

## Effects of pulsatile secretion of growth hormone (GH) on fat deposition in human GH transgenic rats

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Growth hormone (GH) is an endocrine regulator of glucose and lipid metabolism as well as body growth. GH levels are decreased and a unique pulsatile secretory pattern becomes obvious after puberty particularly in males. Coincidentally with this, males tend to deposit body fat. Experimental and clinical evidence has accumulated that obesity is associated with a decrease in GH levels. A strain of transgenic rats has been generated with severe obesity but normal nose-to-tail length, which has low circulating GH levels without pulsatility (human growth hormone (hGH) transgenic rats). The present review mainly focuses on recent and current work analysing the relationship between the occurrence of obesity and low GH levels and/or the absence of GH pulsatility in this transgenic animal model. This model has elevated blood glucose, non-esterified fatty acid, insulin and leptin levels associated with hyperphagia, suggesting that these rats also carry insulin- and leptin-resistant characteristics. hGH transgenic rats were subjected to a pair-feeding treatment to normalize food intake and chronic GH replacement to normalize GH levels. While the pair-feeding for 8 weeks successfully suppressed body-weight gain, the fat pad : body weight ratio remained very similar to freely-eating control hGH transgenic rats, which indicates the hyperphagia is not the sole contributor to the excess fat accumulation in this model. However, continuous elevation of peripheral hGH levels (approximately 2-fold) for 8 weeks by means of a slow-release vehicle resulted in a significant decrease in the fat mass : body weight ratios by 30 %. This GH treatment altered neither food intake nor body-weight gain. Thus, two characteristic phenotypes observed in the hGH transgenic rats, hyperphagia and obesity, seem to be closely related to GH levels and GH secretory pattern. This relationship might be working in the regulation of changes in seasonal body composition in wild animals.

### **Growth hormone: Transgenic rats: Obesity: Body composition**

**Abbreviations:** GH, growth hormone; GHD, growth hormone-deficient; hGH, human growth hormone; IRS, insulin receptor substrate; NEFA, non-esterified fatty acid; NIDDM, non-insulin-dependent diabetes mellitus.

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### Introduction

Growth hormone (GH) is a prerequisite for enhancing body growth until puberty in man and animals. After puberty, however, GH effects on phenotypic expressions become more ambiguous and the physiological significance of GH secretion seems to be underestimated. Various animal models for analysing physiological activities of GH have been developed. Several lines of transgenic animals carrying a GH transgene have been reported (Kopchick *et al.* 1999). Due to the over-expression of the transgene, most of these transgenic animals exhibited the gigantic phenotype with an elongated body length. On the other hand, model animals retaining deficiencies either in GH secretion or expression of GH function show the dwarfish phenotype. These GH-excess and -deficient phenotypes in model animals agree with symptoms in acromegalic or GH-deficient (GHD) human subjects.

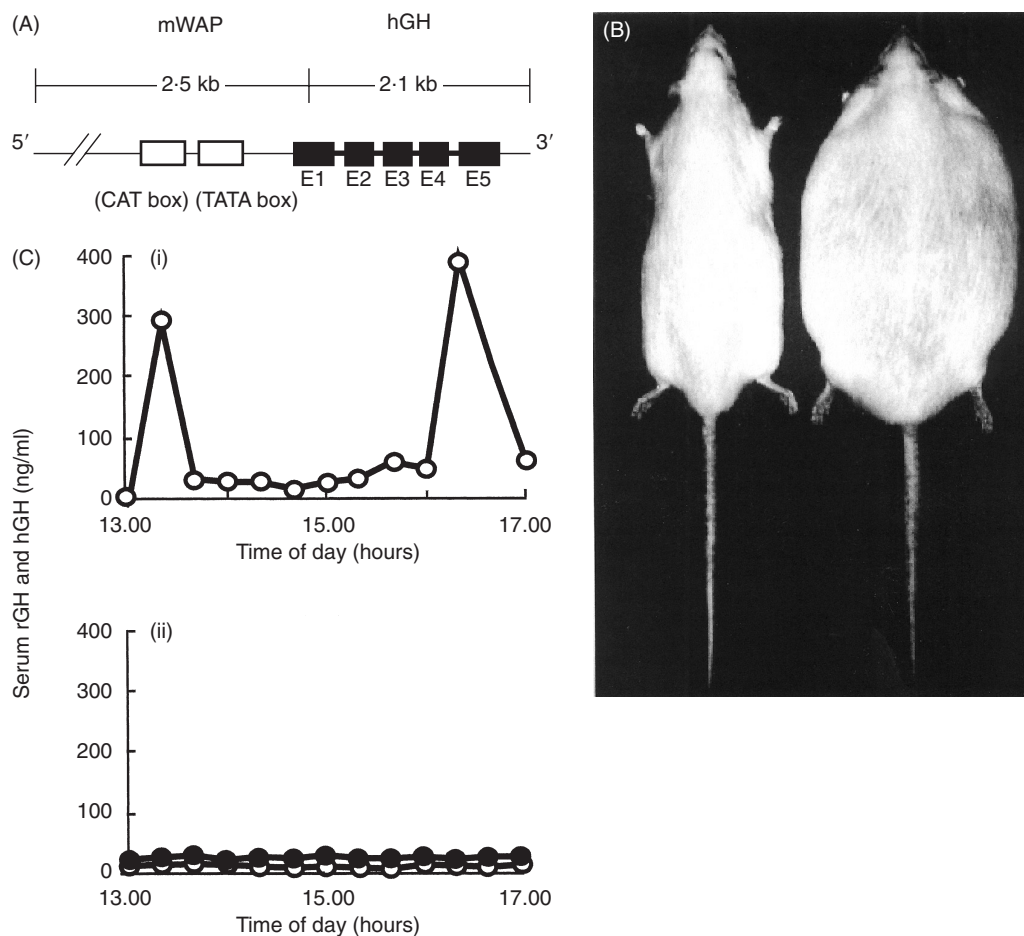
In GHD human adults, frequent occurrence of fat accumulation was reported, which suggests that GH regulates not only body length but also body composition (Davidson, 1987). Compared with human studies in this context, animal studies have not been sufficiently well developed largely due to difficulty in obtaining good animal models. The animal models for GH deficiency have not been used for the obesity research effectively, because their obesity, if any, is usually compensated by a decrease in body growth and they usually do not exhibit an increase in body weight. However, some recent animal models have been characterized by simultaneous low GH levels and obesity. Transgenic mice carrying a chimeric gene of a metallothionein 1a promoter sequence and ovine GH gene have been reported whose ovine GH secretion could be induced by supplementation with Zn but discontinued by the cessation of the supplementation (Eisen *et al.* 1998). Congenital GHD dwarf rats fed on a high-fat diet are another animal model (Clark *et al.* 1996).

Because these animal models express both GH deficiency and obesity symptoms simultaneously, a relationship between GH and obesity was clear. Unfortunately, however, these models have not been utilized effectively to expand the understanding of that relationship until now. The present review highlights some of the authors' recent works towards this goal, by development of a new line of GH transgenic rats expressing both GH deficiency and obesity.

### Characterization of transgenic rats with low circulating growth hormone levels

Recently, we generated two lines of transgenic rats expressing the human GH (hGH) gene (Ikeda *et al.* 1994). As shown in Fig. 1(A), the transgene used consists of mouse whey acidic protein promoter and hGH genome gene. One line had excess circulating hGH and exhibited gigantic phenotypes as expected. The other line, however, had exactly similar body lengths as their non-transgenic littermates and they grew massively fat, contrary to expectations (Ikeda *et al.* 1994, 1998; Fig. 1(B)). We used the heterozygotes in all our experiments.

Circulating hGH levels in these obese transgenic rats were continuously low and endogenous GH secretion from the pituitary was severely suppressed. Thus, pulsatile peaks of peripheral GH levels once in 3–4 h, such as those observed in normal male animals, were completely absent (Fig. 1(C)). Both human and rat GH levels in the peripheral circulation of these transgenic rats coincide with the basal levels of intact animals, though there is no definite information about hGH potency in the rat. The reason for the severe inhibition of endogenous GH secretion could be attributable to the whey acidic protein promoter, which enhances the transcription of the following structure gene in the brain tissue as well as in the mammary glands (Günzburg *et al.* 1991; Tojo *et al.* 1993). Thus, the hGH gene might be expressed in the brain of the transgenic rats and inhibit endogenous GH secretion via a short feedback mechanism.



**Fig. 1** (A), Construct of the mouse whey acidic protein–human growth hormone (mWAP–hGH) transgene introduced into rats. (B), Representative features of a transgenic rat (right) and its littermate (left) at 23 weeks of age. Body weights of the transgenic rat and its littermate were 1.2 kg and 560 g, respectively. (C), Representative individual profiles of serum rat growth hormone (rGH; -○-) and hGH (-●-) changes over 4 h in control male (i) and transgenic rat (ii). (From Ikeda *et al.* 1994, 1998.)

Therefore, the pulsatile pattern of GH secretion was completely abolished in these transgenic animals.

It is known that a gender-dependent GH secretory pattern affects the expression of various types of hepatic steroid metabolic enzymes (Kato & Yamazoe, 1993). Relative abundance of liver microsomal cytochrome P450 isozymes were analysed in our male transgenic rats and were found to be functionally feminized due to absence of male specific GH pulsatility (Takahashi *et al.* 1999). This result also confirms that loss of pulsatile secretory pattern of GH occurs in these transgenic rats.

It should be emphasized again that these transgenic animals do not cease GH secretion completely, and that the low levels of GH secreted are enough to maintain normal body growth. The characteristics of GH in these transgenic rats led us to hypothesize that the cause of the severely fat phenotypes might originate from the loss of GH pulses. In this context, we have analysed the relationship between GH function and obesity.

### Causes of obesity in the human growth hormone transgenic rats

It is widely accepted that GH is an endocrine regulator of protein synthesis and lipolysis as well as of skeletal bone growth (Davidson 1987; de Boer *et al.* 1995). Moreover, there are reports that obesity is associated with a decrease in GH levels. GH-deficient human subjects have an increased body-fat mass, which can be reduced by GH therapy (Davidson, 1987; Salomon *et al.* 1989; Bengtsson *et al.* 1993; de Boer *et al.* 1995). Beneficial effects of GH on obese (Richelsen *et al.* 1994) and obese and diet-restricted subjects have been reported (Snyder *et al.* 1988). In addition, GHD rats and transgenic mice expressing mutant GH exhibit not only dwarfism but also fat accumulation (Turner *et al.* 1998). Obesity develops ultimately under conditions in which energy intake and expenditure are imbalanced. Increased food intake has been demonstrated in several obese animal models including *ob/ob* and *db/db* mice (Levin *et al.* 1996). Interestingly, the circulating GH levels of these animals are depressed (Renier *et al.* 1990; Veldhuis *et al.* 1991; Rasmussen *et al.* 1995), which may also account for their fat accumulation.

As shown in Table 1, the hGH transgenic rats have an increased food intake; in addition, decreased locomotor activity was also observed (Furuhata *et al.* 2000). A significant increase in body weight in hGH transgenic rats compared with control rats ( $P < 0.05$ ) was first discernible at 13 weeks of age and thereafter they developed severe obesity. However, food intake was already augmented at 4 weeks of age and locomotor activity started to decline from 7 weeks of age, indicating that behavioural changes start much earlier than apparent metabolic abnormalities or abnormal body-weight gain. In addition, we observed that significant fat deposition in the epididymal fat pad of the transgenic rats was already evident at 4 weeks of age ( $P < 0.05$ ) (Ikeda *et al.* 1998). Taken together, the transgenic rats consume more food and accumulate excess energy in the fat tissue as early as 4 weeks of age due to the impaired GH secretory pattern caused by the transgene product and a decreased locomotor activity.

In order to elucidate how energy imbalance and impaired GH secretion are involved in fat accumulation and metabolic abnormalities in transgenic rats, we utilized a pair-feeding paradigm and chronic GH replacement (Furuhata *et al.* 2002a). Pair-feeding of the transgenic animals for 8 weeks successfully suppressed body-weight gain and the final body weight was nearly normal (Table 1), indicating that an increase in body weight in the hGH transgenic rats was dependent on an increased energy intake. Although the fat mass in the pair-fed transgenic

**Table 1.** Effects of pair-feeding and human growth hormone (hGH) treatment in transgenic rats (TG) (From Furuhata *et al.* 2002a)

	Control rats	TG	Treatment	
			Pair-feeding	TG + hGH
Food intake	—	↑	↓	→
Growth hormone level	—	↓	→	↑
WAT:BW	—	↑	→	↓
Triacylglycerol	—	↑	↓	→
NEFA	—	↑	→	→
Insulin	—	↑	↓	→
Leptin	—	↑	↓	↓

WAT, white adipose tissue mass; BW, body weight; NEFA, non-esterified fatty acid; →, change compared with Control; →, change compared with TG without treatment.

animals became significantly smaller than that in the transgenic rats fed *ad libitum* ( $P < 0.05$ ), it still was two times heavier than those in the non-transgenic littermates. In addition, the fat pad : body weight ratios were not significantly different between the pair-fed and freely-eating transgenic animals (Table 1). Thus, the fat accumulation observed in the hGH transgenic rats is not solely attributable to an increase in food intake.

Continuous elevation of peripheral hGH levels (approximately 2-fold) for 8 weeks by means of a slowly releasing vehicle resulted in a decrease in the fat mass : body weight ratios by 30 % in the transgenic rats, though it did not affect food intake or body weight (Table 1). In our previous report, intermittent hGH treatment during daytime at 3 h intervals for 1 week was found to normalize completely the body-weight gain during the treatment period (Ikeda *et al.* 1998). Taken together, these results suggest that the severe fat accumulation observed in the hGH transgenic rats can be attributed to a decrease in lipolytic action of GH rather than an enhanced food intake.

Lipolytic action of GH is well documented both *in vivo* (Beauville *et al.* 1992; Kamel *et al.* 2000) and *in vitro* (Goodman & Grichting, 1983; Fielder & Talamantes, 1987), but the signalling mechanism by which GH regulates that process is yet to be fully explored. In adipocytes isolated from obese children after chronic GH treatment, catecholamine-induced lipolysis is increased maximally (Kamel *et al.* 2000). This increased sensitivity of adipocytes to lipolysis is reproduced by terbutaline but not by isoprenaline, indicating that the lipolytic effect of GH is related to the enhancement of the  $\beta_2$ -adrenoreceptor itself and/or its signalling pathway in adipocytes (Kamel *et al.* 2000). GH stimulates lipolysis through an inhibitory action on the G protein  $G_i$ , thereby increasing cAMP accumulation that results in lipolysis (Yip & Goodman, 1999). An involvement of STAT5 proteins in this lipolytic action has also been reported (Fain *et al.* 1999). On the other hand, GH appears to inhibit adipocyte differentiation at a step before the induction of genes required for terminal differentiation, such as peroxisome proliferator activated receptor  $\gamma$  (Hansen *et al.* 1998). Fat accumulation observed in the transgenic rats may not be related to the stimulation of adipocyte differentiation because we could not find an increase in the number of adipocytes in the transgenic rats (Ikeda *et al.* 1998).

### Metabolic abnormalities in transgenic rats

Obesity is frequently associated with metabolic abnormalities and hyperinsulinaemia and hyperlipidaemia were observed in the transgenic rats (Table 1). Pair-feeding of the hGH transgenic rats with control littermates almost completely ameliorated hyperinsulinaemia (Furuhata *et al.* 2002a). By pair-feeding, the weight of the fat pad decreased but still remained significantly heavier than controls ( $P < 0.05$ ). Therefore, hyperinsulinaemia in the transgenic rats was not attributable to excess fat accumulation. Moreover, continuous elevation of peripheral hGH levels (approximately 2-fold) for 8 weeks did not affect serum insulin levels, though this treatment reduced fat pad mass. Therefore, the hyperphagia was likely to have been one of the major reasons for hyperinsulinaemia in the hGH transgenic rats, but excess fat accumulation did not play a major role for the development of hyperinsulinaemia.

Since elevated circulating triacylglycerol levels were normalized by pair-feeding, hypertriglycerolaemia in these rats could also be explained by excessive energy intake (Table 1). However, pair-feeding did not affect serum non-esterified fatty acid (NEFA) levels in the transgenic rats. One possible explanation for this result could be the fact that fat mass was still 2-fold heavier in pair-fed transgenic rats, because it has been shown that circulating NEFA concentration is well correlated with the adipose tissue mass (Bjorntorp *et al.* 1969). hGH treatment, on the other hand, did not significantly affect serum NEFA levels, though the treatment

significantly reduced fat accumulation in the transgenic animals ( $P < 0.05$ ). Presence of high NEFA levels in the hGH-treated rats may be a consequence of lipolysis by hGH, because it is reported that GH treatment of obese human subjects reduces fat accumulation and elevates circulating NEFA levels (Richelsen *et al.* 1994).

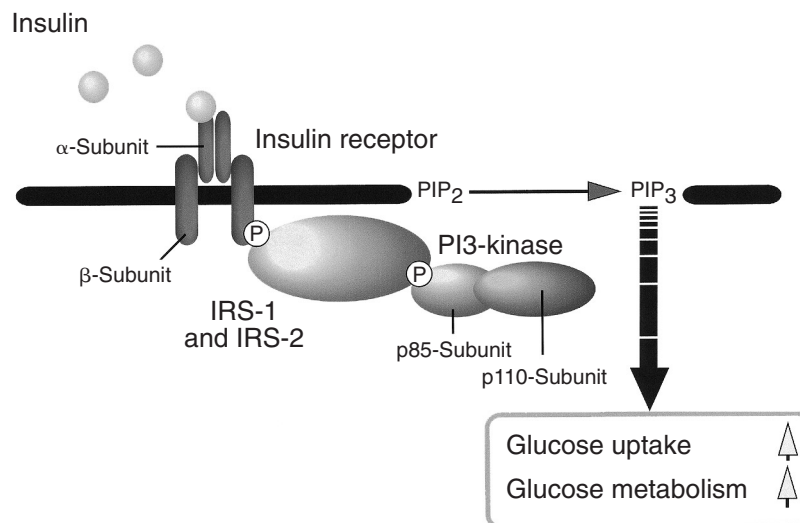
Pair-feeding of the hGH transgenic rats almost completely normalized serum leptin levels and it has been reported that adipocytes pretreated with insulin secrete larger amounts of leptin (Cusin *et al.* 1995; Saladin *et al.* 1995). Fasting reduces leptin levels coincidentally with lowering of insulin levels (Trayhurn *et al.* 1999), which suggests that the decrease in serum leptin levels in the pair-fed hGH transgenic rats was attributable to the lowered serum insulin levels. On the other hand, hGH treatment partially corrected serum leptin levels without affecting serum insulin. In GHD adults, increased circulating leptin levels were observed (Bjarnason *et al.* 1997; Fisker *et al.* 1997) and this hyperleptinaemia could be normalized by GH therapy. Under *in vitro* conditions, GH does not affect adipocyte leptin expression or secretion (Hardie *et al.* 1996) and therefore it is likely that leptin levels in GHD adults would reflect body composition rather than GH status. The primary cause for a decrease in serum leptin levels in the hGH-treated transgenic rats would be a lipolytic action of GH that decreases the fat pad mass.

### Insulin resistance

GH is known to modulate insulin actions (Davidson, 1987) and GHD subjects have long been recognized as exhibiting increased insulin sensitivity. In fact, the occurrence of fasting hypoglycaemia has been demonstrated in GHD children (Wolfsdorf *et al.* 1983) and adults (Merimee *et al.* 1971). Studies using animal models such as dwarf rats (Daugaard *et al.* 1999) and GH receptor-knockout mice (Dominici *et al.* 2000) support this notion. In contrast, several researchers have recently reported observing insulin resistance in GHD subjects. Cuneo *et al.* (1992) reported increased fasting insulin levels in GHD adults. Decreased insulin sensitivity was also shown in GHD adults by the euglycaemic-hyperinsulinaemic clamp method (Johansson *et al.* 1995; Hew *et al.* 1996). In addition, the studies of GHD subjects have shown increased fat accumulation, which is one of the major risk factors for developing non-insulin-dependent diabetes mellitus (NIDDM) with insulin resistance (Kopelman, 2000); insulin resistance can occur in the state of GH deficiency. In contrast with clinical studies, there have been few reports of studies to investigate the relationship between insulin resistance and GH deficiency in animal models. One reason may be that until recently, no good animal models that exhibit both insulin resistance and GH deficiency were available.

The hGH transgenic rats with low circulating levels of GH develop insulin resistance as well as severe obesity. As described earlier, insulin, triacylglycerol and NEFA levels, which are known to be indicators of early diabetes, are all elevated. In addition, an elevation of blood glucose level and glucose intolerance after a bolus injection of glucose are observed in these transgenic rats (Ikeda *et al.* 1998; Furuhashi *et al.* 2002b). Thus, we believe our transgenic rats are useful model animals for investigating the relationship between insulin resistance and impaired GH status.

We have analysed insulin signalling in the liver of the hGH transgenic rats to explore whether insulin resistance could actually be detected and the targets for this purpose are shown in Fig. 2. Insulin signalling is initiated by the activation of insulin receptor tyrosine kinase, leading to the phosphorylation of intracellular receptor substrates, including insulin receptor substrates (IRS)-1 and -2, which contain over twenty potential tyrosine phosphorylation sites (Sun *et al.* 1993, 1995). The possible tyrosine phosphorylation sites are highly conserved



**Fig. 2.** Insulin receptor signalling pathway. Insulin signalling is initiated by the activation of insulin receptor tyrosine kinase, leading to the phosphorylation of intracellular receptor substrates, including insulin receptor substrates (IRS)-1 and -2. The motifs containing phosphorylated tyrosine residues in IRS-1 and IRS-2 could bind to the p85 subunit of phosphatidylinositol 3-kinase (PI3-kinase) and then the p110 subunit of PI3-kinase is activated. PIP, phosphatidylinositol. (From Kanai *et al.* 1993; Cheatham *et al.* 1994; Okada *et al.* 1994.)

between IRS-1 and IRS-2 (Sun *et al.* 1995). The motifs containing phosphorylated tyrosine residues in IRS-1 and IRS-2 serve as docking sites for binding to various proteins having the Src homology-2 domain. Among these Src homology-2 proteins, phosphatidylinositol 3-kinase is considered to be particularly important for the insulin-induced glucose uptake that is observed in muscle and adipose tissue via the translocation of a glucose transporter to the plasma membrane (Kanai *et al.* 1993; Cheatham *et al.* 1994; Okada *et al.* 1994). Furthermore, the activation of phosphatidylinositol 3-kinase was reported to play an important role in insulin-induced glycogen synthesis and suppression of phosphoenolpyruvate carboxykinase gene expression in hepatocytes (Sakaue *et al.* 1995; Shepherd *et al.* 1995; Sutherland *et al.* 1995; Gabbay *et al.* 1996).

We first measured the amount of insulin receptor in the liver of transgenic rats by Western blotting and found that insulin receptor levels were lowered (Furuhata *et al.* 2002b). Insulin-stimulated phosphorylation of insulin receptor was also proportionally suppressed in the transgenic rats, indicating that insulin resistance in the liver is initiated at the receptor level. The amounts of both IRS-1 and -2 proteins in the liver of the transgenic rats were reduced compared with that of controls. Moreover, phosphorylated tyrosine of IRS-1 and -2 and phosphatidylinositol 3-kinase activity associated with these proteins were also decreased. These results suggest that the liver of the hGH transgenic rats is somehow insulin resistant.

One of the candidates that suppresses insulin sensitivity in the transgenic rats is hyperinsulinaemia. There are reports of down regulation of insulin receptors under high circulating insulin levels (Gavin *et al.* 1974; Kahn *et al.* 1978; Vigneri *et al.* 1978; Almira & Reddy, 1979). However, it has also been reported that GH does not directly affect insulin receptor levels either *in vivo* (Dominici *et al.* 1998) or *in vitro* (Lesniak & Roth, 1976; Maloff *et al.* 1980). In the hGH transgenic rats, elevated circulating insulin levels are observed after 12 weeks of age (Ikeda *et al.* 1998) and therefore the decreased insulin receptor levels described earlier could be the result of hyperinsulinaemia rather than from low GH levels.

It has been reported that IRS-1 protein was moderately increased in the liver of streptozotocin-treated rats, in which insulin was virtually absent (Saad *et al.* 1992). Conversely, hyperinsulinaemic *ob/ob* mice and Zucker rats showed reduced IRS-1 (Saad *et al.* 1992) and both IRS-1 and -2 (Anai *et al.* 1998) proteins in the liver, respectively. These findings suggest that IRS-1 and -2 levels are inversely related to circulating insulin levels so that decreased IRS-1 and -2 in the liver of the hGH transgenic rats may be related to hyperinsulinaemia. Because transgenic mice over-expressing GH display normal concentrations of IRS-1 in the liver despite hyperinsulinaemia (Dominici *et al.* 1999), a possibility that other factors, such as changes in the concentrations of glucose or counter-regulatory hormones (including GH), are involved in the regulation of IRS levels cannot be ruled out. We discussed earlier that increased hyperinsulinaemia in the hGH transgenic rats results from an excess energy intake, but it remains to be elucidated whether or not restriction of energy intake restores insulin resistance in the transgenic rats.

Basal levels of tyrosine phosphorylation of IRS-1 in the hGH transgenic rats were significantly lowered. This may be secondary to the decreased IRS-1 protein level. The amount of IRS-2 was also decreased, but no significant difference in basal tyrosine phosphorylation levels of IRS-2 was observed between the transgenic and control rats, suggesting a different regulatory mechanism for the basal tyrosine phosphorylation level of IRS-1 and -2 in the liver. GH has been shown to stimulate tyrosine phosphorylation of IRS-1 *in vivo* (Yamauchi *et al.* 1998; Thirone *et al.* 1999). Further, Dominici *et al.* (1999) reported that GH transgenic mice showed an increased basal tyrosine phosphorylation of IRS-1, which, they suggested, may be one of the causes of insulin resistance in those mice. Thus, altered GH levels, either higher or lower than normal, may affect basal tyrosine phosphorylation of IRS-1 in the liver. These findings suggest that GH has an important role for maintaining insulin sensitivity in the liver. In this context, a cross-talk mechanism between GH and insulin signal transduction can be hypothesized for the development of insulin resistance in the liver.

Serine and threonine phosphorylations in IRS proteins may be also involved in impaired insulin signalling in transgenic rats. It has been demonstrated that serine and threonine phosphorylation of IRS-1 impairs the ability of IRS-1 to associate with the insulin receptor, which inhibits subsequent insulin-stimulated tyrosine phosphorylation (Paz *et al.* 1997). In addition, it has also been reported that serine and threonine phosphorylation in IRS-1 is increased in subjects with insulin resistance and NIDDM (Virkamaki *et al.* 1999) and that cytokines, which induce insulin resistance, promote serine phosphorylation of IRS-1 (Kanety *et al.* 1995; Hotamisligil *et al.* 1996; Peraldi *et al.* 1996). Taken together, serine and threonine phosphorylation in IRS proteins seems to play an important role in the development of insulin resistance.

### Leptin resistance

As described earlier, failure of energy balance causes obesity and metabolic abnormalities. Why does hyperphagia occur in the hGH transgenic rats? Leptin, a 16 kDa peptide hormone, for which the gene was positionally cloned in 1994 (Zhang *et al.* 1994), is an adipocyte-derived hormone that decreases food intake and increases energy expenditure, thereby leading to a marked reduction in body weight (Pellemounter *et al.* 1995, Levin *et al.* 1996). It has been reported that the absence of leptin or functional leptin receptor causes massive obesity and NIDDM, similar to previously known animal models, i.e. *ob/ob* mice or Zucker fatty rats (Zhang *et al.* 1994; Ghilardi *et al.* 1996; Lee *et al.* 1996). Leptin levels correlate with specific estimates of body fat (Hickey *et al.* 1996; Rosenbaum *et al.* 1996) and reducing the obesity

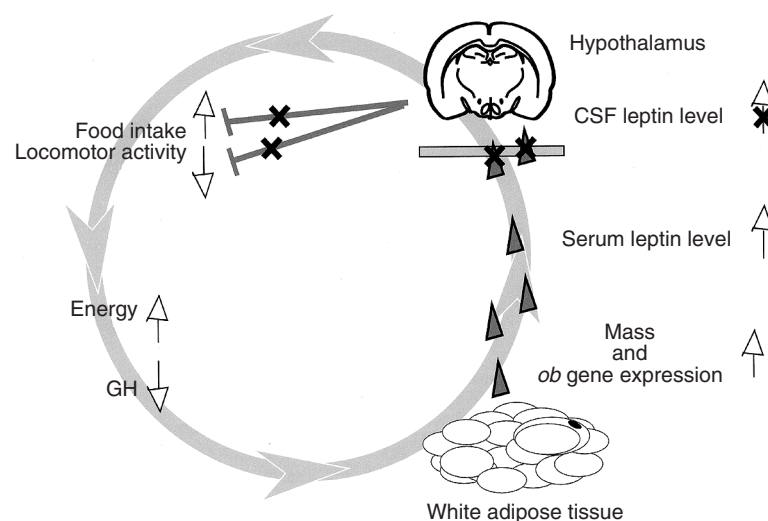


decreases the high leptin concentrations in obese human subjects. In obese individuals, however, high plasma leptin levels do not induce anorexic responses theoretically expected and this inconsistency has led to the concept of leptin resistance (Caro *et al.* 1996; Bjarnason *et al.* 1997). In obese GHD human adults, an increased circulating leptin is observed (Bjarnason *et al.* 1997; Fisker *et al.* 1997; Janssen *et al.* 1997), therefore we analysed our obese and GH-deficient hGH transgenic animals to determine whether or not they were leptin-resistant.

As mentioned already, the transgenic rats have much increased leptin levels, despite which, food intake is enhanced and locomotor activity suppressed. Thus, the hGH transgenic rats are leptin-resistant phenotypically. This conclusion was further supported by the observation that systemic administration of leptin, which decreased food intake and increased locomotor activity in the control rats, did not affect these parameters in the transgenic rats (Furuhata *et al.* 2000). Interestingly, however, direct administration of leptin into the brain was effective to a similar extent in both the transgenic and control rats, suggesting a dysfunction of transmission of peripheral leptin signals to the central nervous system in the transgenic rats.

The biological actions of leptin are thought to be mediated largely through interactions with its receptors that are expressed in the hypothalamus (Tartaglia *et al.* 1995). Because the leptin molecule seems too large to cross the blood–brain barrier, it appears to be transported into the brain by a saturable system (Banks *et al.* 1996; McKinley & Oldheld, 1998). Thus, while there is a correlation between plasma and cerebrospinal fluid levels of leptin, the relationship is logarithmic rather than linear and recently, this relationship has been postulated as one of the causes of leptin resistance in man (Caro *et al.* 1996; Schwartz *et al.* 1996). In the present study (Furuhata *et al.* 2000), we found that even though circulating leptin levels were much higher in the transgenic than in the control rats, cerebrospinal fluid leptin levels were not different. This observation provides experimental evidence that leptin resistance occurs at least partially at the level of transportation of leptin into the brain (Fig. 3).

The leptin receptor is a single transmembrane-spanning protein that resembles gp130, a member of the class I cytokine receptor superfamily (Banks *et al.* 1996). The leptin receptor



**Fig. 3.** Leptin resistance may contribute to the development of obesity in the transgenic rats. Low circulating growth hormone (GH) levels and energy imbalance induce fat accumulation and hyperleptinaemia. However, although circulating leptin level is increased, it could not be transported into the brain efficiently, resulting in excessive energy balance. CSF, cerebrospinal fluid. (From Caro *et al.* 1996; Schwartz *et al.* 1996; Furuhata *et al.* 2000.)

has five or more splice variants expressed in a tissue-specific fashion (Chen *et al.* 1996; Chua *et al.* 1996). Wu-Peng *et al.* (1997) have reported that the leptin receptor itself is one of the candidates for a leptin transporter. It is especially likely that OB-Ra, a short form of leptin receptor, is the transporter molecule accounting for uptake into the brain, since it is expressed in the choroid plexus. Recently, Burguera *et al.* (2000) have reported that the leptin transport system is saturated near physiological concentrations in lean individuals, which implies that the elevated leptin levels observed in obesity can produce no biological effects because the system is already saturated. In this regard, the leptin transport system in the transgenic rats could be already saturated, so that they have no response in appetite to peripheral administration of leptin. The early onset of behavioural changes suggests the early onset of leptin resistance, which is presumably one of the primary causes of obesity in the transgenic rat with low circulating levels of GH.

In our experimental paradigm, where continuous elevation of peripheral hGH levels (approximately 2-fold higher than those in the non-treated transgenic rats) was maintained for 8 weeks, serum leptin levels in the treated transgenic rats were lowered partially but not completely. The possibility cannot be excluded that an alternate method of treatment, such as one simulating pulsatile GH secretion, could have restored leptin resistance more efficiently.

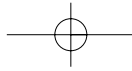
### General considerations

GH is not an essential hormone for survival, since GHD models are not lethal. Moreover, although they are dwarves, it has been reported that they have a longer life span compared with GH normal individuals (Brown-Borg *et al.* 1996). GH, however, induces the growth of bone and muscle directly or indirectly. This effect must have evolutionary advantages particularly for competition for survival among individuals of the same species. In addition, GH must have a function of changing body composition in order to adapt individuals to various natural environments. Wild animals, such as birds before migration, hibernants before hibernation or male fur seals before making a harem, store large amounts of fat in preparation for the future deficiency in food intake. Hibernation is a strategy employed by particular mammals during periods of climatic challenge associated with reduced food availability. During hibernation, metabolism, body temperature and basic behaviours such as eating are greatly reduced and energy must be stored as fat during the pre-hibernatory period, often leading to a doubling of body mass (Mrosovsky, 1976; Young, 1976; Boyer *et al.* 1997; Kunz *et al.* 1998). Because GH pulses are instituted by neural mechanisms including GH releasing hormone and somatostatin neuronal systems, GH pulsatility, at least theoretically, can be stopped by those same neuronal mechanisms, which in turn promotes efficient fat deposition. Based on our observations on hGH transgenic rats, the prevalence of obesity in aged men and women could be attributed to the attenuation of GH pulsatility with age that epidemiological study confirms.

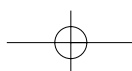
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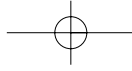
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