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EFFECTS OF LONG-TERM ELEVATED SERUM LEVELS OF GROWTH HORMONE ON LIFE EXPECTANCY OF MICE: LESSONS FROM TRANSGENIC ANIMAL MODELS

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SUMMARY

In this study, we characterize transgenic mice carrying fusion genes, in which the genes coding for human (h) or bovine (b) growth hormone (GH) have been put under the transcriptional control of the mouse metallothionein I (MT) or the rat phosphoenolpyruvate carboxykinase (PCK) promoter as models for investigating the long-term effects of elevated GH on life expectancy. Circulating GH concentrations ranged from 3000 to 900 000 ng/ml, from 320 to 2960 ng/ml and from 34 to 1050 ng/ml in transgenic mice belonging to the MThGH, the PCKbGH and the MTbGH groups, respectively, and were high on a short-, medium-, and long-term basis. As a consequence of excess GH in their serum, GH transgenic mice exhibited drastically reduced life span which was primarily due to severe kidney lesions (glomerular hypertrophy, sclerosis and hyalinosis associated with tubulo-interstitial changes) consistently found in these animals. Alterations of the liver observed in transgenic mice included both hepatocellularmegaly and various degrees of regressive, regenerative and fibrotic changes. In older MTbGH and PCKbGH transgenic mice, hepatocellular neoplasms including both adenoma and carcinoma were frequently found in addition to non-neoplastic changes. Our study points out the suitability of GH transgenic mice to evaluate the effects of various levels of GH in long-term studies without having to take antibody production against the heterologous hormone into account. Findings in GH transgenic animals suggest that the long-term benefits and risks of GH therapy should be carefully evaluated.

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INTRODUCTION

Ageing is an inevitable aspect of living and results from a sequence of events occurring on the molecular, cellular and systemic level of an individual. Molecular theories of ageing mainly assume that the life span of any species is governed by gene-environment interactions, while cellular theories include structural and functional changes of cellular components that occur as a function of age. Systemic theories ascribe ageing of the entire organism to a reduced function of a key system, such as the immune, nervous and endocrine systems [1].

The efficiency of hypothalamo-pituitary signals, e.g. in the growth hormone (GH) cascade, is known to be altered with ageing; basal and GH-releasing hormone-stimulated secretion of GH declines in elderly human beings and in animals as well [2–7]. Substitution of GH is therefore a current therapeutic approach of geriatric medicine [8]. Rudman et al. [9] studied the effects of human GH given three times a week in a dosage of 30 μg/kg body mass in men over 60 years of age. The administration of hGH for 6 months resulted in a significant increase in lean body mass accompanied by a decrease in adipose-tissue mass. Furthermore, the mean concentration of circulating insulin like growth factor I (IGF I) rose to a youthful range during treatment. A pilot study of Kaiser et al. [10] also suggested GH therapy to be an effective way of maintaining and enhancing body weight in malnourished older individuals. In mice, long-term growth hormone treatment (30 μg per mouse twice weekly) was reported to even prolong life expectancy [11].

Since a potentially unlimited supply of GH, free of pituitary contaminants, is provided by recombinant biotechnology [12], a plethora of clinical applications of growth hormone therapy has been found. While the objective of GH treatment has traditionally been to increase the growth rate and adult height of short statured GH-deficient children, wider pediatric indications for GH therapy include Turner's syndrome, skeletal dysplasia, intrauterine growth retardation, delay of pubertal development, chronic illness, catabolic states as well as regeneration and repair processes [13,14]. Symptoms of GH deficiency in adults, like psychological maladjustment, reduced muscle strength and exercise capacity, sub-normal kidney function and cardiovascular problems have also been shown to be positively influenced by GH in short-term treatment trials [15,16].

In spite of these enthusiastic results of GH therapy there is recent concern about ethical and medical risks especially in treating patients that do not meet the classic symptoms of GH deficiency [17,18]. Therefore, the need for animal models which provide evidence for the long-term benefits and risks of elevated GH in an organism arises.

Transgenic mice expressing foreign GH or GH-releasing factor (GRF) genes have been established as valuable tools to study the consequences of long-term elevated circulating growth hormone [19–21]. In this study, we report the effects of species and level of expression of GH transgenes on life expectancy of mice.

MATERIAL AND METHODS

Animals

Transgenic founder animals harbouring fusion genes in which the coding region of the human growth hormone (hGH) gene is transcriptionally controlled by the mouse metallothionein I (MT) promoter were produced by pronuclear microinjection as described elsewhere [19,22]. Zygotes were obtained from superovulated donors belonging to an outbred stock of mice (Pop) based on the NMRI strain [23]. Three independent lines of MThGH transgenic mice were established by mating hemizygous founder animals and subsequently transgenic progeny with non-transgenic Pop mice. Litters therefore statistically consisted of 50% hemizygous transgenic and of 50% non-transgenic mice which served as controls in our experiments. Since transgenic mice belonging to these three lines were not significantly different in any trait investigated (data not shown), data from these animals were pooled and the resulting large group of mice is referred to as MThGH.

Two lines of mice carrying the bovine growth hormone (bGH) gene driven by either the MT or the rat phosphoenolpyruvate carboxykinase (PCK) promoter were established by mating transgenic mice (originally generated [24,25] and kindly provided by T.E. Wagner, The Edison Biotechnology Center, Athens, Ohio, USA) with Pop mice as described above. Four generations of transgenic individuals and controls were included in this study. The presence of the foreign GH fusion genes was detected by Southern or slot blot hybridization [26,27]. Throughout the experiment all animals were kept under identical conventional (non-barrier) conditions (21 ± 1°C; 55 ± 3% relative humidity; 12/12-h light/dark cycle). Altromin 1324 laboratory diet and tap water were supplied ad libitum. Detailed information on the housing conditions including the pathogens in the colony is provided elsewhere [28].

Detection of circulating growth hormone

Blood samples were taken from transgenic mice and controls by orbital puncture under ether anesthesia and were allowed to clot for 1 h at 4°C. Blood samples were taken repeatedly from some animals to investigate the short-, medium- and long-term pattern of GH production in GH transgenic mice. After centrifugation (2 × 10 min, 15 000 × *g*) concentration of hGH in serum samples from MThGH transgenic mice was measured using a commercial immunoradiometric assay (Hybritech Tandem-R® hGH assay) which has been proven to be suitable for this purpose and does not measure GH in serum samples from nontransgenic controls [29]. Serum levels of bGH were quantified by radioimmunoassay [30]. Levels of GH measured in control serum with this assay were below 10 ng/ml.

To determine the molecular forms of foreign GH in the serum of transgenic mice, serum proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and subsequently transferred to Hybond-C nitrocellulose (Amersham, UK) by electroblotting. Immunodetection of hGH and bGH, respectively, was performed according to Amersham's ECL Western blotting protocols using polyclonal primary antibodies raised in rabbits (Fig. 2).

Clinical and pathological investigations

All animals were regularly monitored for clinical symptoms by inspection and recording of body weight. Unhealthy mice which were euthanized for ethical reasons were sacrificed by bleeding under ether anesthesia. The date of euthanasia was used for calculation of life expectancy. Post mortems of sacrificed and spontaneously dead mice were carried out to establish the causes of disease and death. After macroscopic examination, tissue specimens of the internal organs were routinely processed for histological examination [19].

Statistical analysis

Analysis of variance was carried out on the data obtained for serum GH levels and life expectancy from transgenic mice and controls. Data obtained for serum GH concentrations were transformed to common logarithms before analysis to approximate normal distribution of values. The statistical model employed contained group, sex and the interaction group × sex as fixed effects. The General Linear Models (GLM) procedure (SAS, version 6.0) was used to calculate least squares means (LSM) and standard errors (S.E.) of LSM. LSM obtained for males and females belonging to different groups were compared using Student *t*-tests.

TABLE I

EFFECTS OF GROUP AND SEX ON SERUM GH LEVELS AND LIFE EXPECTANCY OF GH TRANSGENIC MICE (ANALYSIS OF VARIANCE)

Dependent variable	*Fixed effect*	*D.F.*	*Mean square*	*F value*	*PR > F*
Log serum GH concentration[a]	Group	2	130.04	662.98	0.0001
	Sex	1	0.70	3.58	0.0594
	Group × sex	2	0.51	2.59	0.0765
Life expectancy	Group	3	3182229.65	218.43	0.0001
	Sex	1	40431.30	2.78	0.0967
	Group × sex	3	29445.53	2.02	0.1107

[a]Values were transformed to common logarithms to approximate normal distribution.

RESULTS

Expression of GH fusion genes in transgenic mice

Analysis of variance revealed GH levels in the serum of transgenic mice to be significantly influenced by group, whereas sex and the interaction group × sex reached the borderline of statistical significance (Table I). Circulating GH concentrations ranged 3000–900 000 ng/ml, 320–2960 ng/ml and 34–1050 ng/ml in trans-

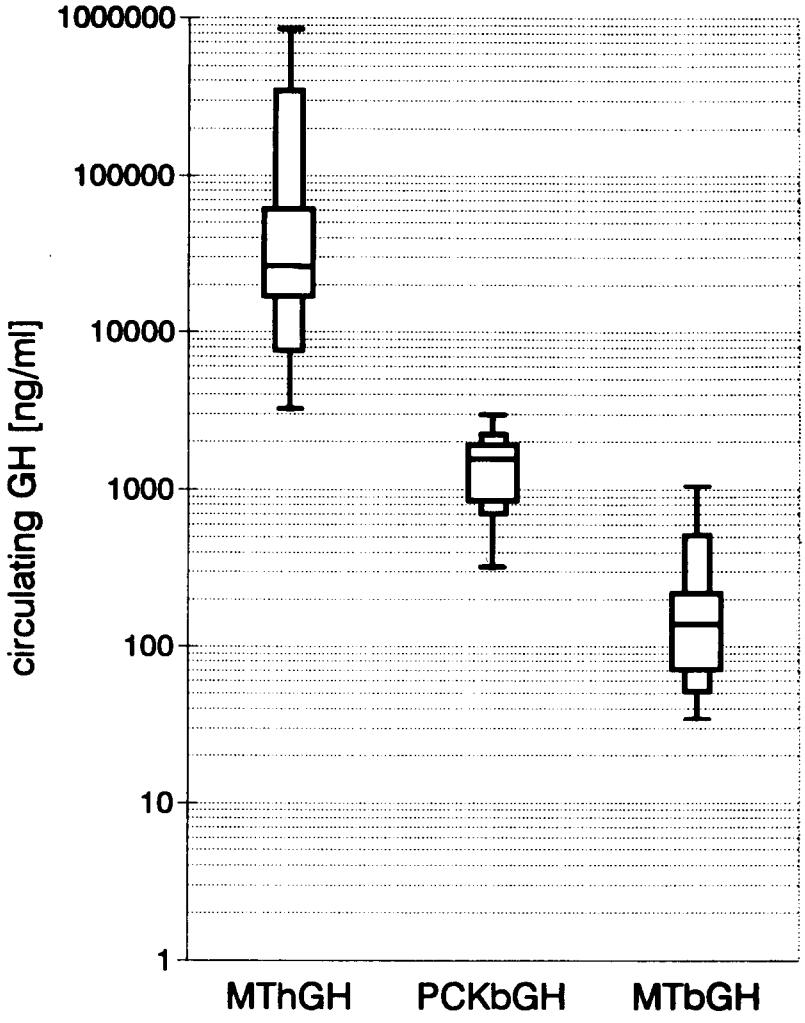

Fig. 1. Schematic plots of GH concentrations in serum samples from MThGH, PCKbGH and MTbGH transgenic mice. The bottom and top edges of the narrow and the wide boxes are located at the sample 10th and 90th percentiles and the sample 25th and 75th percentiles, respectively. The center horizontal line is drawn at the sample median. Minimum and maximum values are also shown.

TABLE II

CONCENTRATIONS OF CIRCULATING GH (ng/ml) IN GH TRANSGENIC MICE

Group of mice	*Male*			*Female*			*♂:♀ within group*
	n	*LSM*	*S.E.*	*n*	*LSM*	*S.E.*	
MTbGH	22	204[a]	1.2	21	98[a]	1.2	$P < 0.05$
PCKbGH	25	1447[b]	1.2	26	1253[b]	1.2	N.S.
MThGH	124	36183[c]	1.1	110	37531[c]	1.1	N.S.

Least squares means (LSM) of logarithmic GH levels were compared using Student *t*-tests. The table shows retransformed data. Values with different superscripts are significantly different within sex ($P < 0.001$). N.S., not statistically significant.

TABLE III

LONG-, MEDIUM- AND SHORT-TERM VARIATION OF CIRCULATING GH IN GH TRANSGENIC MICE

Experimental procedure	*Animal*			*Circulating GH (ng/ml)*		
	No.	*Sex*	*Age* (days)[a]	*Mean*	*S.D.*	*CV*
Long-term trial: 8 blood samples	12	♂	91	39 433	26 636	68%
taken in weekly intervals from	14	♂	91	19 500	3302	17%
MThGH transgenic mice	18	♀	91	24 671	12 219	50%
	20	♀	91	33 375	11 779	35%
Medium trial: 7 blood samples	74	♂	144	21 686	1918	9%
taken in intervals of 1–3 days	76	♂	144	37 817	9937	26%
from MThGH transgenic mice	84	♀	144	40 786	16 662	41%
	85	♀	144	36 486	10 708	29%
Medium-term trial: 6 blood samples	38	♂	115	165	61	37%
taken in intervals of 1–3 days	39	♂	115	245	70	29%
from MTbGH transgenic mice	40	♂	115	124	57	46%
	42	♀	115	56	11	20%
	44	♀	115	68	20	29%
Medium-term trial: 6 blood samples	15	♂	143	1812	259	14%
taken in intervals of 1–3 days	17	♂	143	2121	548	26%
from PCKbGH transgenic mice	20	♀	143	1856	309	17%
	23	♀	143	1710	838	49%
	25	♀	143	1065	344	32%
Short-term trial: 8 blood samples	26	♂	162	38 963	5033	13%
taken in intervals of 1 h from	31	♂	139	12 250	4245	35%
MThGH transgenic mice	32	♀	139	24 213	3683	15%
	33	♀	139	14 200	2816	20%

[a]Age at the beginning of the experiment; S.D., standard deviation; CV, coefficient of variation.

genic mice belonging to the MThGH, PCKbGH and MTbGH group, respectively (Fig. 1). Least squares means (LSM) calculated for serum GH levels were significantly different when groups were compared within sex ($P < 0.001$; Table II). A comparison of males and females within groups revealed male MTbGH transgenic mice to exhibit significantly higher serum GH concentrations than their female counterparts ($P < 0.05$), whereas the remaining two transgenic groups did not show a significant sex-related difference with respect to this parameter (Table II). In order to judge the pattern of circulating GH, serum samples from transgenic mice were taken repeatedly in intervals of 1 h to 1 week over a period of 8 h to 8 weeks. Circulating levels of GH were continuously high on a long-, medium- and short-term basis (Table III). Coefficients of variation calculated for GH values measured in serum samples from individual animals ranged over 17–68%, 9–49% and 13–35% in the long-, medium- and short-term trials, respectively.

Western blot analysis revealed solely 22 kDa bGH to be present in serum samples from MTbGH and PCKbGH transgenic mice whereas serum samples from MThGH transgenic mice contained various molecular forms of immunoreactive hGH (mainly 22 kDa, but also 26 kDa, 20 kDa and 16 kDa; Fig. 2).

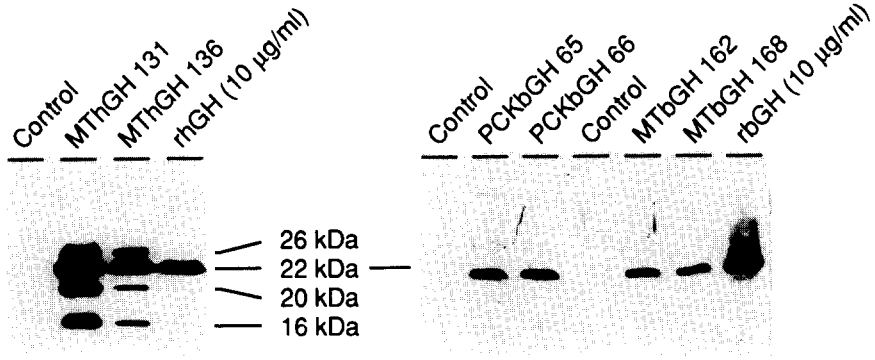

Fig. 2. Western blot analysis of serum samples from MThGH, PCKbGH and MTbGH transgenic mice. Serum samples (5 µl) were diluted 1:10 (MThGH) or 1:2 (PCKbGH, MTbGH) with 5 mM Tris–HCl (pH 6.8) and subsequently mixed with an equal volume of 2× sample buffer (5% glycerol, 10% 2-mercaptoethanol, 4% SDS and 10 mM Tris–HCl, pH 6.8), boiled (5 min) and loaded on a 5% stacking/15% separating SDS-polyacrylamide gel (5 µl/slot). Electrophoresis was performed in a Mini-Protean® II Dual Slab Cell (Bio-Rad) for 5 min at 100 V and then for 45 min at 180 V. Proteins were transferred to Hybond-C nitrocellulose (Amersham) using a horizontal semi-dry electroblotting system (Sartorius). Immunodetection of hGH and bGH, respectively, was performed according to Amersham's ECL Western blotting protocols. Briefly, the blots were blocked in 5% bovine serum albumin (BSA) in PBS-0.1% Tween 20 (PBS-T) for 2 h at 37°C. After washing (3 × 10 min in PBS-T) the filters were incubated in 1:1000 dilutions of a rabbit-anti-hGH (UCB) and a rabbit-anti-bGH antiserum (a kind gift from D. Schams), respectively (12 h, 37°C). After washing (see above) the blots were soaked for 1 h in a 1:1000 dilution of horseradish peroxidase-labelled goat-anti-rabbit-antibodies (UCB), washed again and incubated in ECL detection reagents (Amersham) for 60 s. The blots were exposed to Kodak X-OMAT™ scientific imaging film for 2 s. Developing and fixing chemicals were also obtained from Kodak. Serum samples from nontransgenic littermates served as negative and recombinant (r) hGH (Lilly) and bGH (Monsanto) as positive controls.

TABLE IV

LIFE EXPECTANCY (DAYS) OF GH TRANSGENIC MICE AND CONTROLS

Group of mice	*Male*			*Female*			♂:♀ *within group*
	n	*LSM*	*S.E.*	*n*	*LSM*	*S.E.*	
Control	32	627[a]	21	40	559[a]	19	N.S.
MTbGH	31	316[b]	21	18	348[b]	28	N.S.
PCKbGH	23	307[b]	25	36	257[c]	20	N.S.
MThGH	85	163[c]	13	83	153[d]	13	N.S.

Least squares means (LSM) were compared using Student *t*-tests. Values with different superscripts are significantly different within sex ($P < 0.001$). N.S., not statistically significant.

Life expectancy of GH transgenic mice versus controls

The fixed effect 'group' significantly influenced life expectancy as shown by analysis of variance (Table I). LSM and standard errors (S.E.) of LSM calculated for life expectancy of GH transgenic mice and controls are summarized in Table IV. A comparison of groups within sex revealed controls to reach the highest age followed by

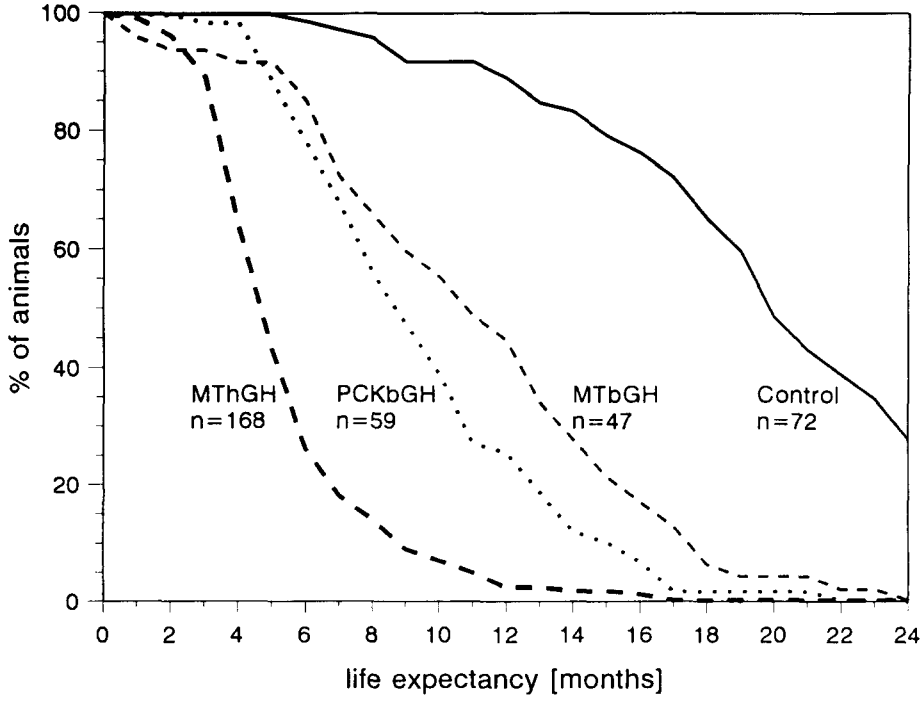

Fig. 3. Life expectancy of MThGH, PCKbGH and MTbGH transgenic mice as compared to non-transgenic littermates.

MTbGH, PCKbGH and MThGH transgenic mice. These differences were statistically significant ($P < 0.001$) except for the difference between MTbGH and PCKbGH transgenic males. None of the groups displayed a significant sex-related difference in longevity. One year survival rates corresponded to 90% in controls, 44% in MTbGH, 25% in PCKbGH and 3% in MThGH transgenic mice (Fig. 3). Maximum life expectancies were 17, 18 and 24 months in the MThGH, PCKbGH and MTbGH groups, respectively, whereas 28% of the controls reached ages greater than 2 years. There was no significant correlation between life expectancy and serum GH levels within groups (data not shown).

Clinical and pathological findings

The majority of MThGH transgenic mice displayed severe health afflictions at very early ages. The first symptoms of morbidity were a rough coat and a reduction of spontaneous motility. A dramatic loss of body weight was recorded by regularly weighing the animals and was obvious when the contours of the spinal cord became visible through the coat. Post mortems confirmed bad nutritional condition: most animals were cachectic with only a few mice possessing marginal mesenterial fat deposits. Apart from a general enlargement of inner organs, severe organ damage was found with pathomorphological changes of kidneys (glomerular hypertrophy, sclerosis and hyalinosis associated with tubulo-interstitial changes), liver (hepatocellularmegaly and various degrees of regressive, regenerative and fibrotic changes) and heart (multifocal myocardial fibrosis) being predominant. We have described our findings in detail elsewhere [19,20] and were able to confirm them using a larger number of animals in the present study. MTbGH and PCKbGH transgenic mice showed similar clinical symptoms and pathomorphological changes as did MThGH mice. Detailed post mortems were carried out on 18 (13♂, 5♀) MTbGH and on 20 (10♂, 10♀) PCKbGH transgenic mice. The majority of these animals (15 MTbGH and 16 PCKbGH transgenic mice) demonstrated severe kidney lesions which were identical to those observed in MThGH transgenic mice. In seven bGH transgenic mice the degree of kidney lesions appeared less severe and liver dystrophy ($n = 3$), intestinal occlusion ($n = 2$), adenocarcinoma of lungs ($n = 1$) and severe pneumonia ($n = 1$) were considered as the primary cause of death in these animals. Furthermore, various stages of hepatic changes mirroring those seen in MThGH transgenic mice were consistently observed in both groups of bGH transgenic mice. Apart from non-neoplastic alterations, hepatocellular neoplasms including both adenoma and carcinoma were found in 14 of 21 bGH transgenic mice older than 11 months. Myocardial fibroses were seen in four MTbGH and in eight PCKbGH transgenic mice.

DISCUSSION

Suitable model systems for investigating physiological and possible side effects of a hormonal substance are prerequisitory to estimate the safety of prolonged

therapeutical application of the particular hormone. A number of attempts have been made to simulate the consequences of high levels of homologous or heterologous GH in animals.

The growth promoting activity of GH has impressively been demonstrated already several decades ago by injection of pituitary-derived GH into intact, hypophysectomized and thyroidectomized rats [31–35]. More recent studies point out the influence of different regimens of application (intermittent injection versus continuous infusion) on the biological efficiency of GH [36,37]. However, all experiments involving treatment of animals with heterologous GH may be disturbed by immunological reactions. A neutralizing effect of hGH antibodies on GH stimulated body weight gain was found in hGH injected hypophysectomized female rats after a 17–20-day treatment period [38]. Similarly, 39 out of 40 female rats which received 0.5, 3.3, or 25 I.U. of biosynthetic hGH per kg body mass per day developed antibodies to hGH after 3 weeks [39]. Khansari and Gustad [11] investigated the effects of GH on immune function of aged mice and injected 15-month-old Balb/c males twice a week subcutaneously with 30 μg of human GH. While applicating a foreign antigen over a period of 13 weeks and after a lag period of 4 weeks, for another 6 weeks, the authors did not observe any antibody response. This information was, however, given as preliminary observation. The main effect seen in this study was a prolonged life expectancy of the GH treated mice.

In a transcaryotic gene therapy experiment, Chang et al. [40,41] grafted rats with genetically modified autologous fibroblasts which produced human growth hormone. All recipient animals developed extremely high titres of antibodies against the circulating human growth hormone within two weeks of grafting. This reaction was, after a delay of 1 month, even observed when immunologically immature neonatal rats were used as recipients. These results underline that reliable long-term studies on the physiological function of a postnatally applicated foreign GH are not possible without using immunosuppressants.

Another approach of producing high levels of homologous growth hormone in the serum of rats is the transplantation of functioning pituitary tumours. However, these tumours as a rule do not only secrete GH but also other hypophysial peptides (e.g. adrenocorticotropic hormone, prolactin) [42,43]. Alterations observed in these animals therefore cannot exclusively be interpreted as an effect of GH. Furthermore, catabolic or toxic changes by the tumor-bearing condition have to be taken into consideration [43].

Transgenic technology, however, allows the introduction of a known gene coding for a normally foreign antigen into the genome of an animal. The former antigen is synthesized by the transgenic animal as an authentic self molecule, synthesis commonly following the developmental, metabolic and tissue-specific regulation of the particular promoter sequence employed.

We used transgenic mice carrying gene constructs in which the human or bovine GH genes were put under the transcriptional control of the MT or the PCK promoter.

Metallothionein genes are expressed both pre- and postnatally in a variety of tissues in animals as well as humans [44,45]. Similarly, expression of GH genes driven by the MT promoter has been found to start from embryonic day 13 onward in transgenic mice which will thereby develop immunological tolerance to the foreign peptides [46–48]. The level of expression of MT driven genes in transgenic mice can be drastically increased by addition of heavy metal ions to the drinking water [46].

The gene coding for cytosolic PCK (GTP; EC 4.1.1.32), a pace-setting enzyme in gluconeogenesis, is expressed in multiple tissues and regulated in a complex tissue-specific manner [49,50]. In the liver, for example, cyclic AMP, glucocorticoids, thyroid hormone, prolactin and retinoic acid are inducers, and insulin and diacylglycerol are inhibitors [50]. The expression of the bGH structural gene under the transcriptional control of 460 base pairs of the PCK 5′ regulatory sequences mirrors the expression of the endogenous PCK with respect to both tissue specificity and developmental pattern. While the predominant site of PCKbGH transgene expression is the liver [24], bGH-specific mRNA is also present in kidney, adipose tissue, intestine and mammary gland [25]. Expression starts at day 19 of fetal development and rises approximately 200-fold after birth [51]. The transcriptional activity of the PCKbGH fusion gene in transgenic mice is responsive to dietary regulation. Diets high in carbohydrate markedly depress the activity of the PCK promoter by increasing the level of insulin in the serum, while starvation, or a diet devoid of carbohydrate but high in protein stimulates transgene expression [24]. In spite of the relatively late onset of bGH synthesis as compared to MT promoter driven GH transgene expression, PCKbGH transgenic mice do not seem to produce neutralizing anti-bGH-antibodies as evidenced by (i) the drastically increased growth rate of PCKbGH transgenic mice up to an age of at least 4 months (Ref. 24; unpublished data), (ii) the continuously elevated serum bGH levels and (iii) the close similarity of pathological lesions observed in PCKbGH and MTbGH transgenic mice.

The levels of expression of foreign GH genes observed in different lines of transgenic mice investigated in this study differ by several orders of magnitude. This is consistent with a considerable variation of data pertaining to the expression of GH transgenes in mice reported in the literature which have recently been summarized elsewhere [19,20]. Among the variety of factors that influence transgene expression the site of transgene integration in the genome plays an important role [52]. It is therefore possible to breed special lines of transgenic mice expressing foreign GH at a certain order of magnitude that best meets the requirements for answering a distinct experimental question. For example, therapeutical doses of GH in humans vary usually within the range 2–16 mg/m^2 per week which is up to 8-fold in excess of the normal production rate of 2–4 mg/m^2 per week [53,54]. Our MTbGH transgenic mice expressing bGH at low levels can therefore be considered as suitable models to mimick the therapeutical use of GH, while transgenic mice exhibiting high concentrations of foreign GH in their serum may help to recognize possible side effects of GH treatment more clearly and at an earlier age.

In addition to the variation of serum GH concentrations between various trans-

genic lines and various transgenic animals within the lines, we have studied the time course of MTGH transgene expression in individual mice. All animals investigated displayed continuously high levels of circulating GH with minor fluctuations which did not show any regular periodicity. By contrast, physiological GH secretion in mice follows a strongly pulsatile pattern resulting in low baseline serum levels and peaks with GH concentrations up to 100 ng/ml every 2.5 h and 1.4 h in males and females, respectively [55]. Mean serum GH concentrations calculated for male and female mice corresponded to 10 ± 3 and 16 ± 3 ng/ml, respectively.

As investigated by Western blot analysis, MThGH transgenic mice synthesize both monomeric forms of GH (22 kDa and 20 kDa, a product of alternative splicing [56]) which are predominant in human serum. Stewart et al. [57] have generated transgenic mice expressing only one, either the 22 kDa or the 20 kDa hGH variant by modifying the splice acceptor region of the third exon of the hGH gene. The origin of 26 kDa and 16 kDa hGH immunoreactive peptides in the serum of our MThGH transgenic mice cannot exactly be elucidated. Possible reasons for GH heterogeneity including posttranscriptional events, posttranslational processing, postsecretory events, metabolic conversions and laboratory artifacts have recently been reviewed by Baumann [58]. In serum from MTbGH and PCKbGH transgenic mice we found only 22 kDa bGH.

Apart from a dramatic promotion of body, skeletal and organ growth [28, 59–64], a variety of pathological alterations have been found in GH and in GRF transgenic mice. Both excess production of foreign GH and a stimulation of murine GH synthesis by expression of a GRF transgene regularly result in alterations of kidney and liver. This observation suggests that renal as well as hepatic alterations are not autocrine or paracrine effects of ectopically produced GH, but result from chronic elevation of circulating GH [61,65]. The variety of pathological alterations found in mice expressing various transgenes of the GH family have recently been reviewed [21]. Morphological and clinico-chemical investigations indicate renal failure to be the primary cause of the shortened life-span of these animals [19,61,66]. The pattern of nephropathological changes is the same for MThGH, MTbGH and PCKbGH transgenic mice. However, we found the degree of renal alterations to be more severe at an earlier age in our MThGH transgenic mice than in their bGH transgenic counterparts. Studies carried out by us and other groups to evaluate the development of renal alterations in GH transgenic mice revealed initial glomerular changes including both significant glomerular enlargement and progressive glomerulosclerosis [19,61,65–67]. End-stage renal lesions were characterized by a marked atrophy of nephrons, sclerosis and hyalinosis of remnant glomeruli and pronounced tubulocystic alterations [19,20].

In addition to renal lesions, hepatic alterations were consistently found in GH transgenic mice. Apart from non-neoplastic liver changes, we found hepatocellular tumours including both adenoma and carcinoma in aged bGH transgenic mice, thereby confirming our previous findings [68]. The occurrence of these tumours was

age-dependent and clearly related to bGH transgene expression as demonstrated by the absence of such tumours in the nontransgenic littermate controls. Similar to our findings pertaining to bGH transgenic mice, MToGH transgenic mice have been reported to develop age-related hepatocellular tumours at a very high frequency [69].

Deleterious consequences to health of chronically elevated serum GH were not only seen in mice. Transgenic pigs carrying MThGH, MTbGH and MTGRF fusion genes also exhibited a reduced life expectancy [70]. Clinical and pathological examination of the MTbGH transgenic pigs revealed high incidence of fertility problems, gastric ulcers, cardiomegaly, dermatitis, renal disease and degenerative joint disease. This joint pathology known as *osteochondrosis dissecans* was also observed in a pig expressing a MLVrGH transgene [71] and in PCKbGH transgenic pigs [72]. In the latter group, however, these disorders occurred later in life relative to MTbGH transgenic pigs. The authors suggested that the delayed onset of negative effects may be due to the use of the PCK promoter which is activated postnatally rather than prenatally as it is the case for the MT regulatory sequences. Treatment of pigs with high doses of pGH was also associated with systemic abnormalities such as liver and kidney degeneration, edema and joint problems [73–75].

Taken together our results and the findings of other authors indicate that (i) GH transgenic mice are unique models offering the possibility to study the consequences of various levels of foreign GH in immunocompetent animals on a long-term basis, (ii) continuously elevated circulating GH in mice is detrimental to health and even shortens life-span and (iii) the long-term risks and benefits of GH therapy in human beings should be carefully evaluated.

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