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(54) Title: METHOD FOR TREATING INTESTINAL DISEASES (57) Abstract A method for the treatment of disorders in gut function in animals including humans, which method includes administering to a patient to be treated an effective amount of a mammalian insulin-like growth factor-I (IGF-I) or a peptide analogue thereof.		

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METHOD FOR TREATING INTESTINAL DISEASES

This invention relates to insulin-like growth factor-I (IGF-I) and its analogues. More particularly, the invention relates to the use of IGF-I and its analogues to treat disorders in gut function, e.g. relating to the effects of intestinal diseases.

IGF-I is a small protein that has been shown to stimulate the growth of cells in culture. Animal growth is also stimulated in pituitary-deficient, normal and catabolic states. Kidney function is also improved. These studies have led to the interpretation that IGF-I may be usefully applied in humans:

- (1) to treat growth hormone deficiencies;
- (2) to suppress the loss of body protein in catabolic states following burns, infection or other trauma; and
- (3) as a treatment for patients suffering from renal diseases.

A number of human diseases result either in the subject having a lesser amount of gut tissue than required for normal digestion or absorption, or alternatively a diseased but normal-length gut in which digestion or absorption is impaired. Examples of human diseases that fit these two categories include short-gut syndrome, chronic ulcerative gut diseases, inflammatory gut diseases such as colitis and Crohn's disease, and necrotising enterocolitis in infants.

IGF-I is not known in the prior art to increase the growth or function of the cell types that comprise the abdominal gut, viz the stomach, duodenum, jejunum, ileum, cecum and colon. It is these regions of the gut that are affected in the diseases described above. Gut tissues do contain receptors for IGF-I (for example see: M. Laburthe et al, Am. J. Physiol. 254, G457, 1988; D.J. Pillion et al., Am. J. Physiol. 257, E27, 1989; H. Werner et al, Proc. Natl. Acad. Sci. USA 86, 7451, 1989), and are known under certain conditions to synthesise IGF-I or IGF-I messenger RNA (for example see: P.K. Lund et al., J. Biol. Chem. 261, 14539, 1986; A.J. D'Ercole et al.,

Pediatr. Res. 20, 253, 1986; H.-A. Hansson et al., Histochemistry 89, 403, 1988; W.L. Lowe, Jr., J. Clin. Invest. 84, 619, 1989).

IGF-I has previously been administered to dwarf
5 mice, hypophysectomized rats, diabetic rats, starved mice
and rats, normal rats, mini poodles and normal human
subjects. The levels of IGF-I have also been increased by
insertion of an IGF-I-expressing transgene in mice. In
10 most of these studies IGF-I treatment has led to an
increase in body growth. It has been noted that an
undesirable side effect of high doses of IGF-I is
hypoglycemia. However, in none of the reported studies
was there any indication of an IGF-I effect on the
abdominal gut. For example in one investigation where
15 over expression of IGF-I was produced by transgenesis and
led to substantial body growth in transgenic mice, the
fractional weight of the duodenum was the same as in
IGF-deficient animals (R.R. Behringer et al.,
Endocrinology 127, 1033, 1990). In another study the
20 administration of low doses of IGF-I to suckling rats did
not alter the weights of gastrointestinal organs (G.P.
Young et al., Digestion 46S2, 240, 1990). In particular,
there exists no prior report on the increase of gut weight
following IGF-I administration.

25 It is accordingly an object of the present
invention to overcome or at least alleviate, one or more
of the difficulties related to the prior art.

The first aspect of the present method concerns
the treatment of animals, including humans, with disorders
30 in gut function by using mammalian, preferably human IGF-I.

Accordingly in a first aspect of the present
invention there is provided a method for the treatment of
disorders in gut function in animals, including humans,
which method includes administering to a patient to be
35 treated an effective amount of mammalian insulin-like
growth factor-1 (IGF-I) or a peptide analogue thereof.

By the term "disorders in gut function", as used
herein, we mean disorders in one or more of the stomach,
duodenum, jejunum plus ileum, and colon. Such disorders

may relate to intestinal diseases and/or surgical treatments. Thus where reference is made herein in general terms to the treatment of intestinal diseases, it is to be understood that we include the treatment of
5 non-diseased intestines, part of which have been surgically removed and where increased growth of the residual intestine may be advantageous.

The term "peptide analogue", as used herein, includes one or more of the peptide analogues referred to
10 in applicant's International Patent Applications PCT/AU86/00246 and PCT/AU88/00485, or fusion proteins derived therefrom as described in International Patent Application PCT/AU90/00210, referred to above, the entire disclosures of which are incorporated herein by reference.

15 An effective amount of mammalian IGF-I or an analogue of IGF-I is defined as that needed to increase the weight of gut tissue by more than approximately 20% above the weight of gut tissue prior to treatment.

Surprisingly IGF-I and its analogues have now
20 been found by the applicants to increase the weight of the stomach, duodenum, jejunum plus ileum, and colon as well as the total gut weight. Of particular significance is the fact that these effects on growth have been found to occur in animals under a range of conditions, including

- 25 (a) in animals in which gut function had previously been markedly compromised by the surgical removal of a substantial portion of the jejunum plus ileum
(b) animals to which glucocorticoids have been administered in order to produce a catabolic state
30 (c) animals in which diabetes had been induced by the drug streptozotocin, a condition that led to gut growth so that the effects of IGF-I were above an already-stimulated growth state, and
(d) animals which had compromised kidney function.

35 Since glucocorticoids are administered to humans as a current treatment for the inflammation that characterizes several gut diseases, the present discovery that gut growth can be partially restored by IGF-I or its analogues even in the presence of glucocorticoids is both

surprising and advantageous.

In a preferred aspect of the present invention a peptide analogue to mammalian, preferably human, IGF-I is administered. Preferably the peptide analogue is an
5 analogue wherein from 1 to 5 amino acid residues are absent from the N-terminal of mammalian IGF-I. Preferably 3 amino acid residues are absent therefrom. Such peptide analogue has been designated des(1-3)IGF-I.

Alternatively the peptide analogue may be an
10 analogue wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of mammalian IGF-I, and optionally replaced by a different amino acid residue. Preferably the analogue is one wherein the glutamic acid residue is replaced by an arginine residue.

15 More preferably the peptide analogue has an N-terminal sequence selected from

Val-Leu-Cys-

Arg-Leu-Cys-

Gly-Leu-Cys-

20 Gly-Thr-Leu-Cys-

Gly-Pro-Arg-Thr-Leu-Cys-

Gly-Pro-Gly-Arg-Leu-Cys-

Gly-Pro-Gly-Gly-Leu-Cys-

Gly-Pro-Gly-Thr-Leu-Cys-

25 Gly-Pro-Gln-Thr-Leu-Cys-

Gly-Pro-Lys-Thr-Leu-Cys-

Gly-Pro-Leu-Thr-Leu-Cys-

with the Cys residue shown being that normally at position 6 from the N-terminal.

30 In a further alternative aspect, the peptide analogue is a fusion protein including

a first amino acid sequence including approximately the first 100 N-terminal amino acids of methionine porcine growth hormone, or a fragment thereof;
35 and

a second amino acid sequence of mammalian insulin-like growth factor-1, or an analogue thereof, joined to the C-terminal of the first amino acid sequence.

Preferably the first amino acid sequence includes

approximately the first 46, more preferably the first 11, N-terminal amino acids of methionine porcine growth hormone, or fragment thereof.

The IGF-I analogues include des(1-3)IGF-I, specified in the co-owned International application PCT/AU86/00246, which specification is included herein by reference and had been shown to increase the growth of cultured cells at lower dose rates than required for IGF-I. In the development of the present invention, des (1-3)IGF-I has surprisingly been shown to show this increased potency in vivo, in the treatment of disorders in gut function, since lower concentrations of the analogue than of IGF-I itself produce comparable increases in the growth of gut tissue.

The IGF-I analogues also include a fusion protein MpGH(11)VN/R³IGF-I (abbreviated to LR³), specified as Example 13 in the co-owned International Application PCT/AU90/00210, which specification is included herein by reference, and had been shown to increase the growth of cultured cells at lower dose rates than required for IGF-I. In the present invention, LR³ has also surprisingly been shown to show increased potency in the treatment of disorders in gut function, since lower concentrations of the analogue than of IGF-I itself produce comparable increases in the growth of gut tissue.

Although the method in particular applies to the treatment of human subjects with IGF-I or an analogue of IGF-I, it can also be applied veterinarily to animals with intestinal diseases.

Accordingly, in a further aspect, the present invention provides a pharmaceutical or veterinary composition for the treatment of disorders in gut function including:

- (a) an effective amount of a mammalian, preferably human, IGF-I or peptide analogue thereof; and
- (b) a pharmaceutically or veterinarily acceptable diluent, carrier or excipient therefor.

In a preferred form, the present invention provides a pharmaceutical or veterinary composition

wherein the IGF-I or the analogue of IGF-I is present in amounts sufficient to provide a dose rate of approximately 10 to 2000, preferably 100 to 1000 micrograms/kg body weight/day.

5 In a further preferred aspect of the present invention, there is provided a method for the treatment of disorders in gut function which method includes administering to a patient to be treated a pharmaceutical or veterinary composition including

10 (a) an effective amount of a mammalian, preferably human, IGF-I or peptide analogue thereof, and

(b) a pharmaceutically or veterinarily acceptable diluent, carrier or excipient therefor;

intravenously, subcutaneously, intramuscularly or
15 enterally at a dose rate of approximately 10 to 2000, preferably 100 to 1000 micrograms/kg body weight/day. Treatment may continue for a period of approximately 1 to 60 days, preferably approximately 5 to 30 days.

The dose rate, dose intervals and treatment
20 period may be adjusted to the degree of intestinal disease and the route of administration. Caution should be taken that blood glucose is monitored so that hypoglycemia can be prevented.

The dose rates and intervals for the
25 administration of des(1-3)IGF-I and related analogues may be set at levels proportionally adjusted to the relative potency of the analogue to that of IGF-I itself. For example the levels for des(1-3)IGF-I or LR³ will be proportionally less than for the full IGF-I peptide in
30 accordance with the increased potency of des(1-3)IGF-I or LR³. Dose rates of 50 to 500 micrograms of des(1-3) IGF-I LR³ /kg body weight/day are preferred.

In a still further aspect of the present invention there is provided the use of a mammalian
35 insulin-like growth factor-1 (IGF-I) or a peptide analogue thereof for the manufacture of a pharmaceutical or veterinary preparation for the treatment of disorders in gut function.

The pharmaceutical or veterinary preparations may

be prepared utilising conventional techniques.

The benefits and parameters of the present invention will now be more fully described with reference to the accompanying examples. It should be understood, however, that the following description is illustrative only and should not be taken in any way as a restriction of the generality of the foregoing description.

EXAMPLE 1

Effects of IGF-I and des(1-3)IGF-I on stomach growth in the growth hormone-deficient lit/lit mouse

At 6 weeks of age lit/lit mice were housed individually and weighed on a daily basis, and by 8 weeks of age it had been established that body growth had virtually ceased.

Mice were then randomised into 5 groups according to body weight and sex and were injected daily for 20 days with

- (a) 120 μ l of a sterile solution containing one part of HCl (10 mmol/l) and nine parts of potassium phosphate (50 mmol/l), NaCl (150 mmol/l) and 0.1% human serum albumin at pH 7.4 (diluent),
- (b) 120 μ l of diluent containing 3 μ g IGF-I,
- (c) 120 μ l of diluent containing 3 μ g des(1-3)IGF-I,
- (d) 120 μ l of diluent containing 30 μ g IGF-I, or
- (e) 120 μ l of diluent containing 30 μ g des(1-3)IGF-I.

Each dose was administered subcutaneously as two injections, one at 9-10 am, the other at 4-5 pm. The animals were weighed and their lengths measured at 7 day intervals.

On day 21 the animals were killed by an anaesthetic overdose and tissues removed for weighing. The body weights, animal lengths (including tail) and stomach weights are shown in the following Table 1. Values are means $\pm$ standard errors with statistical significance from diluent-treated control animals shown as *P<0.05; **P<0.01. The numbers of animals are given in parentheses.

Since the initial body weights were 10 grams for each group, the daily dose rates were approximately

equivalent to 300 µg and 3000 µg of each peptide per kg body weight.

Stomach weights, expressed as a percentage of the diluent group, were 105% and 123% with 300 µg and 3000 µg/kg body weight/day of IGF-I respectively, and 110% and 123% with 300 µg and 3000 µg/kg body weight/day of des(1-3)IGF-1 respectively.

EXAMPLE 2

Effects of IGF-I and des(1-3)IGF-I LR³ on gut weights in dexamethasone-treated rats

Male Hooded Wistar rats, weighing on average 152 g (range 138-164 g) and maintained in metabolism cages, had Alzet model 2001 osmotic pumps inserted subcutaneously within the scapular region under ether anaesthesia. One pump delivered dexamethasone at 20 micrograms/d and the other either

- (a) 0.1M acetic acid as diluent;
- (b) IGF-I at 111 µg/d;
- (c) IGF-I at 278 µg/d;
- (d) IGF-I at 695 µg/d;
- (e) des(1-3) GF- at 44 µg/d;
- (f) des(1-3)IGF-I at 111 µg/d;
- (g) des(1-3)IGF-I at 278 µg/d;
- (h) LR³ at 44 µg/d;
- (i) LR³ at 111 µg/d;
- (j) LR³ at 278 µg/d.

Animals were maintained in the metabolism cages for 7 days with daily measurements of body weight, food and nitrogen intake and nitrogen excretion. After this period the animals were killed by exsanguination under anaesthesia and the gastro-intestinal tract from stomach to colon was removed and separated into stomach, duodenum, jejunum plus ileum, cecum and colon. All regions were cleared of food or fecal contents and weighed.

The body weights and the weights of the different regions of the gastrointestinal tract are given as means ± standard errors in Table 2. Statistical significance from the diluent-treated group is shown as *P<0.05; **P<0.01; ***P<0.001. There were six animals in each

group.

The weights of the total gut from stomach through colon is depicted in Figure 1 as a fraction of total body weight in the format of dose-response curves for IGF-I, des(1-3)IGF-I and LR³.

For a midpoint region of the ileum and the colon a portion was cut longitudinally and scraped to remove the mucosal layer. The weight of this layer was expressed as a percentage of the total mucosa plus muscularis. The protein contents of the same regions of the ileum were measured and expressed as mg per gram wet weight of tissue. These values are given as means $\pm$ standard errors in Table 3. Statistical significance from the diluent-treated animals was not achieved using ANOVA ($P > 0.05$).

This example demonstrates marked increases in the weights of different regions of the gut of dexamethasone-treated rats following the administration OF IGF-I, des(1-3)IGF-I or LR³. The effects are dose dependent and are greater for des(1-3)IGF-I or LR³ at doses equivalent to IGF-I.

The increased growth occurs predominantly through an expansion in the cross-sectional area of the gut because the length of each region is either not increased or increased only slightly.

It is evident from Figure 1 that the increase in gut weight is proportionally above that occurring for body weight.

Both mucosal and muscularis regions of the jejunum and colon show increased growth because the percent by weight accounted for by the mucosa is not affected by IGF treatment.

Growth occurs by an increase in tissue protein since the percent protein content of the jejunum and colon is not changed by IGF treatment.

EXAMPLE 3

Quantitative histology of the duodenum from rats in Example 2

The mid region of the duodenum from certain animals in Example 1 was fixed in Bouin's fixative,

dehydrated, embedded, transverse sections cut and stained with haematoxylin and eosin for quantitative histological analysis. All animals in groups (a), (c), (g) and (j) of Example 2 were processed and measurements of villus height, crypt depth, mucosal area, submucosal area, muscularis externa area and total cross-sectional area were obtained. For each duodenum, 30 villus heights, 30 crypt depths and 8 area measurements were averaged to obtain representative values. Means $\pm$ SEM (N=6) are shown in Table 4.

This example establishes that the growth of the duodenum produced by IGF-I, des(1-3)IGF-I or LR³ at dose rates of 278 μ g/d is accompanied by statistically significant ($P < 0.01$) increases in cross-sectional area. The increase is predominantly in the mucosal area, although the muscularis layer is also increased. The villi that make up much of the mucosa and play a key role in digestion and absorption, are also increased in height.

EXAMPLE 4

Effects of IGF-I and des(1-3)IGF-I on gut weights in rats treated following partial resection of the jejunum plus ileum

Male Sprague Dawley rats, weighing on average 175 g (range 160-193 g) and maintained in metabolism cages, had Alzet model 2001 osmotic pumps inserted as in Example 2. The dose ranges of growth factors were 170 μ g/d for IGF-I and for des(1-3)IGF-I as well as a higher dose rate of 425 μ g IGF-I/d. At the same time as the pumps were inserted subcutaneously, and using tribromethanol in amylene hydrate anaesthesia and aseptic techniques, the jejunum plus ileum was exposed through a mid-line incision. The mid 80% of these parts of the intestine was removed starting 10 cm from the ligament of Treitz and finishing 10 cm from the ileo-cecal valve. The intestine and abdominal cavity were bathed frequently in sterile saline containing penicillin (1000 U/ml). To further guard against possible infection, the animals were injected with 0.6 ml of procaine penicillin before surgery and again 4 days later. The animals were returned to

their metabolic cages and allowed free access to food and water. Body weights, food intakes, nitrogen intakes and nitrogen outputs were measured daily.

After 7 days of treatment, the animals were
5 killed by exsanguination under anaesthesia and the
gastro-intestinal tract from stomach to colon was removed
and separated into stomach, duodenum, residual jejunum
plus ileum, colon and cecum. All collected regions of the
gut were cleared of food or fecal contents and weighed.
10 The body weights and the weights of the different regions
of the gut are given as means $\pm$ standard errors in Table
5. Statistical significance from the diluent-treated
group is shown as * $P < 0.05$; ** $P < 0.01$. There were 7 animals
in the diluent and des(1-3)IGF-I groups, six in the group
15 with the low dose of IGF-I and five in the group with the
high dose of IGF-I.

The example shows that des(1-3)IGF-I and a 2.5
fold higher dose of IGF-I produce pronounced growth
effects on the gut. A dose of IGF-I equal
20 to that of des(1-3)IGF-I gave no statistically-significant
effects.

The total gut weight less the residual jejunum
and ileum, expressed as a percentage of the diluent group,
was 113% with 170 μ g IGF-I/d, 131% with 425 μ g IGF-I/d,
25 and 125% with 170 μ g des(1-3)IGF-I/d.

EXAMPLE 5

Effects of IGF-I, des(1-3)IGF-I and MpGH(11)VN/R³IGF-I (LR³) on gut growth in diabetic rats

Male Hooded-Wistar rats weighing approximately
30 150 g were injected with streptozotocin intra-peritoneally
at a dose of 70 mg/kg and transferred to metabolic cages.
Diabetes was confirmed by blood glucose measurements.
After 7 days (average body weight 162 g) the animals were
implanted with osmotic pumps in exactly the same way and to
35 deliver exactly the same doses as in Example 1. After 7
days treatment the animals were killed and the weights of
the following gut organs measured: stomach, duodenum,
ileum plus jejunum and colon. These values are shown in
Table 6 and the total gut weights in Figure 2. Each value

is the mean $\pm$ SEM for six animals. Statistical significance (ANOVA; least significant difference) is shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus diluent-treated diabetic rats.

5 These results demonstrate that IGF-I and lower doses of des(1-3)IGF-I or LR³ produce substantial growth effects on the stomach, ileum plus jejunum, colon and total gut weights. Effects on the duodenum are somewhat less.

10 The response of IGFs in the diabetic rats is especially important since even untreated animals already have heavier gut weights as a result of the hyperphagia associated with this condition. For example the weight of ileum plus jejunum was 5.08 ± 0.29 g in another group of
15 animals in which the diabetes was treated by insulin administration. This is lower than the diluent group in Table 5, notwithstanding the fact that the body weight of the insulin-treated animals was much heavier (231 ± 8 g).

EXAMPLE 6

20 Effects of IGF-I and des(1-3)IGF-I on gut weights in rats following partial nephrectomy

Partial renal failure was produced in Sprague Dawley male rats (95-125 g) by a 2-stage sub-total nephrectomy. This was performed via flank incisions, by
25 ligating terminal branches of the left renal artery to give ischemia of at least half of the left kidney (Day 0), with a right nephrectomy being undertaken one week later, at which time the right jugular vein was also cannulated (Day 7). Partially nephrectomized rats were selected on
30 day 14 for inclusion in the treatment period of the study on the basis of detectable proteinuria, and of increased urine volume and serum urea levels at least twice those of the sham-operated control animals. On Day 16 treatment was commenced by means of mini-osmotic pumps (Alzet Model
35 2001, Alza Co., Palo Alto, California) which were implanted subcutaneously in the dorsal thoracic region under halothane anesthesia. Nephrectomized rats were randomly allocated to 4 treatment groups: diluent treated (0.1M acetic acid), low dose IGF-I treated ($170 \mu\text{g/d}$),

high dose IGF-I treated (425 µg/d), or des(1-3)IGF-I treated (170 µg/d). The average body weight at time of pump insertion was 193 g. Animals were killed on Day 23 and gut weights measured as in Example 2. These are shown in Table 7. Statistical significance from diluent-treated animals is indicated by *P<0.05, **P<0.01.

This example demonstrates that gut weights are increased by 7 days treatment with IGF-I or des(1-3)IGF-I in rats that are compromised by partial nephrectomy.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

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TABLE 1
Growth effects of IGF-I and des(1-3)IGF-I over a 20-day treatment period of lit/lit mice

Treatment Group	Body Weight (g)	Animal Length (mm)	Stomach Weight (mg)
Diluent (8)	10.20±0.19	124±2	64.3±3.0
IGF-I, 3 µg/d (8)	10.75±0.28	127±1	67.8±2.1
30 µg/d (6)	11.25±0.41	132±1*	79.0±3.0**
des(1-3) IGF-I, 3 µg/d (8)	10.76±0.31	127±2	70.6±2.5
30 µg/d (6)	11.33±0.42	132±1	79.3±4.4**

TABLE 2
Gut weights (g), lengths (cm) and body weights (g) of dexamethasone-treated rats
also treated with IGF-I, des(1-3)IGF-I or LR³

Treatment	Body Weight	Stomach Weight	Duodenum	
			Weight	Length
Diluent	131±3	0.87±0.03	0.50±0.02	7.2±0.4
IGF-I, 111 µg/d	130±4	0.93±0.03*	0.55±0.03	6.9±0.3
278 µg/d	143±4*	1.09±0.02***	0.70±0.02***	7.6±0.2
695 µg/d	158±2***	1.17±0.02***	0.84±0.02***	8.0±0.2*
des(1-3)IGF-I,				
44 µg/d	135±3	0.98±0.02**	0.63±0.03**	7.6±0.3
111 µg/d	144±5*	1.06±0.02***	0.68±0.02***	7.6±0.2
278 µg/d	151±2***	1.14±0.02***	0.86±0.03***	8.7±0.3***
LR ³				
44 µg/d	135±1	0.95±0.01*	0.61±0.02**	7.3±0.3
111 µg/d	140±4	1.07±0.02***	0.75±0.03***	7.6±0.4
278 µg/d	150±3***	1.16±0.02***	0.86±0.03***	8.3±0.2**

TABLE 2 - CONTINUED

Ileum + Jejunum		Colon	
Weight	Length	Weight	Length
3.07±0.22	70.8±4.1	0.68±0.05	11.0±0.4
3.60±0.19	71.8±2.6	0.64±0.02	12.0±0.3
4.30±0.21***	71.7±3.1	0.92±0.04***	13.3±0.6
5.22±0.23***	86.1±3.6	0.93±0.05***	12.7±0.6
3.80±0.25*	72.1±3.5	0.72±0.05	11.3±0.8
4.44±0.24***	71.8±2.6	0.86±0.04**	13.4±0.5
5.09±0.21***	73.0±4.1	0.91±0.04***	11.3±0.8
3.93±0.24**	72.4±3.8	0.75±0.03	12.3±0.2
4.67±0.18***	75.8±3.3	0.89±0.05**	12.5±0.8
5.20±0.33***	79.5±4.1	0.98±0.05***	12.9±0.6

TABLE 3

Fractional weights and protein contents of the jejunum in the mucosal layers in the jejunum and in the colon dexamethasone-treated rats treated with IGFs

Treatment	Jejunum			Colon	
	Mucosa (% of total)	Mucosa Protein (mg/g weight)	Muscularis Protein (mg/g weight)	Mucosa (% of total)	
Diluent	55.1±3.3	124±5	169±4	30.5±2.5	
IGF-I, 111 µg/d	58.8±2.2	117±6	151±4	29.6±1.7	
278 µg/d	62.7±1.5	135±5	161±5	31.7±1.7	
695 µg/d	61.1±2.8	136±5	159±2	28.8±1.2	
des(1-3)IGF-I, 44 µg/d	53.3±3.9	134±5	161±5	31.1±1.7	
111 µg/d	59.6±2.3	140±5	162±4	27.5±2.2	
278 µg/d	55.3±3.6	140±4	154±6	32.5±2.5	
LR ³ , 44 µg/d	56.8±4.2	119±10	147±4	30.2±2.8	
111 µg/d	60.7±3.3	139±4	168±6	29.6±2.1	
278 µg/d	54.2±3.2	135±6	159±5	30.6±2.2	

TABLE 4
Sections from rats treated with IGF-I des(1-3)IGF-I, LR³ or diluent

Measurement	Treatment Group			
	Diluent	IGF-I	des(1-3)IGF-I	LR ³
Villus height (mm)	0.65±0.02	0.76±0.03	0.73±0.03	0.84±0.03***
Crypt depth (mm)	0.20±0.01	0.23±0.01	0.23±0.00	0.23±0.01
Villus : crypt ratio	3.3 ±0.2	3.4 ±0.2	3.3 ±0.1	3.7 ±0.2
Mucosal area (mm ²)	5.67±0.44	7.72±0.56**	8.03±0.58**	8.29±0.56***
Sub-mucosal area (mm ²)	0.42±0.04	0.51±0.04	0.52±0.03	0.51±0.04
Musc. externa area (mm ²)	1.20±0.15	1.51±0.05*	1.54±0.06**	1.41±0.12
Total area (mm ²)	7.29±0.61	9.74±0.59**	10.10±0.62**	10.21±0.66**

*P<0.05, **P<0.01, ***P<0.001 versus diluent

TABLE 5

Duodenum weight (mg)	Diluent	IGF-I 170µg/d	IGF-I 425 µg/d	des(1-3) IGF-1 170 µg/d
Body weight (g)	171.6 ± 4.5	173.4 ± 5.9	190.9 ± 4.2	184.1 ± 5.6
Stomach weight (mg)	980 ± 75	1089 ± 47	1236** ± 26	1136* ± 52
Duodenum weight (mg)	1067 ± 75	1179 ± 88	1572** ± 81	1542** ± 102
Residual Jejunum + Ileum weight (mg)	1984 ± 118	2217 ± 244	2288 ± 60	2259 ± 150
Colon weight (g)	1067 ± 162	1305 ± 164	1330 ± 65	1250 ± 127
Total gut weight less residual jejunum & ileum (g)	4.28 ± 0.27	4.83 ± 0.34	5.59** ± 0.13	5.37** ± 0.24

TABLE 6

Gut weights (g) of diabetic rats and body weights (g)

	Body Weight	Stomach	Duodenum	Ileum + Jejunum	Colon
Diluent	172±6	1.01±0.04	0.72±0.09	6.10±0.49	0.95±0.02
IGF-I, 111 µg/d	186±4	1.13±0.04	0.74±0.08	6.21±0.37	1.05±0.07
278 µg/d	198±8*	1.19±0.06*	0.91±0.05	7.41±0.32*	1.09±0.05
695 µg/d	210±8***	1.44±0.08***	0.94±0.06*	8.17±0.32***	1.18±0.05**
des(1-3) IGF-I, 44 µg/d	185±6	1.23±0.05**	0.82±0.11	6.83±0.38	1.02±0.05
111 µg/d	194±7*	1.27±0.06**	0.89±0.07	7.97±0.20**	1.16±0.08**
278 µg/d	212±8***	1.49±0.07***	1.02±0.09**	8.69±0.40***	1.41±0.03***
LR ³ 44 µg/d	190±7	1.14±0.06	0.85±0.07	7.58±0.48*	1.05±0.04
111 µg/d	204±6**	1.38±0.04***	0.88±0.11	7.83±0.53**	1.25±0.07***
278 µg/d	213±8***	1.48±0.08***	0.91±0.06	8.47±0.46***	1.50±0.04***

TABLE 7

Gut weights (g) in partially-nephrectomised rats treated with IGF-I or des(1-3)IGF-I

Treatment	Stomach	Duodenum	Ileum + Jejunum	Colon	Total
Diluent	1.08±0.04	0.51±0.02	3.78±0.19	0.83±0.03	6.21±0.22
IGF-I, 170 µg/d	1.19±0.03	0.63±0.03*	4.70±0.27**	0.97±0.03*	7.50±0.30**
425 µg/d	1.25±0.06*	0.75±0.07**	5.11±0.22**	1.05±0.07**	8.16±0.27**
des(1-3) IGF-I					
170 µg/d	1.16±0.06	0.66±0.03*	4.48±0.15*	0.95±0.06	7.24±0.10**

1. A method for the treatment of disorders in gut function in animals including humans, which method includes administering to a patient to be treated an effective amount of a mammalian insulin-like growth factor-I (IGF-I) or a peptide analogue thereof.
2. A method according to Claim 1 wherein the IGF-I or peptide analogue thereof is administered in an amount of from approximately 10 to 5000 microgram/kg body weight/day for a period of approximately 1 to 60 days.
3. A method according to Claim 1, which method includes administering to a patient an effective amount of human insulin-like growth factor-I.
4. A method according to Claim 1, which method includes administering to a patient an effective amount of a peptide analogue of mammalian insulin-like growth factor-I, wherein from 1 to 5 amino acid residues are absent from the N-terminal thereof.
5. A method according to Claim 3, wherein 3 amino acid residues are absent from the N-terminal thereof.
6. A method according to Claim 4, wherein the peptide analogue is administered at a dose rate of approximately 50 to 500 micrograms/kg body weight/day for a period of approximately 1 to 60 days.
7. A method according to Claim 1, which method includes administering to the patient an effective amount of a peptide analogue of mammalian insulin-like growth factor-I, wherein at least the glutamic acid residue is absent at position 3 from the N-terminal thereof, and is optionally replaced by a different amino acid residue.
8. A method according to Claim 7, wherein the glutamic acid residue is replaced by an argenine residue.
9. A method according to Claim 7, wherein the peptide analogue has an N-terminal sequence selected from
 - Val-Leu-Cys-
 - Arg-Leu-Cys-
 - Gly-Leu-Cys-
 - Gly-Thr-Leu-Cys-
 - Gly-Pro-Arg-Thr-Leu-Cys-
 - Gly-Pro-Gly-Arg-Leu-Cys-

Gly-Pro-Gly-Gly-Leu-Cys-
Gly-Pro-Gly-Thr-Leu-Cys-
Gly-Pro-Gln-Thr-Leu-Cys-
Gly-Pro-Lys-Thr-Leu-Cys-
5 Gly-Pro-Leu-Thr-Leu-Cys-

with the Cys residue shown being that normally at position 6 from the N-terminal.

10. A method according to Claim 6 wherein the peptide analogue is administered at a dose rate of approximately
10 50 to 500 micrograms/kg body weight/day for a period of approximately 1 to 4 days.

11. A method according to Claim 1, which method includes administering to a patient an effective amount of a peptide analogue of mammalian insulin-like growth
15 factor-I which is a fusion protein including

a first amino acid sequence including approximately the first 100 N-terminal amino acids of methionine porcine growth hormone, or a fragment thereof; and

20 a second amino acid sequence of mammalian insulin-like growth factor-I, or an analogue thereof, joined to the C-terminal of the first amino acid sequence.

12. A method according to Claim 10, wherein the first amino acid sequence includes approximately the first 46,
25 more preferably the first 11 N-terminal amino acids of methionine porcine growth hormone, or fragment thereof.

13. A method according to Claim 10, wherein the peptide analogue is administered at a dose rate of approximately 50 to 500 microgram/kg body weight/day for a
30 period of approximately 1 to 60 days.

14. A pharmaceutical or veterinary composition for the treatment of disorders in gut function, including

(a) an effective amount of a mammalian insulin-like growth factor-I or peptide analogue thereof; and
35 (b) a pharmaceutically or veterinarily acceptable diluent carrier or excipient therefor.

15. A pharmaceutical or veterinary composition according to Claim 14, wherein the insulin-like growth factor-I or analogue thereof is present in amounts

sufficient to provide a dose rate of approximately 10 to 5000 microgram/kg body weight/day.

16. A method for the treatment of disorders in gut function, which method includes administering to a patient
5 to be treated a pharmaceutical or veterinary composition including

(a) an effective amount of a mammalian insulin-like growth factor-I or analogue thereof; and

(b) a pharmaceutically or veterinarily acceptable
10 diluent carrier or excipient therefor

intravenously, subcutaneously, intramuscularly or enterily at a dose rate of approximately 10 to 5000 microgram/kg body weight/day for a period of approximately 1 to 60 days.

17. Use of a mammalian insulin-like growth factor-I (IGF-I) or a peptide analogue thereof for the manufacture of a pharmaceutical or veterinary preparation for the treatment of disorders in gut function.

18. A method according to Claim 1 substantially as
20 hereinbefore described with reference to any one of the Examples.

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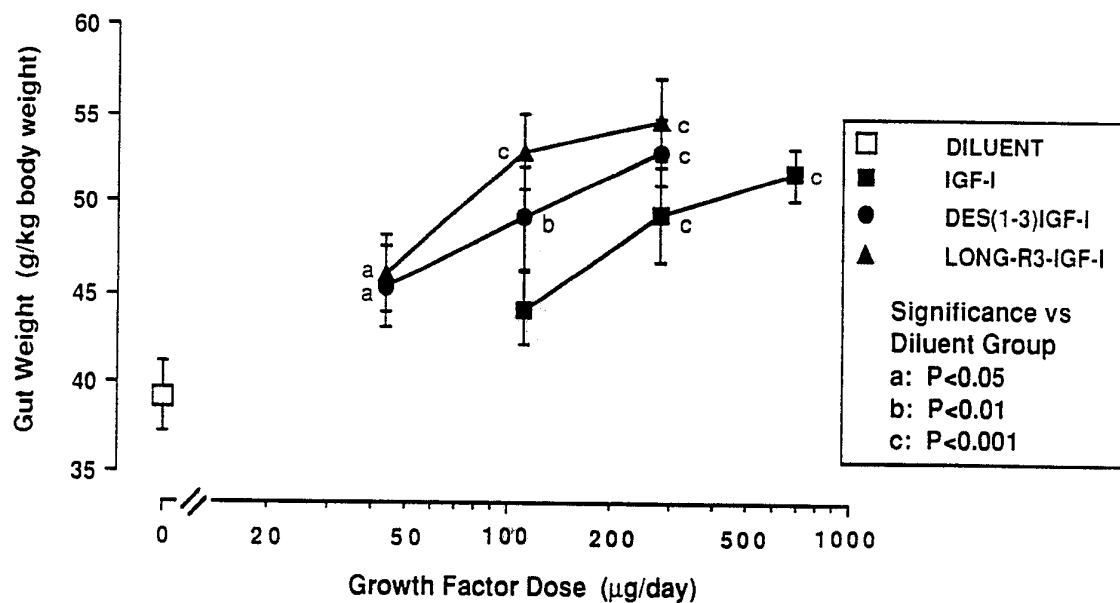
35

FIGURE 1

Effects of IGF-I, des(1-3)IGF-I and LR³ on total gut weight in dexamethasone-treated rats. Letters indicate statistical significance from diluent group (ANOVA): (a) $P < 0.05$; (b) $P < 0.01$; (c) $P < 0.001$.

FIG. 1. GLUCOCORTICOID-TREATED RATS:
TOTAL GUT WEIGHT AS A PROPORTION OF BODY WEIGHT

Dose Responses for IGF-I, Des(1-3)IGF-I and Long-R3-IGF-I



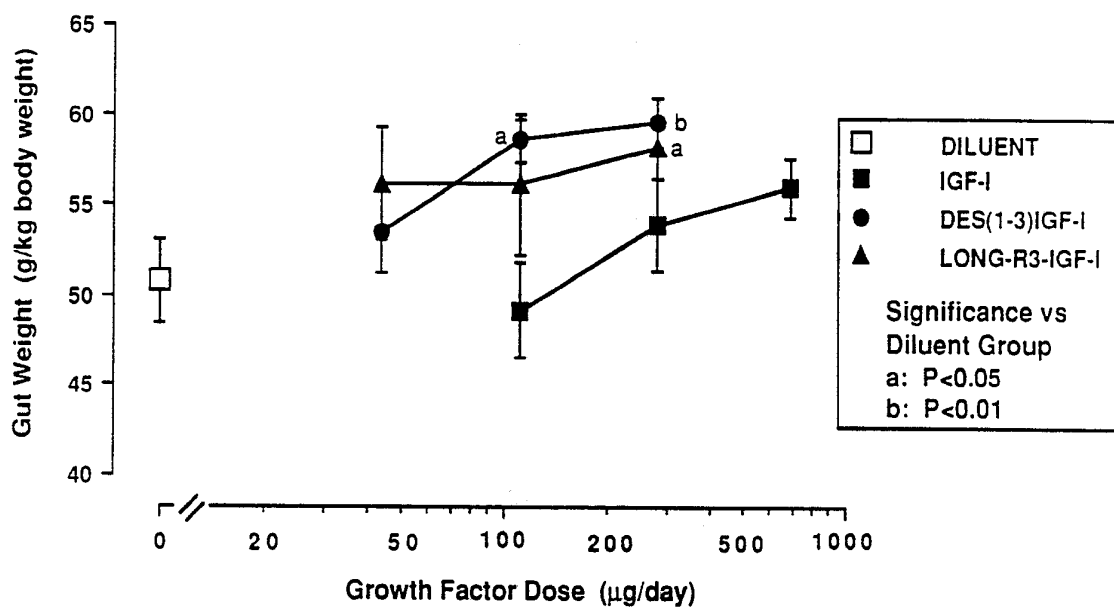
2/2

FIGURE 2

Total gut weights (stomach to colon) of diabetic rats treated with IGFs for 7 days. Letters indicate statistical significance (ANOVA) from diluent group : (a) $P < 0.05$; (b) $P < 0.01$; (c) $P < 0.001$.

**FIG. 2. DIABETIC RATS:
TOTAL GUT WEIGHT AS A PROPORTION OF BODY WEIGHT**

Dose Responses for IGF-I, Des(1-3)IGF-I and Long-R3-IGF-I



INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 91/00031

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int. Cl. ⁵ A61K 37/36		
II. FIELDS SEARCHED		
Minimum Documentation Searched 7		
Classification System	Classification Symbols	
IPC ⁵	A61K 37/36	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8		
III. DOCUMENTS CONSIDERED TO BE RELEVANT 9		
Category*	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
X	EP,A1, 0308386 (KABIVTRUM AB) 22 March 1989 (22.03.89)	(14, 15, 17)
* Special categories of cited documents: 10		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 1 May 1991 (01.05.91)	Date of Mailing of this International Search Report 13 MAY 1991	
International Searching Authority Australian Patent Office	Signature of Authorized Officer J.P. PULVIRENTI <i>J.P. Pulvirenti</i>	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 91/00031

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document
Cited in Search
Report

Patent Family Members

EP 308386

EP 308386

SE 8703625
