rates of protein and ash deposition had not reached a plateau in the E117L and M11 mice, indicating that they had not fully matured by this age (143).

ORGAN DAMAGE Elevated GH was implicated years ago in diabetes-associated proliferative retinopathy, when hypophysectomy was shown to inhibit further development of, and even to reverse, existing retinopathy at the same time that it lowered insulin requirements in individuals with diabetes (4, 135). However, hypophysectomy often worsened renal problems because it led to

rapid increases in renal plasma flow and glomerular filtration rate, which, in turn, outweighed any advantage to the kidney in reducing GH-associated microvascular disease (135). In GH transgenic mice, an unequivocal relationship has emerged between elevated levels of circulating GH and kidney damage; in fact one of the most consistent findings in GH transgenic mice is kidney damage (Tables 1 and 2) (151). Furthermore, whereas both bGH and hGHRH transgenic mice develop severe glomerular sclerosis spontaneously by about 6 months of age, IGF-I transgenic mice develop enlarged glomeruli, but without lesions in the kidney (47, 115). GH antagonist dwarf mice fail to develop renal damage when they are made diabetic with streptozotocin, whereas streptozotocin-treated nontransgenic mice show clear signs of glomerulosclerosis (27, 28). Thus, antagonism of GH action interferes with the development of glomerular lesions. bGH-M8 (E117L-G119R-A122D) dwarf mice also are protected from kidney damage (160), providing further evidence that, in general, GH antagonists protect against this effect of GH. bGH-G119K mice also were protected from ischemia-induced neonatal retinopathy (134). We carried out studies to determine whether enhanced growth and kidney damage were separable effects. Both the bGH-E117L (giant) transgenic mouse and the bGH-M11 (L121P-E126G: normal size) mouse develop glomerular sclerosis (160). Thus different regions of GH appear to regulate growth and kidney damage.

Other organ anomalies have been reported. Liver lesions, including sclerosis and dysplasia, have been noted in oGH, bGH, and hGH transgenic mice (107, 115). IGF-I transgenic mice have skin anomalies, including thickened adipose layers and disrupted collagen bundles (115). Although the only ovarian anomaly reported is reduced size, the transgenic mice showing this (bGH, rGH, and hGH) also show reduced fertility associated with altered prolactin secretion, which causes luteal failure (23, 24, 66, 67, 102). The infertility in bGH transgenic females occurs whether the TRE is MT or PEPCK (102). It is interesting that oGH transgenic mice do not develop this infertility problem (107). Similarly, hGHRH transgenic mice retain fertility (67). Finally, although not as well characterized, male GH transgenic mice have some reduction in fertility (115).

METABOLISM Linear growth ceases in adulthood but GH continues to play a role in physiology, and one function of major importance is the modulation of metabolism. Although there is evidence both in vitro and in vivo that GH can have insulin-like effects, anti-insulin effects are the principal physiological actions of GH (114). Thus, GH promotes lipolysis while helping to maintain normal circulating glucose levels, whereas insulin fosters lipogenesis and glucose uptake (139). GH is released in response to hypoglycemia

and fasting, whereas insulin is released in response to meal consumption or to high circulating glucose (20). It should be noted, however, that in rats, fasting causes a decrease in GH, and thus species differences must be taken into account (17, 114). One anabolic function that is shared by GH and insulin is increased amino acid uptake and protein synthesis, simultaneous with decreased protein breakdown (121). GH, along with epinephrine and glucocorticoids, is released in response to many stressors. Under these circumstances, the latter two hormones exert major anti-insulin actions, and GH likely helps to offset the muscle catabolism caused by glucocorticoids, fostering the utilization of fatty acids for energy production and sparing both protein and glucose. As noted above, chronic elevations in circulating GH, as seen in acromegaly, often lead to the development of hyperinsulinemia, impaired glucose tolerance, insulin resistance, and sometimes frank diabetes (136). Elevated cholesterol and triglyceride levels, both of which are atherogenic, also occur in acromegaly.

In contrast to the severe consequences of excessive GH in adulthood, relatively modest changes have been reported to accompany adult-onset GH deficiency. Nevertheless, these changes may be of clinical significance. Indeed, some researchers believe that aging-associated decline in GH level may contribute to decreases in lean body mass and increases in adipose tissue, long considered unavoidable aspects of aging. Twelve men aged 61 to 81, with IGF-I concentrations of less than 350 U/liter, were treated with synthetic human GH for 6 months. They experienced increases in lean body mass and lumbar vertebral bone density and decreases in adipose tissue mass, along with IGF-I level increases to those in the normal range (500–1500 U/liter) of young adults (123). Young adults may also suffer negative consequences of reduced GH. One review cited epidemiological evidence of reduced life expectancy in hypopituitary adults, due either to pituitary adenoma or as secondary to other diseases, despite apparently sufficient replacement with cortisone, thyroxine, sex hormones, and vasopressin (38). In addition, GH deficiency in younger adults has been associated with psychosocial problems, reduced muscle strength, and reduced exercise capacity, all of which improved with short-term GH treatment (38). Finally, obese women treated with GH experienced a reduction in body fat and an increase in fat-free mass without restricting calorie intake (130).

In general, GH deficiency leads to increased adiposity and decreased lean body mass, the most dramatic effects being in GH-deficient dwarfs (94). A small number of adult growth hormone-deficient dwarfs had severely impaired glucose tolerance and dyslipidemia, which worsened over the course of about 10 years (94, 95). Despite developing glucose intolerance as severe as that seen in type 2 diabetes, these individuals had a very low incidence of coronary heart disease and demonstrated neither retinopathy nor other organ damage usually associated with elevated glucose levels. These effects are dramatic, but

dwarfs experience extremely low GH levels throughout development, not just in adulthood. Studies of the more modest deficiencies seen during aging have yet to provide a clear picture regarding the use of GH replacement therapy to maintain young adult GH levels during midlife and aging (44, 101, 145).

GH transgenic animals could be useful in characterizing the advantages and disadvantages of sustained elevations or deficiencies in GH levels. As seen in acromegalics, hGH and bGH transgenic mice are hyperinsulinemic, particularly when MT is the TRE (92, 115). When driven by the PEPCK TRE, GH levels tend to be inversely related to insulin levels (91). In addition to hyperinsulinemia, female GH and GHRH transgenic mice have elevated cholesterol, a pattern similar to that which occurs in type 2 diabetes (115). In this same report, IGF-I transgenic mice have low levels of insulin, but elevated triglyceride levels, a profile seen in type 1 diabetes that may result from the lack of expression of lipoprotein lipase secondary to insufficient insulin.

Despite hyperinsulinemia, GH transgenic mice always have normal fasting blood glucose levels. This has been interpreted to be a reflection of insulin resistance (92). MT-bGH transgenic mice also have impaired glucose tolerance accompanied by reductions in GLUT4 mRNA (92). Taken together, these changes have been interpreted as demonstrating that the bGH transgenic mouse is a model of acromegaly. Yet, although they show some metabolic perturbations, we know of no reports that MT-bGH mice develop overt diabetes. In fact, rats and mice appear to be resistant to the diabetogenic effects of bGH, which are clearly apparent in dogs (114). Human acromegalics also develop severe insulin resistance, and as many as 50% develop overt diabetes. The reason for the apparent discrepancy in diabetogenesis between the bGH-transgenic mouse and the human acromegalic is not clear. However, there are some possible explanations for this differential susceptibility to diabetes. First, acromegalics experience GH excess only in adulthood. bGH-transgenic mice are exposed to supraphysiological levels of GH beginning at birth or even prenatally, depending on the TRE used. Another important consideration is the fact that hGH exerts both mammotropic and somatotropic effects, whereas bGH exerts only somatotropic effects. Comparing changes in glucose metabolism in hGH transgenic and bGH transgenic mice could address this issue. Perhaps hGH will turn out to be diabetogenic, although bGH is not.

It is likely that diet contributes to the development of insulin resistance and type 2 diabetes in humans, as the vast majority of individuals who develop this disorder are also overweight. We know of no published studies in which diet has been modified in GH transgenic mice in order to examine the contribution of diet to the development of insulin resistance and diabetes. McGrane et al (92) did carry out dietary manipulations in PEPCK-GH mice, but the focus of the study was on the regulation of transgene expression through manipulation

of the TRE. A 1-week exposure to a very high (80%) carbohydrate diet led to a complete suppression of bGH in these mice, whereas a subsequent 1-week exposure to a high protein (64%), carbohydrate-free diet caused a huge increase in bGH expression. These changes in bGH expression were due totally to effects on the PEPCK TRE. High insulin levels induced by the high carbohydrate diet would have turned off PEPCK TRE expression because PEPCK is involved in gluconeogenesis; however, a high-protein, carbohydrate-free diet would cause ketogenesis, which is normally found during fasting and would trigger gluconeogenesis and, thus, PEPCK TRE expression.

We have assessed the effects of a high-fat diet on glucose regulation in bGH-G119K dwarf mice (13). Dwarf mice develop glucose intolerance spontaneously by 8 months of age. Their fasting blood glucose concentrations are normal, whereas their fasting insulin is low, a pattern inconsistent with insulin resistance. We are currently testing the hypothesis that a deficiency in glucose-stimulated insulin release from the pancreas, rather than reduced tissue responsiveness to insulin, causes impaired glucose tolerance. High-fat diets exacerbated already-impaired glucose tolerance and caused dramatic weight gain in male dwarf mice. Female dwarf mice and nontransgenics were equally susceptible to diet-induced weight gain and impaired glucose tolerance. We are now studying adipose tissue from these animals and plan to extend these studies to determine whether the bGH-G119K mice, like human dwarfs, develop pathological lipid profiles and, more important, if they are protected from atherogenesis despite the development of obesity and insulin resistance (94). GHR-KO mice also appear to develop impaired glucose tolerance, although not as severe as that seen in the dwarf mice (39). In addition, we have been assessing glucose homeostasis in MT-bGH (giant) mice. Unexpectedly, 4-month-old bGH transgenic mice have normal glucose tolerance, in contrast to the previous finding that their glucose tolerance was impaired (92). However, consistent with these findings, our bGH mice have normal fasting blood glucose concentrations and elevated fasting insulin concentrations. We are currently investigating the discrepancy in the findings.

CONCLUDING REMARKS

With the generation of different animal models in which genes implicated in GH action have been altered, the field is set for using these animals to test hypotheses regarding the interaction between nutrition and the molecular basis of the biological activity of GH. The effect of GH and food and nutrient intake on in vivo intracellular signaling molecules can now be established. We believe that the use of these in vivo models will help to define endocrine, biochemical, molecular, and physiological principles of GH action as it applies to nutrition.

ACKNOWLEDGMENTS

John J. Kopchick and this work were supported by grants from the following organizations: Ohio University, the Sensus Corporation, the Central Ohio Diabetes Association, the Juvenile Diabetes Foundation International, and the Human Growth Foundation.

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