

INCREASING ENDOGENOUS GROWTH HORMONE

This invention belongs to the fields of animal husbandry and biochemistry, and provides an improved
5 method of increasing the growth hormone concentration in the blood of economic animals by administering growth hormone releasing factor in a superior manner.

An important part of the recent explosion of research in peptide chemistry and the effect of protein
10 hormones on all life forms has been research in growth hormone. The study of the production of growth hormone in various animals has revealed that a relatively small peptide known as growth hormone releasing factor has a major role in the production and secretion of growth
15 hormone in all the species which have been studied. Interestingly, endogenous growth hormone releasing factor (GRF), is a very similar peptide in all of the species whose GRF has been sequenced. It is a peptide of 43 or 44 amino acids in all known species, and the
20 acid terminal of the peptide is amidated in all known species except the rat. The sequence of amino acids in the various endogenous GRF's is quite similar; indeed, bovine GRF is identical to caprine GRF.

The following nomenclature is used in this
25 document. The term "growth hormone releasing factor" (GRF) is used to refer to any peptide which functions to increase the production and release of growth hormone in an economic mammal. The term "endogenous GRF" is used to refer to GRF naturally produced by an animal. In
30 referring to synthetically or recombinantly produced

GRF's, an initial is used to indicate the species whose GRF has been duplicated; e.g., "h" for human, "b" for bovine, etc. When a GRF is the acid form, the fact is stated; it is amidated otherwise. The term "analog" is
5 used to refer to peptides which function as GRF's, but have less amino acids than the endogenous GRF, or a different sequence. Synthetic GRF's containing less amino acids than the endogenous GRF are indicated by a number; e.g. "hGRF29" indicates a GRF made up of the
10 first 29 amino acids of human endogenous GRF.

The benefit of increasing the growth hormone level in an economic mammal is now well established. The most conspicuous known benefit is the increase in milk production by a dairy cow when growth hormone is
15 increased. Improved growth rates and feed efficiency by pigs and sheep having increased growth hormone levels have been reported in the literature. The same beneficial effects have not yet been reported in cattle, but it must be realized that growth hormone and GRF are
20 still scarce and expensive, and it is believed that no cattle feeding trials with either agent have yet been done. For some years the growth rate of abnormally small children has been increased by direct administration of growth hormone, at great expense because of the
25 difficulty of obtaining the hormone, and no comparable amount of study has as yet been devoted to the use of growth hormone in economic mammals.

The most extensively studied GRF, of course, is the human. It has been found that the endogenous
30 human GRF peptide can be extensively modified without

destroying its effectiveness in increasing the production and secretion of growth hormone. Human GRF analogs of 23 to 40 amino acids have been made, in both the amidated and acid-terminated forms, and found to be
5 active. Further, various changes have been made in the endogenous peptide, such as the interchange of histidine, 3-methylhistidine or N-acetyltyrosine in place of tyrosine at the 1-position of the peptide. Those substances are also effective.

10 It is perhaps not surprising, in view of the similarity of the endogenous GRF's of different species, that they typically are effective across species. For example, Kraft et al., Domestic Animal Endocrinology 2, 133-39 (1985) showed that endogenous human GRF and a
15 human GRF analog having 40 amino acids and a free acid terminal (hGRF40 acid) were active in rats, Rhesus monkeys, rabbits, sheep, cattle and chickens, as well as in humans.

In the human, it appears that GRF is produced
20 both by the pancreas and by the hypothalamus. Identical peptides are produced by both organs. The earliest GRF work was done with peptide produced by human pancreatic tumors, but it has now been clearly shown that the normal pancreas, as well as the hypothalamus, produces
25 the same peptide, which is endogenous human GRF.

There is an extensive literature on GRF and its relationship with the production and secretion of growth hormone. The following articles are mentioned as giving an overview, and an entry into the literature.

Ling et al., Ann. Rev. Biochem. 54, 403-23 (1985)
Baird et al., Neuroendocrinology 42, 273-76 (1986)
Kensinger et al., Fed. Proc. 45, 280 (1986)
Wehrenberg et al., Endocrinology 114, 1613-16 (1984)

5

Patents on synthetic bovine and ovine GRF have recently appeared: Brazeau et al., U.S. 4,585,756, and Bohlen et al., U.S. 4,605,643.

10 The discovery of human GRF led immediately to research into its use in growth hormone-deficient children. Research in GRF to increase growth hormone in economic animals followed soon after. Difficulty and confusion followed.

15 It was soon learned that, in the normal animal of any species, the concentration of growth hormone in the bloodstream varies in an episodic manner. Apparently, the secretion of growth hormone into the bloodstream is pulsatile. The frequency and amplitude of pulses varies with age and from male to female and from time to
20 time within the day, and increases with stress. It was soon found that the administration of a single dose of GRF would reliably increase concentration of growth hormone in the bloodstream for a short period of time, but that the maintenance of increased growth hormone was
25 not a simple matter.

For example, Vance et al., J. Clin. Endo. Metab. 60, 370-75 (1985), infused hGRF40 for 6 hours in normal men, and gave a large bolus injection of GRF after 5.5 hours of infusion. The total amount of growth
30 hormone released during the experimental period was not

different from control for any dose of GRF; a growth hormone response to the infusions was obtained, but the responses to the bolus doses were lessened so that the total was not different from control men who received
5 only the bolus dose.

Vance is typical of numerous articles which suggest that the amount of growth hormone which a normal animal can secrete is in some manner limited, and therefore that the administration of GRF cannot increase
10 the total secretion of growth hormone into the blood-stream over a relatively extended period of time.

Further articles showing declining growth hormone response, as GRF administration is continued, include the following.

15

Gelato et al., J. Clin. Endo. Metab. 61, 223-28 (1985), administered hGRF at 1 mcg/kg-hr intravenously in adult men. Growth hormone level fell after an initial increase, and
20 response to a bolus dose at the end of the 4-hour infusion was markedly less than in control men.

Losa et al., Acta. Endo. 107, 462-70 (1984), infused 100 mcg/hr of hGRF to 11
25 healthy people for periods of 2 or 5 hours. Plasma growth hormone levels immediately increased and then fell. The response to a bolus dose at the end of infusion was less than the response to an initial bolus. Other
30 patients were given a 50 mcg bolus dose every

2 hours, and the growth hormone response fell at each successive dose.

5 Vance et al., J. Clin. Invest. 75, 1584-90 (1985), administered hGRF40 to 6 normal men at 2 ng/kg-min for 24 hours, and then administered a large bolus dose. The secretion of growth hormone was increased by the GRF infusion, but response to the 24-hour bolus was greater in the controls. The overall
10 total growth hormone was the same in treated and control men.

Wehrenberg et al., Endo. 114, 1613-16 (1984), administered hGRF to rats at 15 mcg/hr for 24 hours. Plasma growth hormone rose
15 within 2 hours of the start of infusion, and fell after 6 hours. At the end of the infusion, the rats did not respond to a bolus dose of GRF.

Goldman et al., Clin. Res. 32, 266A (1984), infused GRF (source not stated) for 6
20 hours intravenously in normal men at 10 ng/kg-min. All patients showed an increase in serum growth hormone, peaking at from 1 to 4 hours. The level then fell but not as far as
25 control levels. The growth hormone response to a bolus dose during the infusions was half as large as a pre-infusion response.

Baile et al., Fed. Proc. 43, 2019 (1984),
30 injected sheep with 0.065 nmol/kg of GRF (source not stated). The growth hormone

response fell at each injection. The same result was obtained at 0.016 nmol/kg. Six-hour intravenous infusions of GRF gave dose-related responses within 1 hour, but all responses fell by approximately half as the infusion went on.

On the other hand, some authors assert that they have demonstrated continued effect from administration of GRF, with no indication of exhaustion of the pituitary.

Takano et al., Endo. Japan 32, 287-93 (1985), gave 40 mcg/hr of hGRF intravenously to normal men for 20 hours, and followed with an intravenous bolus of 2 mcg/kg. The total growth hormone secreted during the infusion was 4 times that in a control period, and most patients responded to the final bolus.

Al-Raheem et al., J. An. Sci. 61, (Abstract 113) (1985), infused a number of GRF analogs intravenously to steers for 6 hours. The plasma growth hormone level increased and remained elevated through the 6-hour period.

Thorner et al., N.E.J. Med. 312, 4-9 (1985), gave GRF subcutaneously to growth hormone-deficient children in doses of 1 to 3 mcg/kg, every 3 hours over a period of 6 months. The patients responded with increased growth hormone levels for the 6-month period.

Thorner et al., In Recent Progress in Hormone Research 42, 589-640 (1986), pointed out at page 612 that some patients with pancreatic tumors have greatly increased GRF production, which results in long-continued abnormally high production of growth hormone.

Moseley et al., J. Endo. 104, 433-39 (1985), administered GRF44 (source not stated) to steers for 5 days, intravenously, at the rate of 3.6 mg/day. The growth hormone response did not diminish as the 5-day infusion went on.

Hart et al., J. Endo. 105, 189-96 (1985), gave ewes injections of hGRF every 2 hours for 4 days. The ewes responded with increased plasma growth hormone and milk yield.

Petitclerc et al., Therapeutic Agents Produced by Genetic Engineering, symposium May 29-30, 1985, M.E.D.S.I., Paris, 343-58, administered intravenous doses of GRF (source not stated) twice a day for 10 days to dairy cows. The dose was 0.2 nmol/kg per dose. Growth hormone response was observed at each dose and milk yield increased by 4.8%. In another experiment, hGRF or hGRF29 was injected intravenously at the same dose every 4 hours for 10 days, and milk yield increases of 19.6% and 16.1% were observed.

The literature of GRF indicates a widespread belief that continuous administration of GRF is less effective than periodic or pulsatile administration. For example, Clark et al., Nature 314, 281-83 (1985),
5 gave hGRF29 to female rats, as a continuous infusion at the rate of 8 mcg/day, or in 8 pulses per day of 1 mcg each. The treatments were continued for 12 days. The rats which received the pulsed doses exhibited increased growth and growth hormone content in the pituitary.
10 Those responses were not seen in the continuously dosed animals.

In the literature, both intravenous and subcutaneous administration of GRF have been used. One comparison of those methods of administration, as well
15 as intranasal administration, is in Thorner et al., Recent Progress in Hormone Research 42, 621-23 (1986). Thorner administered hGRF40, and observed that 30-fold more GRF was needed when given subcutaneously than when given intravenously. The intranasal dose was 300 times
20 the intravenous dose.

Others, on the other hand, have published work indicating that subcutaneous and intravenous administration have more closely comparable results. For example, McCutcheon et al., J. Dairy Sci. 67, 288-96 (1984),
25 reported that the growth hormone response of 1 mg of hGRF29, administered subcutaneously to cows, was about one third the response of the same dose, administered intravenously. McCutcheon was confirmed by Johke et al., Endo. Japan 31, 55-61 (1984), who found that the
30 growth hormone response of subcutaneously administered

hGRF in cattle was 37% of the response to an intravenous administration.

Thus, the general teaching of the literature on GRF is that the most preferred method of administration is intravenous, and that most scientists have found it advisable to administer GRF in a pulsatile manner. By and large, continuous administration has not been accepted. Where continuous administration has been used, it has been by the intravenous route.

The present invention provides a method of increasing endogenous growth hormone concentration in the blood of an economic mammal which comprises administering an effective amount of a growth hormone releasing factor to the animal subcutaneously in a substantially continuous manner for an economically significant period of time. *The amount is preferably from 6 to 12 mg/day.*

The present invention is of use in economic mammals generally, of which the most highly preferred animal is the dairy cow. Bovines, or cattle, constitute the most preferred class of mammals, and cattle and pigs are also a preferred class. Cattle, pigs and sheep are a third preferred class. The invention is also of use in other economic mammals, including goats, camels, horses and the like, but use in such animals is of less immediate importance.

As was explained above, GRF from one species will increase growth hormone in other species. Accordingly, in practicing this invention, one need not use only GRF of the species being treated. It is preferred to administer GRF of the same species - for example, to

administer ovine GRF to sheep. However, the benefit of the invention will be obtained when GRF of any mammalian species is administered to any economic mammal. For example, human GRF may be given to pigs, sheep or
5 cattle, bovine GRF may be given to sheep or pigs, porcine GRF may be given to cattle or sheep, and so forth, according to economic considerations or convenience, with confidence that the benefit of the invention will be obtained.

10 Of course, GRF isolated from animal organs may be used, but it is much more practical to prepare it synthetically or by recombinant methods. Both synthetic and recombinant production of peptides are now conventional and numerous types of GRF have been prepared
15 thereby. See U.S. Patents 4,585,756 and 4,605,643 for a compilation of references on the subject.

It has been shown that not only the complete GRF's, but also many analogs of GRF, are effective to increase the production and release of growth hormone.
20 For example, the 23, 27, 29, 30, 31, 34, 37 and 40-amino acid analogs of human GRF have all been made and found to be effective. However, the first 29 amino acids appear to provide most of the activity. Further, both amidated and acid-terminated analogs and complete GRF's
25 have been shown to be effective.

Accordingly, it is believed to be common knowledge in the art that complete GRF's, and GRF analogs, in both amidated and acid-terminated forms, will increase growth hormone in the homologous species
30 and in other species as well. Thus, in the practice of

this invention, the term "growth hormone releasing factor (GRF)" is used to include all mammalian GRF's, and all effective analogs of those GRF's, in both amidated and acid forms. Those of skill in the art know
5 how to recognize effective analogs, and how to titrate the dose of a given type of GRF to obtain the optimum result in a given species.

The essence of the invention is administration in a substantially continuous manner for a long period
10 of time. By the term "substantially continuous" is meant administration in a manner which maintains a substantially constant level of GRF in the treated animal. It is possible to obtain substantially continuous administration by frequent pulses of GRF. Since the
15 half-life of GRF in the body is quite short - 48 minutes in man - pulsatile administration must be very frequent if its effect is to be substantially continuous. For example, GRF could be administered pulse-wise, at intervals in the range of 15 minutes or less, within the
20 concept of the present invention. Pulses at intervals of more than about 30 minutes would undoubtedly fail to produce a substantially continuous effect.

It is preferred, however, to administer GRF in the present invention by means which as closely as
25 possible approximate truly continuous administration. The most preferred method of administration is by means of an implanted osmotic or mechanical pump, or an implanted diffusion device which allows the GRF peptide to escape into the body, essentially one molecule at a
30 time.

The invention is defined as including the administration of GRF for an economically significantly lengthy period of time. By this phrase is meant the administration of GRF for a period of time long enough
5 that the economic benefit of increasing growth hormone becomes evident. Most preferably, administration of GRF is continued throughout a stage of the treated mammal's life. For example, in the case of dairy cattle, administration would most preferably be continued throughout
10 a period of lactation. In the case of a beef animal, administration would preferably be continued throughout the growing or the finishing stage of the animal's maturation.

Thus, the most preferred period of time for
15 use of the invention is at least about 90 days. Another preferred period of time is at least about 30 days, since, it is believed, that period of time will always be long enough for the effect of GRF to become evident and create an economic benefit in the treated animal.
20 It is believed that administration of GRF in the present invention should always be continued for at least 13 days, in order to assure that the benefit of the invention is obtained.

The specific benefit of the invention is
25 described as increased growth hormone in the blood of the treated mammal. It appears that growth hormone does not become effective in the body until it is secreted from the producing glands and circulates in the bloodstream. Thus, the benefit of the present invention is
30 stated in terms of growth hormone in the bloodstream,

rather than in terms of the mere production of growth hormone.

The mechanics of administering GRF in the practice of the present invention may be accomplished in many ways. First, the peptide may be diluted to a convenient volume, preferably in water, and injected subcutaneously with a mechanical pump. Such pumps were used in the operating examples shown below, and are available in a number of different types. All that is necessary is a permanently affixed needle under the skin in any convenient location. The same effect is obtained with an implanted battery - or chemically - driven pump, which is installed permanently under the animal's skin.

A rather convenient slow-release form of GRF can be obtained by preparing a formulation of the peptide in an oil-wax mixture. Similar formulations were taught many years ago, as by U.S. 2,493,202, for the administration of penicillin. Davis et al., J. Dairy Sci. 66, 1980-82 (1983), shows the administration of growth hormone to sheep in an oil-wax composition. Such compositions, in general, comprise in the range of 5-10% wax in a vegetable oil. Suitable waxes include carnauba wax, beeswax and the like, and the most commonly used oils are peanut or sesame oil. The concentration of GRF in the formulation, and the amount of formulation to inject, of course, are readily calculated from the desired daily dose of GRF.

Further, GRF may be administered in the practice of this invention in the form of microcapsules. The microencapsulation of drugs and other substances has

been the subject of research for many years. The following references are mentioned for the convenience of the reader; veterinary pharmacists will be aware of the following and numerous other references concerning microencapsulation.

Goosen et al. microencapsulated living tissue or cells for implantation, using capsules formed of semipermeable membranes. Alginates were preferred. U.S. Patent 4,487,758. To much the same effect are the publications of Damon Corporation, such as U.S. Patents 4,352,883 and 4,409,331.

Polylactic and polyglycolic acids have been used to form microcapsules. U.S. Patents 4,479,911 and PCT Publication 83/03061.

Perhaps the most widely used type of microcapsules are those comprising cellulose esters such as cellulose acetate or butyrate. Typical publications include U.S. Patents 3,954,678 and 3,859,228 and British Patent 1,297,476.

A further, particularly convenient method of administration is by means of a diffusion-driven implantable device. Such devices are now in extremely wide use in feedlot cattle, usually to administer a steroid. The concept is to mingle the active ingredient with a polymer in which the peptide is soluble, and to rely on diffusion of the peptide through the polymer to achieve the desired release rate. Compositions designed for making diffusion implants for peptides have been recently described. U.S. Patent 4,452,775 describes a matrix composed of cholesterol with appropriate binding

and lubricating agents. U.S. Patent 4,526,938 teaches a hydrogel-forming polymer which is said to release GRF, among other peptides, in a reliable and predictable manner.

5 Still further, the use of osmolality-driven pumps in veterinary pharmacy is now widespread. In particular, Alza Corporation is noted for constructing osmolality-powered devices. Such devices may be used in the practice of the present invention. In general, such
10 devices use a semipermeable membrane to separate a reservoir of active ingredient from the body and to control the rate of release of active ingredient.

 The beneficial increase of growth hormone, which is the benefit of the present invention, is
15 brought about by administering an effective growth hormone-increasing amount of GRF to the animal. In general, effective amounts of GRF are in the range from about 0.5 to about 3 mg/day for sheep, goats or pigs, and in the range from about 3 to about 12 mg/day for
20 cattle. The doses and dose ranges are discussed in this document in terms of the daily dose, but the reader must understand that the daily dose is to be administered substantially continuously throughout the 24 hours. More preferred dose ranges are from about 1 to about 2
25 mg of GRF per day for sheep, pigs or goats, and from about 4 to about 8 mg/day for cattle. The knowledgeable reader will understand that research in GRF is still intense, and more potent GRF analogs may well be discovered. The preferred doses of more potent analogs, of
30 course, will be smaller than those stated here. The

most beneficial dose for a given animal will vary with its size, its state of health and nourishment, the desired growth rate or milk yield, and the identity of the GRF to be used. Trivial experiments are used to
5 titrate various GRF dose rates and find the optimum dose per day.

The following operating example of the present invention is given to assure that the reader fully understands the invention and the manner in which
10 it is carried out.

Example 1

The animals in this experiment were Hereford
15 steers weighing about 600 lb each. There were 4 steers per treatment. The animals were acclimated to metabolism crates, and were cannulated with Silastic^(R.T.M.) cannulae in 1 jugular vein, and subcutaneously over the ribs. Four animals each were infused subcutaneously with 6
20 mg/day and 12 mg/day of hGRF, obtained from recombinant synthesis, which differed from the natural peptide in having serine in place of methionine at position 27. The third group of 4 steers were infused with physiological saline only.

25 The infusions were continued for 13 days. There was a control period of 5 days before infusion began. The animals were fed 12 lb/day of a high energy, high-protein corn-based ration, in 2 equal feedings about 12 hours apart.

Blood samples were taken from each animal in the mornings, 2 samples shortly prior to and 2 samples shortly after feeding. Sampling was continued for a day after the end of infusion. The blood samples were
5 heparinized and centrifuged, and the plasma was analyzed for bovine growth hormone.

Total urine was also collected throughout the experimental period and total urinary nitrogen excreted was measured and reported in the following table. The
10 reduced urinary nitrogen in the treated animals shows that they retained more protein than did the controls.

The growth hormone results are reported in Table I as means of all of the analyses run, averaging the pre- and post-feeding assays on each day. Some of
15 the animals on 6 mg/day of GRF lost their bleeding cannulae toward the end of the experiment, and the later results for those animals are based on only a few results. The data are reported as ng/ml.

Table I

<u>Plasma Growth Hormone, ng/ml</u>				
5	Rel. <u>Hours</u>	<u>Control</u>	GRF <u>6mg/day</u>	GRF <u>12mg/day</u>
	-16	20.1	16.0	10.5
	Start Infusion			
10	5	4.4	25.7	14.8
	28	5.8	15.8	32.1
	52	10.8	23.9	20.9
	76	4.2	16.7	25.7
	100	15.4	18.0	25.3
15	124	6.5	13.5	16.1
	148	7.6	13.0	22.3
	172	8.7	25.7	22.2
	195	9.0	20.6	40.1
	220	5.4	19.0	41.4
20	244	5.0	35.0	18.2
	268	10.9	21.1	41.2
	292	8.0	8.8	30.9
	Stop Infusion			
	318	6.8		23.4
25	336	12.9		21.0

Urinary Nitrogen, g/day

	<u>Rel.</u> <u>Hours</u>	<u>Control</u>	GRF <u>6mg/day</u>	GRF <u>12mg/day</u>
5				
	-60	68.7	69.2	69.5
	96	69.4	63.2	64.3
	192	71.4	62.9	61.4
	312	71.1	65.2	61.3
10	0-312	70.4	63.9	62.4



CLAIMS

1. A method of increasing endogenous growth hormone concentration in the blood of an economic mammal^{as herein defined} which comprises administering an effective amount of a growth hormone releasing factor to the animal subcutaneously in a substantially continuous manner^{as herein defined,} for an economically significant period of time, ^{as herein defined.}
2. A method of Claim 1 wherein the mammal is a bovine, a pig or a sheep.
3. A method of Claim 2 wherein the mammal is a dairy cow.
4. A method of any one of Claims 1 to 3 wherein the growth hormone releasing factor is identical to the mammal's endogenous growth hormone releasing factor.
5. A method of Claim 4 wherein the growth hormone releasing factor is bovine growth hormone releasing factor.
6. A method according to any one of the preceding claims wherein the amount of growth hormone releasing factor is from ~~about~~ 6 to ~~about~~ 12 mg/day.
7. A method according to any one of the preceding claims wherein the time of administration is at least ~~about~~ 30 days.
8. A method according to any one of the preceding claims wherein the time of administration is at least ~~about~~ 90 days.
9. A method according to any one of the preceding claims wherein the time of administration is throughout a stage of the mammal's life, ^{as herein defined.}