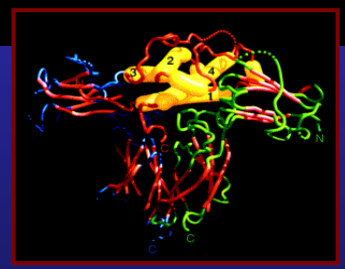
TESTS ANTIDOPING PER IL GH

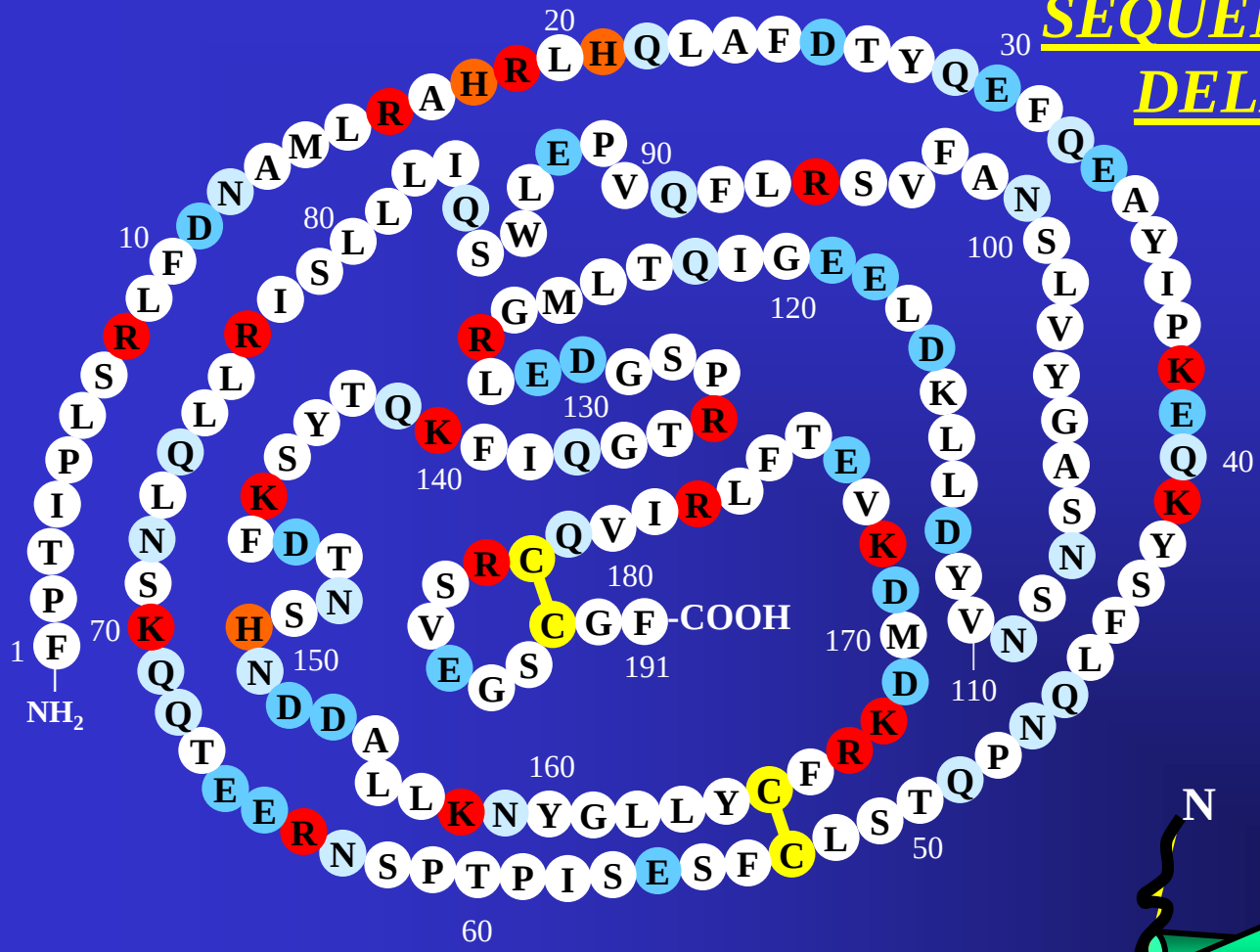


Eugenio E. Müller

Dipartimento di Farmacologia Medica
Università degli Studi di Milano

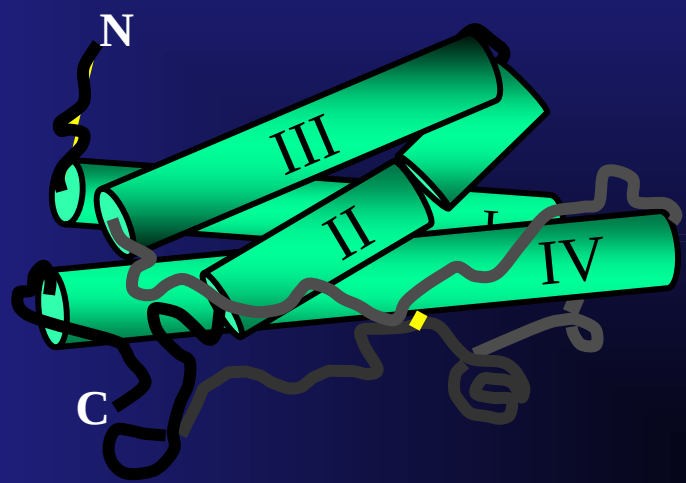
Campobasso, 06 Aprile 2005

SEQUENZA E STRUTTURA DELL'ORMONE DELLA CRESCITA



Sequenza primaria dell'ormone della crescita umano

Struttura cristallina del GH suino



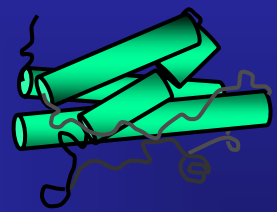
GENE DELLA SOMATOTROPINA

26 191 aa

PRESOMATOTROPINA

(217 aa)

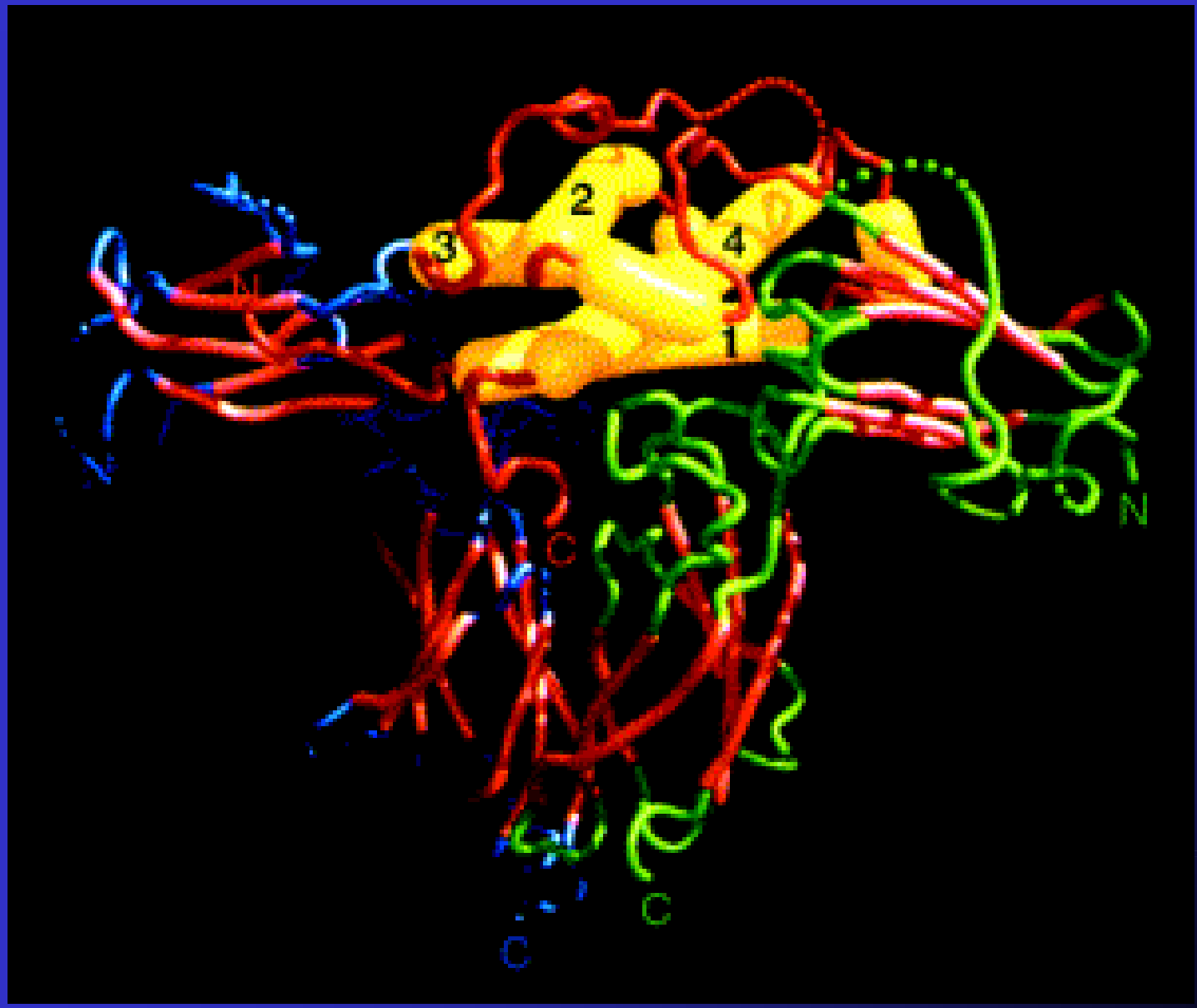
SP GH

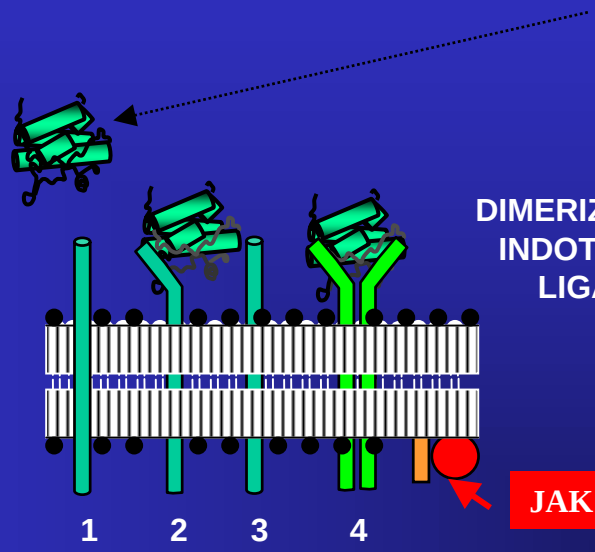


(191 aa)

FORME VARIANTI DELL'ORMONE SOMATOTROPO

VARIANTI	PERCENTUALI
22 KD	76%
20 KD	16%
GH acidico	8%
GH monomerico	55%
GH dimerico	27%
GH oligomerico	18%
GH 22 KD complessato	45%
GH 20 KD complessato	25%

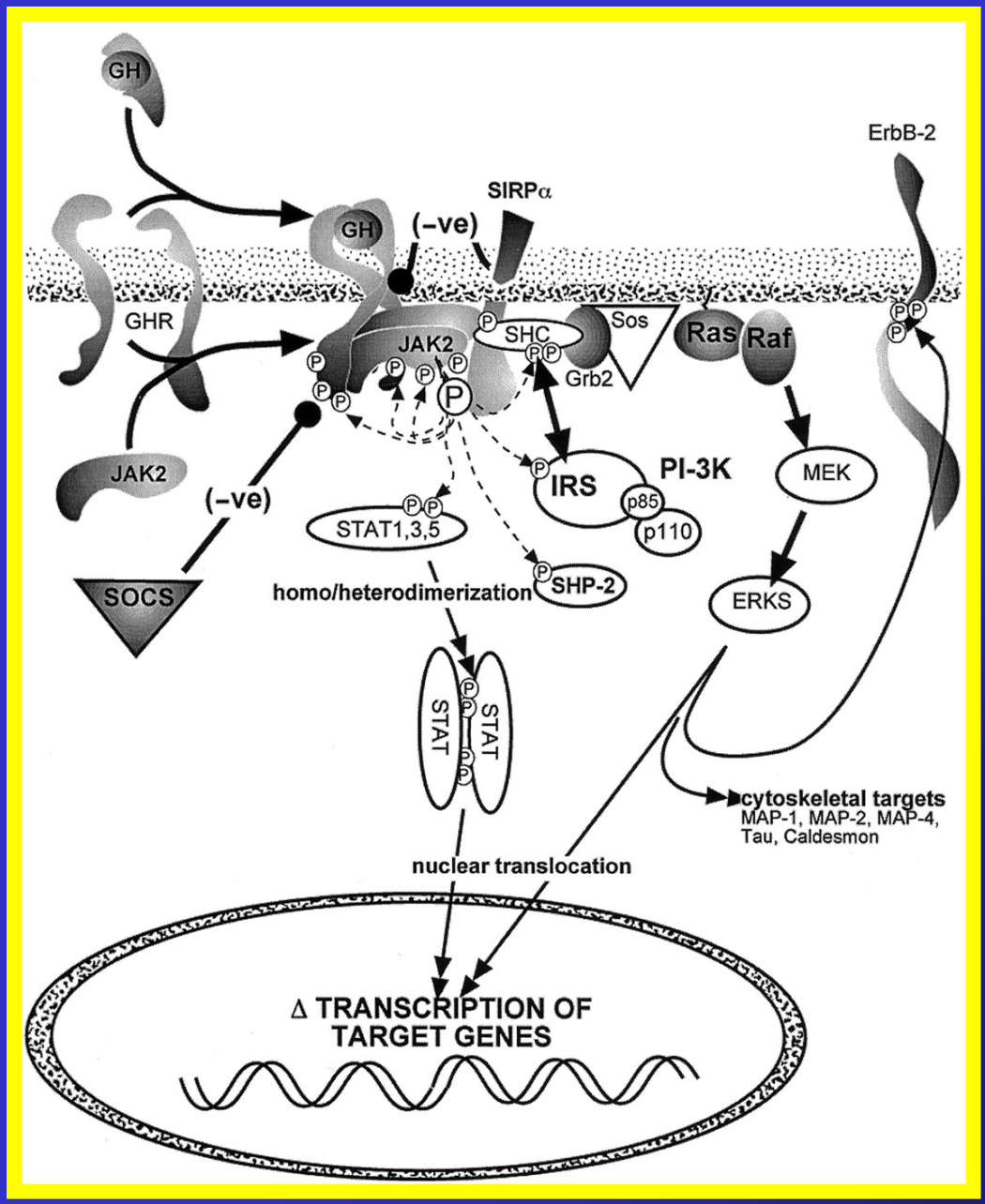

2
3
4
1
N
C
C
C
N



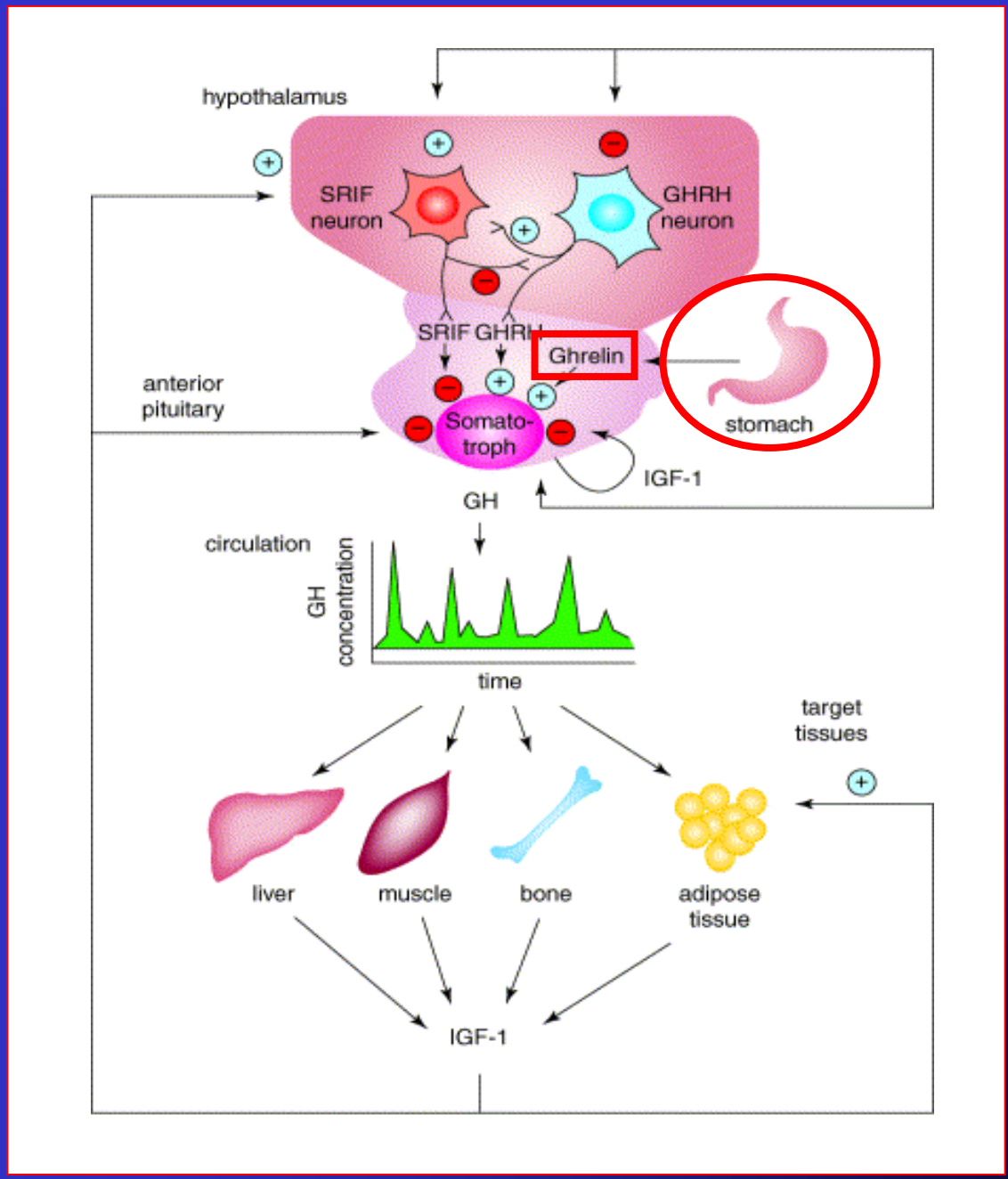
DIMERIZZAZIONE INDOTTA DAL LIGANDO

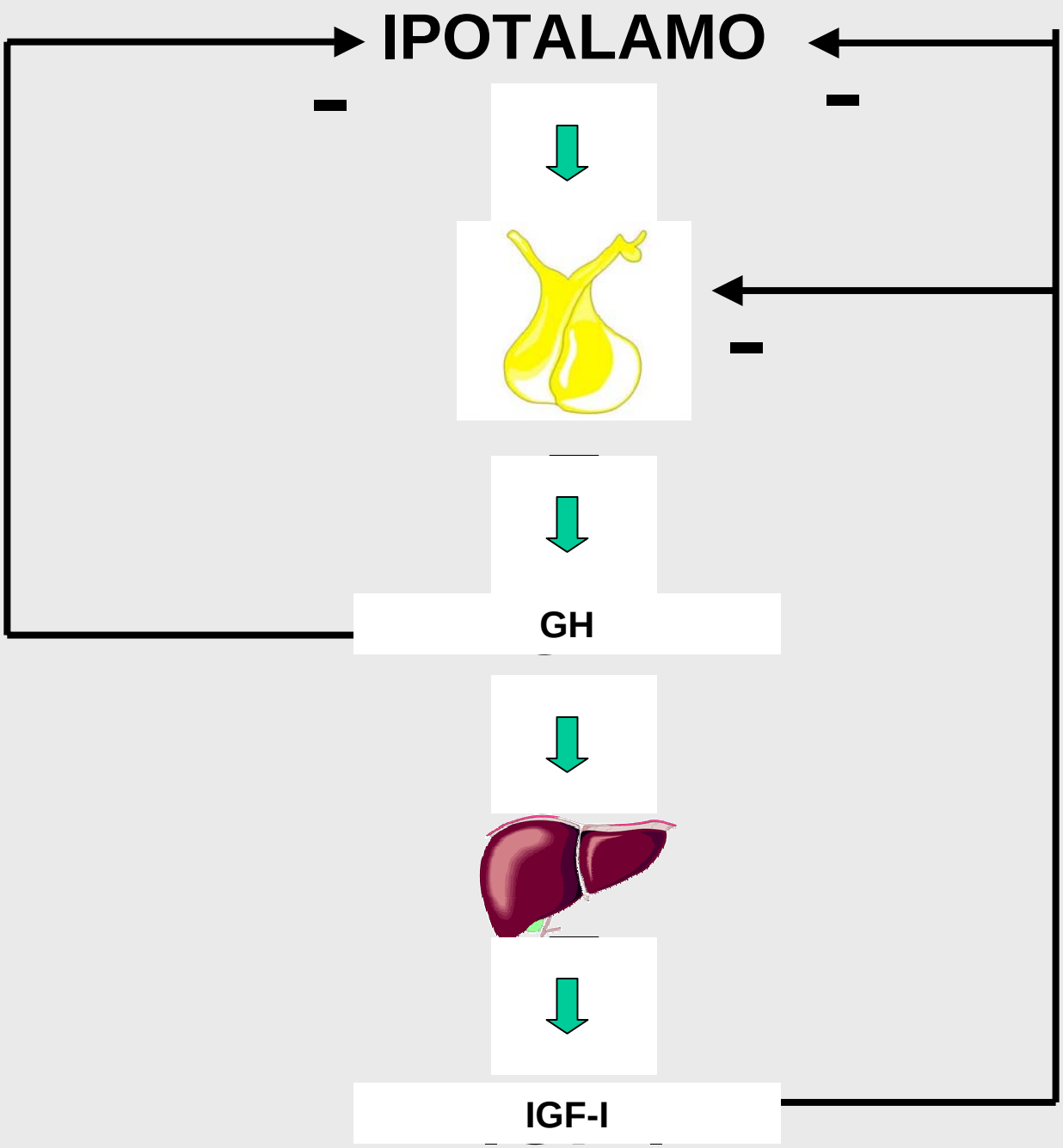
JAK TIROSIN KINASI

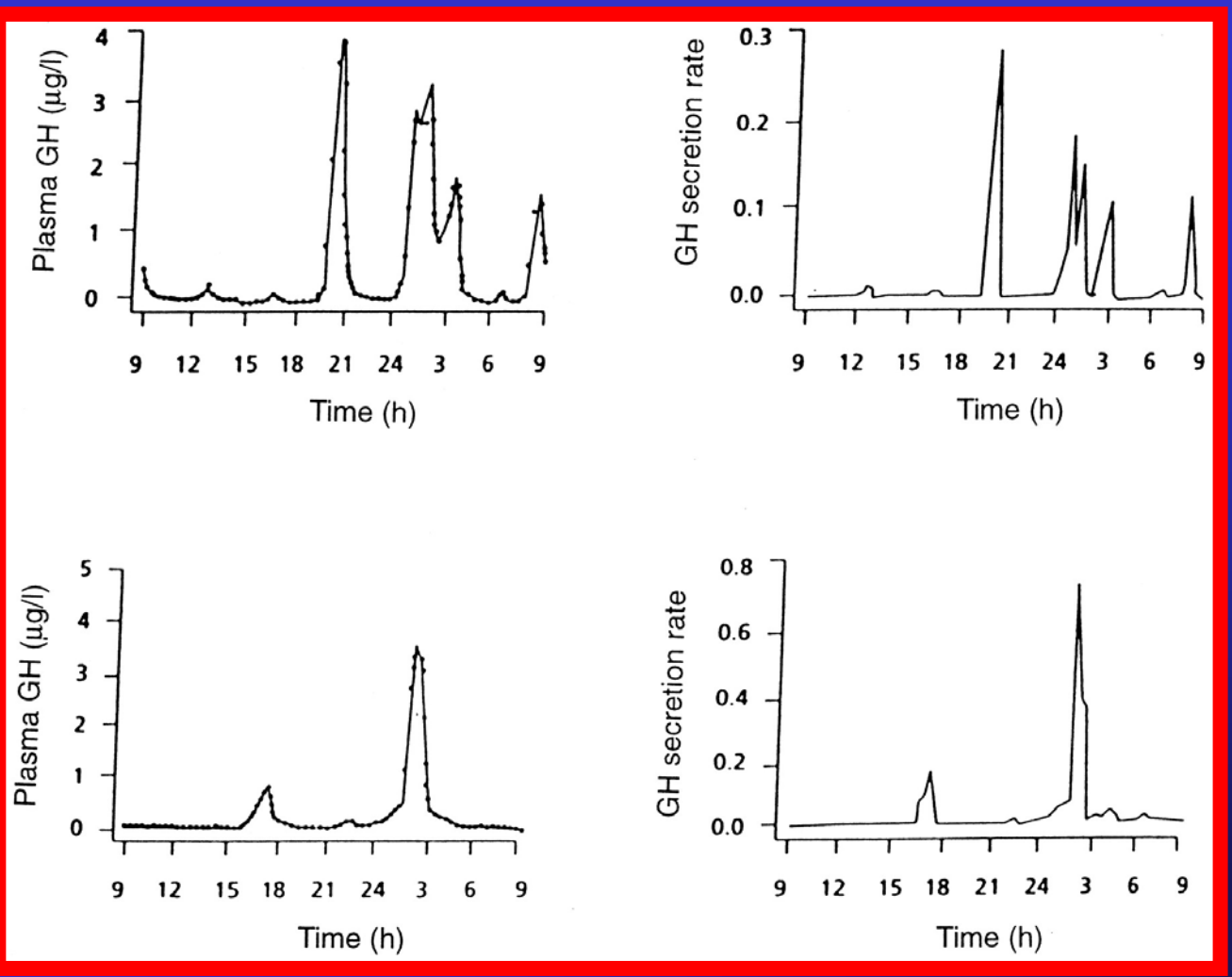
EFFETTI FISIOLOGICI



Signaling pathways used by GH. JAK, Janus kinase; STAT, signal transducers and activators of transcription; SOCS, suppressors of cytokine signaling; SHP2, protein tyrosine phosphatase.


hypothalamus
SRIF neuron
GHRH neuron
SRIF GHRH
Ghrelin
stomach
anterior pituitary
Somato-troph
IGF-1
GH
circulation
GH concentration
time
target tissues
liver
muscle
bone
adipose tissue
IGF-1


IPOTALAMO
-
-
-
GH
IGF-I



Profili della secrezione dell'ormone della crescita (GH) nelle 24-h in donne (*riquadri in alto*) e uomini (*riquadri in basso*). I *riquadri a sinistra* riportano le concentrazioni sieriche di GH nel tempo e la curva ottenuta con l'analisi di deconvoluzione. I *riquadri a destra* mostrano l'entità di secrezione calcolata con questa analisi [Da van den Bergh *et al*.].

GHS milestones

1977 **synthesis of peptidyl GHS**

1995 **synthesis of non peptidyl GHS**

1996 **GHS-R cloning**

1999 **ghrelin, a natural GHS-R ligand**

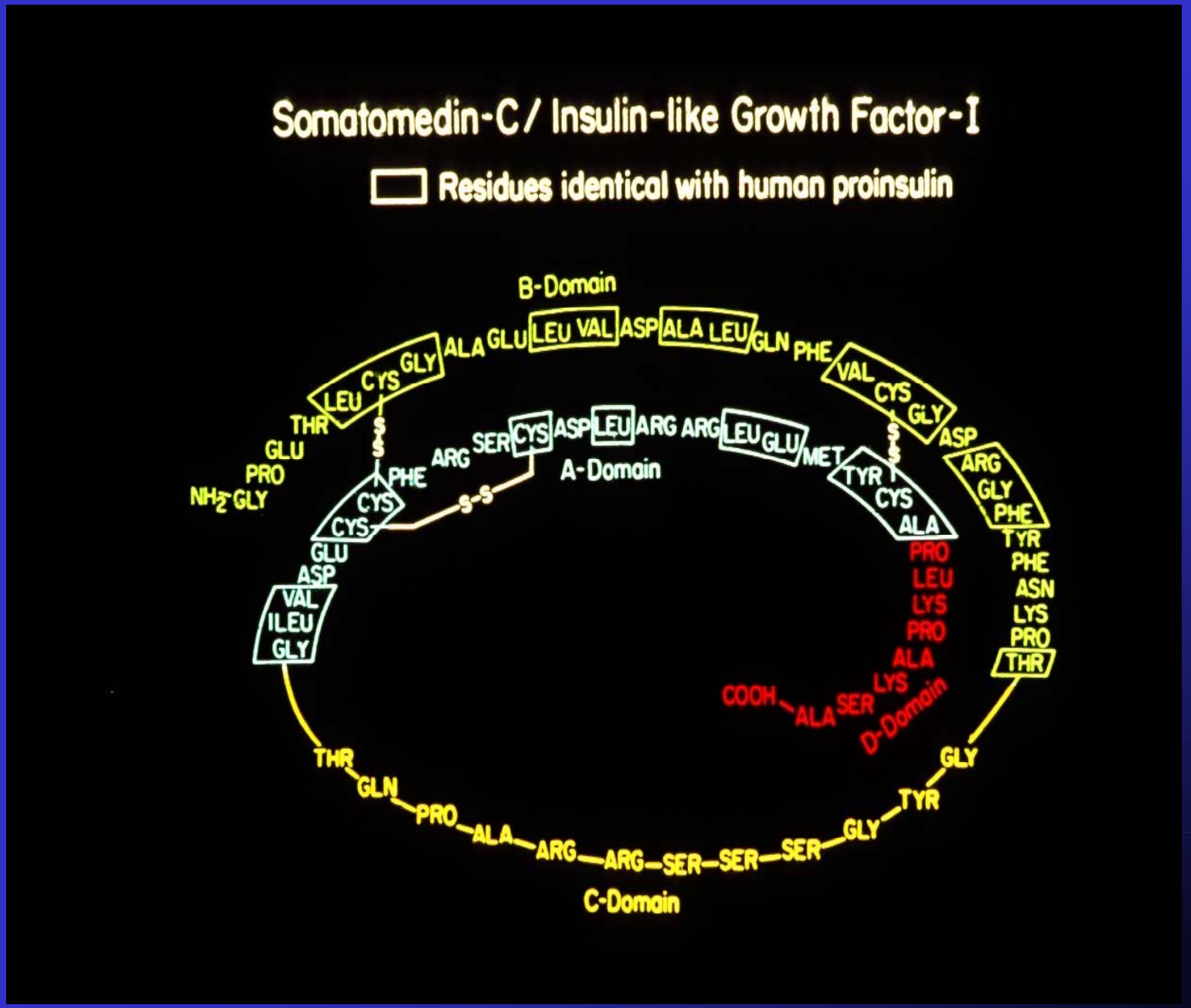
Synthetic GH Secretagogues

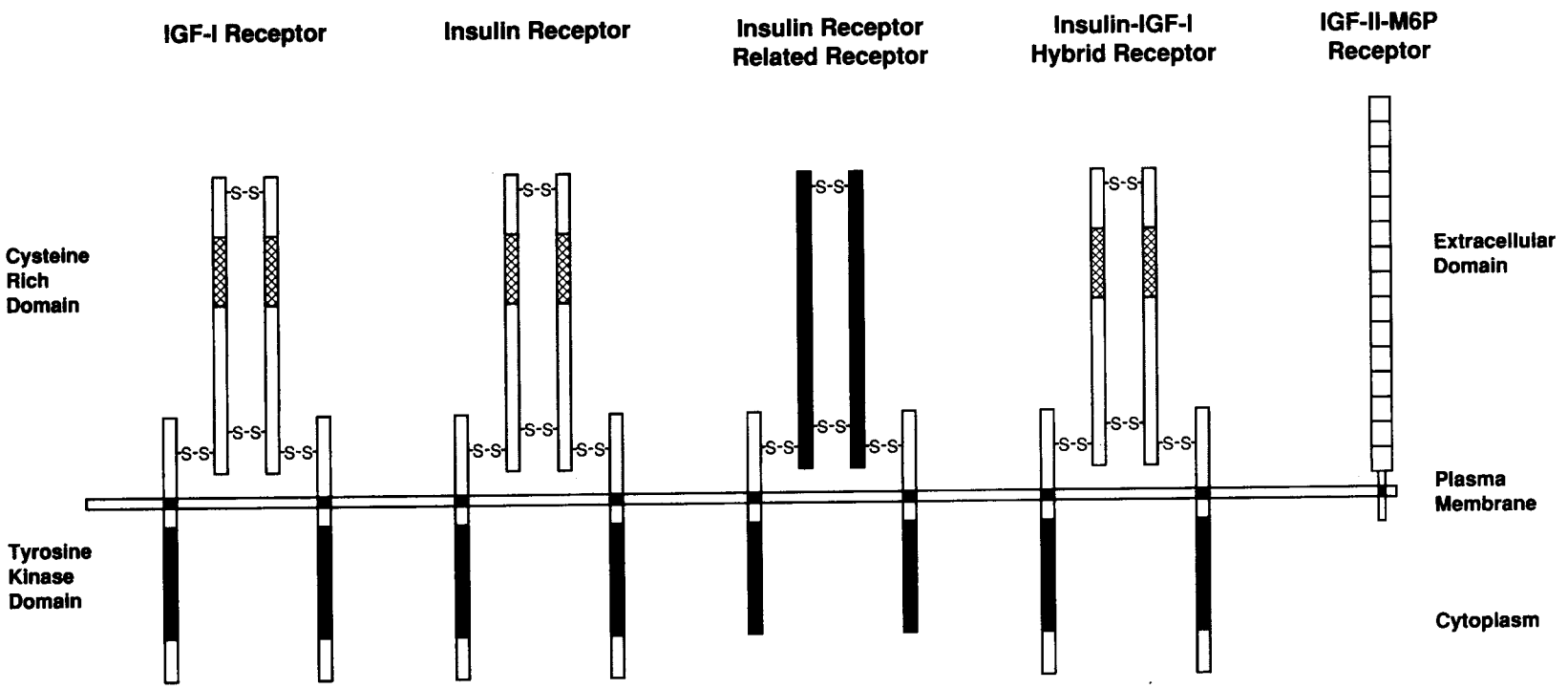
* Peptidyl GHS (GHRPs)		* Non-Peptidyl GHS
- (D-Trp2)-metENKH	**1977**	
- **GHRP-6**	**1984**	
- GHRP-1	**1991**	
- **hexarelin**	**1992**	- L-629,429
- **GHRP-2**	**1993**	
	1994	- L-692,885
	1995	- **MK-0677**
- EP-51389	**1996**	
- ipamorelin	**1998**	
	1999	- NN-703
	2000	- CP-424,391
	2001	- SM-130686
	2002	- EP-01572

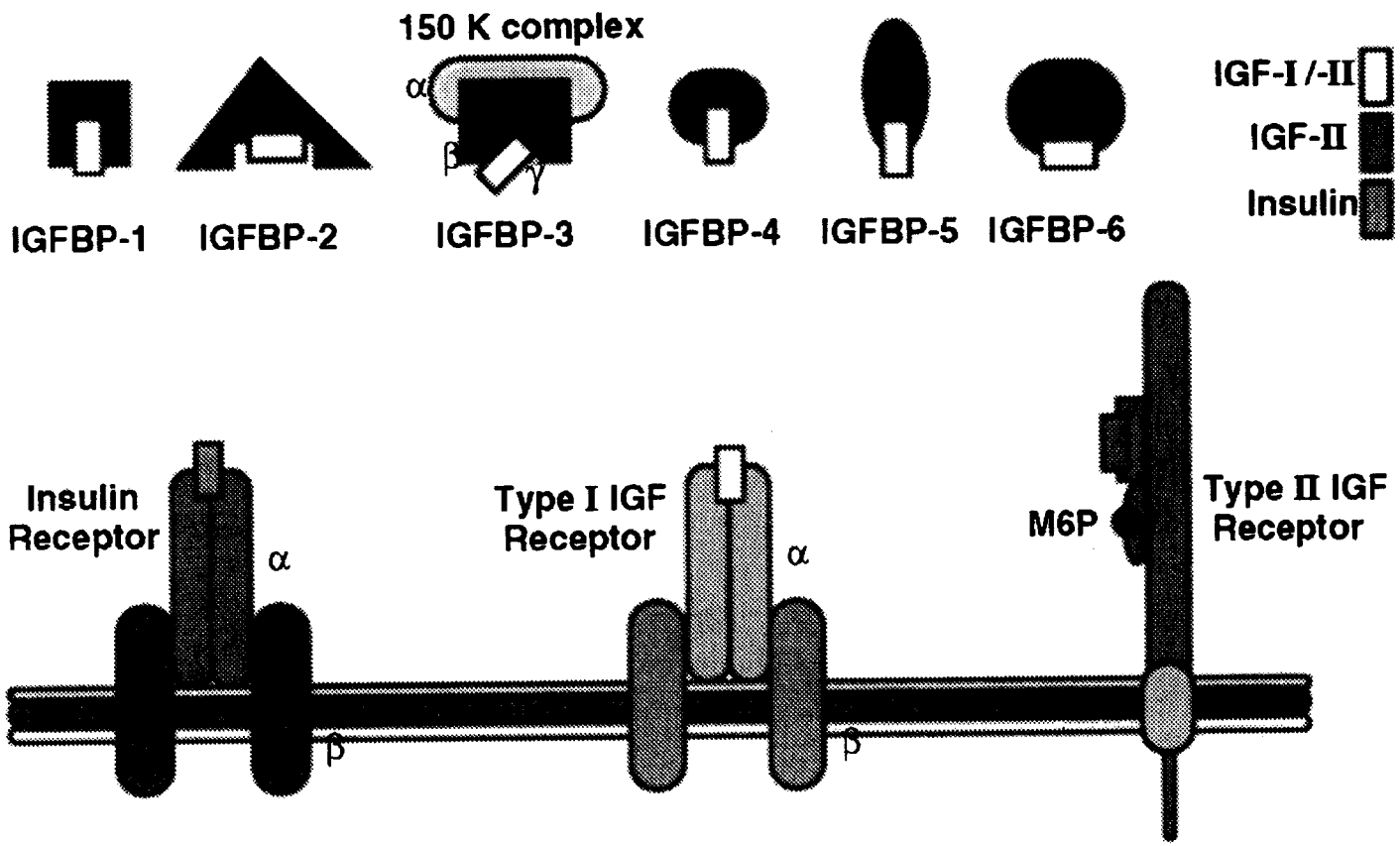
Nature 1999 Dec 9;402(6762):656-60

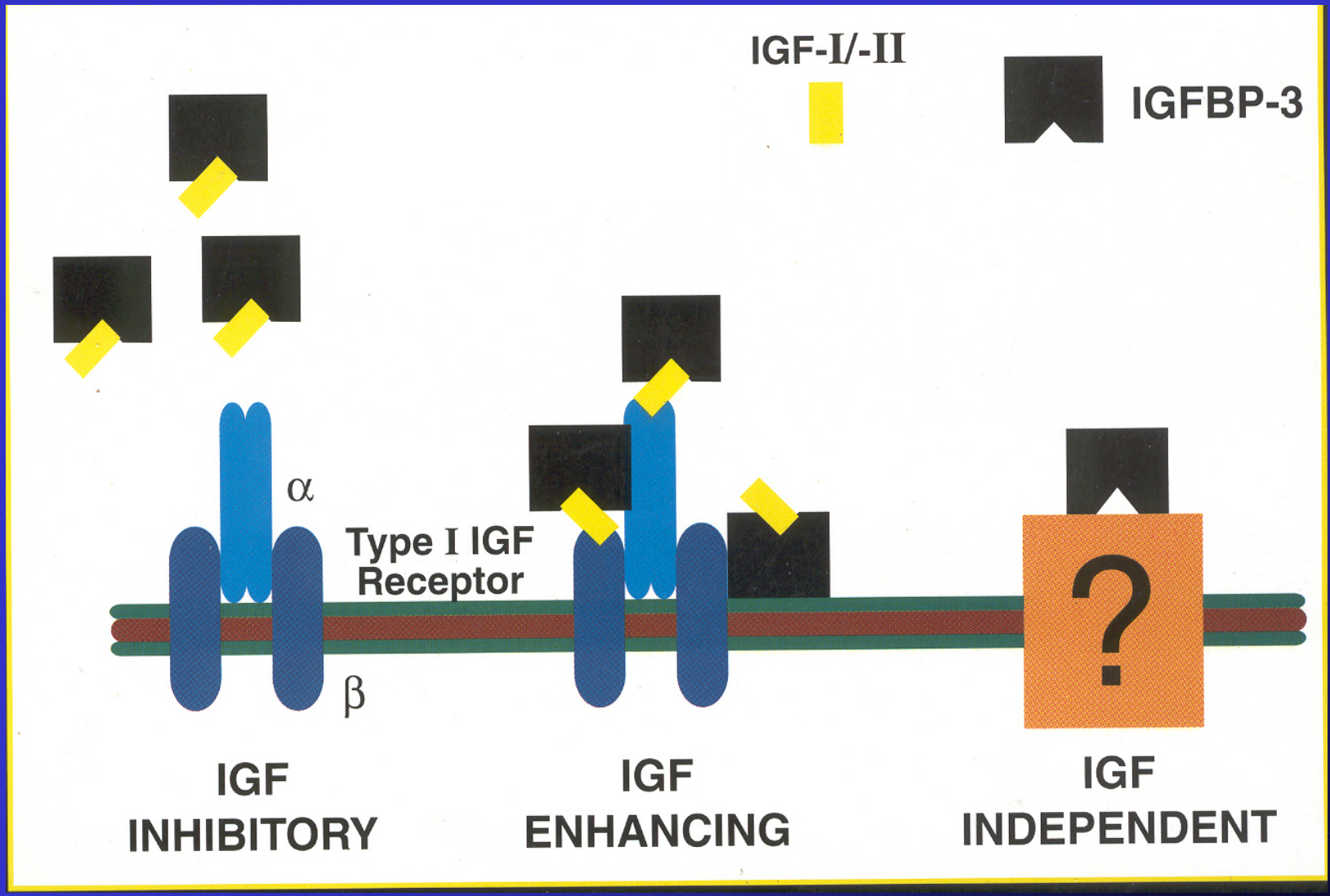
Ghrelin is a growth-hormone-releasing acylated peptide from stomach.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K.


Somatomedin-C / Insulin-like Growth Factor-I
Residues identical with human proinsulin
B-Domain
A-Domain
C-Domain
D-Domain
NH2-GLY PRO GLU THR LEU CYS GLY ALA GLU LEU VAL ASP ALA LEU GLN PHE VAL CYS GLY ASP ARG GLY PHE TYR PHE ASN LYS PRO THR GLY TYR GLY SER SER SER ARG ARG SER PRO ALA GLN THR GLU ASP VAL ILEU GLY CYS CYS PHE ARG SER CYS ASP LEU ARG ARG LEU GLU MET TYR CYS ALA PRO LEU LYS PRO ALA LYS SER ALA COOH


IGF-I Receptor
Insulin Receptor
Insulin Receptor Related Receptor
Insulin-IGF-I Hybrid Receptor
IGF-II-M6P Receptor
Cysteine Rich Domain
Tyrosine Kinase Domain
S-S
Extracellular Domain
Plasma Membrane
Cytoplasm


150 K complex
α
β
γ
IGF-I /-II
IGF-II
Insulin
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
Insulin Receptor
α
β
Type I IGF Receptor
α
β
M6P
Type II IGF Receptor


IGF-I/-II
IGFBP-3
α
Type I IGF
Receptor
β
?
IGF
INHIBITORY
IGF
ENHANCING
IGF
INDEPENDENT

THE VARIOUS FACTORS AFFECTING THE GH SECRETION

Factors	Causes of increased GH	Causes of decreased GH
Physiological	Exercise	Hyperglycemia (post-prandial)
	Stress	Free fatty acid increment
	Sleep	
	Hypo-glycemia	
	Amino acid intake	
Pathological	Acromegaly	Somatostatin ectopic production
	GHRH ectopic production	Obesity
	GH ectopic production	Hypothyroidism
	Anorexia nervosa	Hyperthyroidism
	Protein and amino acid depletion	
Pharmacological	GHRH	Somatostatin effects
	Dopaminergic, α-adrenergic and serotoninergic agents	Other hormone effects
		β-Adrenergic agonists
	Other hormone effects (ACTH, estrogens, etc.)	Neurotransmitters effects
		Serotonin and dopamine effects
	Serotonin precursors	
	Hypo-glycemia (post-insulin)	

CODICE ANTIDOPING

Appendice A - Lista delle classi di sostanze vietate e dei metodi proibiti

CLASSI DI SOSTANZE VIETATE

...

E. Ormoni peptidici, sostanze ad azione mimetica e analoghi

Le sostanze vietate della classe (E) includono gli esempi seguenti ed i relativi analoghi, nonché le sostanze ad azione mimetica:

1. Gonadotropina corionica (hCG) esclusivamente per gli uomini;
2. Gonadotropine ipofisarie e di sintesi esclusivamente per gli uomini;
3. Corticotropine (ACTH, tetracosactide)
4. **Ormone della crescita (hGH)**
5. Fattore di crescita insulino-simile (IGF-1) e tutti i rispettivi "fattori di rilascio" e loro analoghi
6. Eritropoietina (EPO)
7. Insulina:

La presenza di una concentrazione anomala di ormone endogeno appartenente alla classe (E) o dei suoi marcatori diagnostici nelle urine di un atleta costituisce un'infrazione, a meno che non sia stato comprovato in via definitiva che il fenomeno è dovuto esclusivamente ad una condizione *fisiologica o patologica*.

Indagati Rosolino, Bellutti, Idem, Abbagnale e Trillini

Ormone GH: valori fuori norma

Le notizie diffuse da due quotidiani nazionali infangano gli ori olimpici azzurri


CORRIERE DELLA SERA

IL CORAGGIO DEL MEDIATORE

Arafat-Barak, si tratta per il vertice

Minacce dei terroristi islamici, timori di attentati in Occidente

«Cerchi tuo marito? L'ho appena ucciso»

Milano paralizzata, l'ora delle accuse

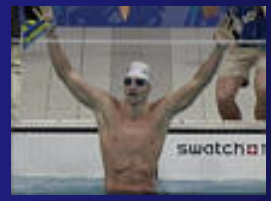
TROPPI ANNI D'INERZIA

Valori fuori norma nel sangue per cinque ori di Sydney

FLEXA

E' il caso del giorno: Corriere della Sera e Manifesto hanno oggi pubblicato un elenco di atleti (Rosolino, Bellutti, Idem, Trillini e Abbagnale), tutte medaglie d'oro a Sydney, ai quali nel corso di vari esami eseguiti prima delle Olimpiadi sono stati riscontrati valori anomali del famoso e famigerato e temutissimo **ormone della crescita**...

"E' solo una strumentalizzazione per un problema che non esiste", ecco la risposta di Antonella Bellutti, "sono dati che non hanno alcuna valenza, finalizzati solo a rovinare l'immagine degli atleti, che ormai è rovinata. Sono senza parole, è da una vita che faccio la lotta al doping"...



Potenziali discipline sportive che usano h-GH

Azione	Sport
Promozione della crescita lineare	Sport altezza–specifici (pallacanestro, pallavolo, nuoto di fondo)
Anabolica	Sport di potenza (sollevamento peso, lotta, velocità su pista, lancio del peso, nuoto di velocità)
Lipolitica	Sport sensibili al peso (sollevamento peso, lotta, pugilato)
Cardiotonica	Sport di resistenza (fondo su pista, nuoto di resistenza)
Deposizione di collagene	Cicatrizzazione delle ferite in ogni sport

Modalità di esaltazione dell'azione del GH

- somministrazione esogena di GH
  - · di origine estrattiva
  - · di origine biosintetica
- somministrazione di sostanze capaci di aumentare la secrezione di GH endogeno
  - ➢ GHRH
  - ➢ GHRP/GHS
  - ➢ vasopressina
  - ➢ clonidina
  - ➢ propranololo
  - ➢ aminoacidi (arginina, lisina, ornitina)
  - ➢ GHB

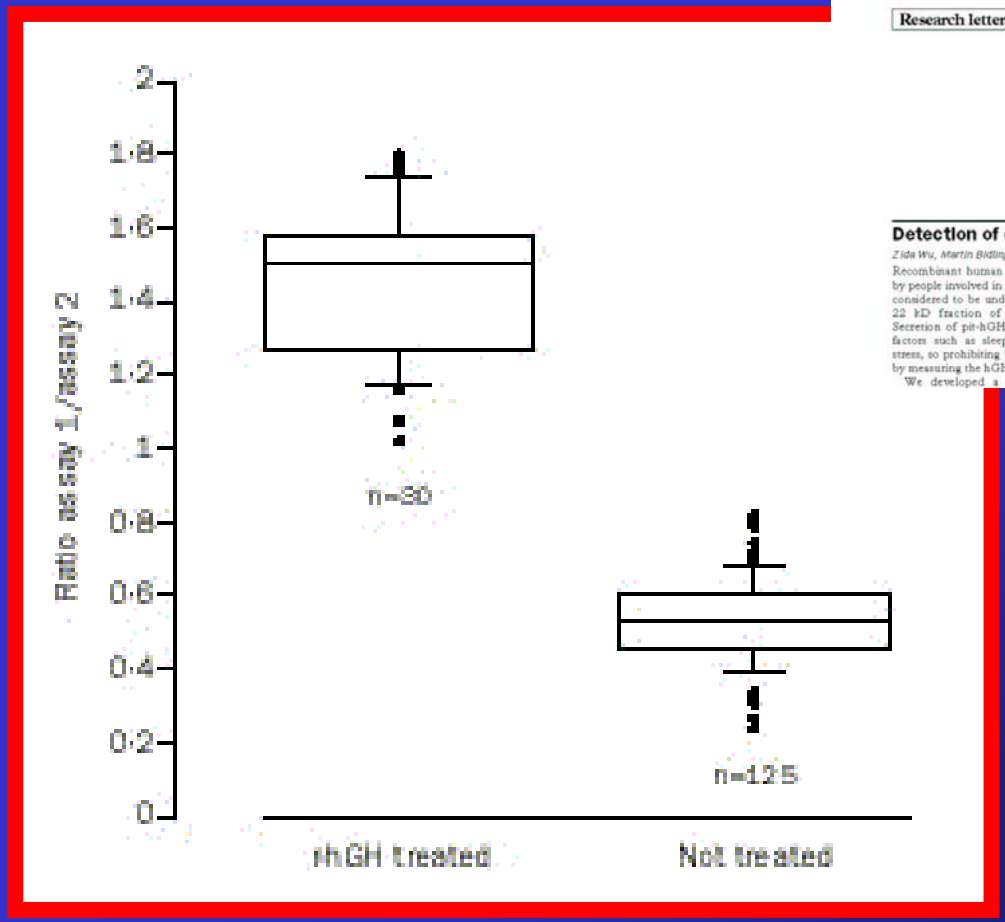
The Lancet

Volume 341(8847) Mar 20, 1993 pp 768-769

Self-administration of cadaveric growth hormone in power athletes.

[Letters to the Editor]

Deyssig, Roman; Frisch, Herwig.
Department of Endocrinology, University Children's Clinic, 1090 Wien, Austria.



Research letters

Detection of doping with human growth hormone

Zida Wu, Martin Bidlingmaier, Rolf Dall, Christian J Strasburger

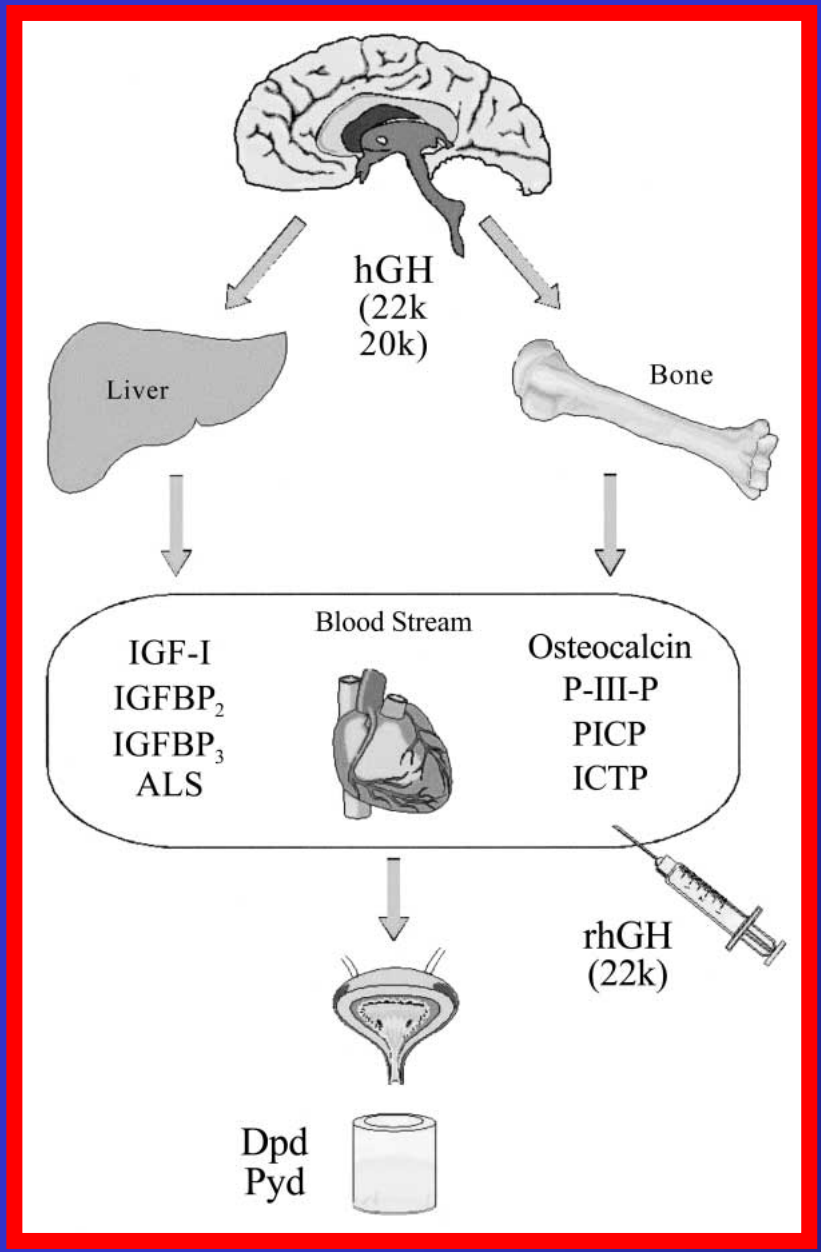
Recombinant human growth hormone (r-hGH) is misused by people involved in sport.[1] Once injected, r-hGH has been considered to be undetectable because it is identical to the 22 kD fraction of pituitary-derived hGH (pit-hGH). Secretion of pit-hGH fluctuates and is regulated by many factors such as sleep, nutrition, exercise, and emotional stress, so prohibiting the detection of r-hGH administration by measuring the hGH concentration.[2]

We developed a method based upon the fact that

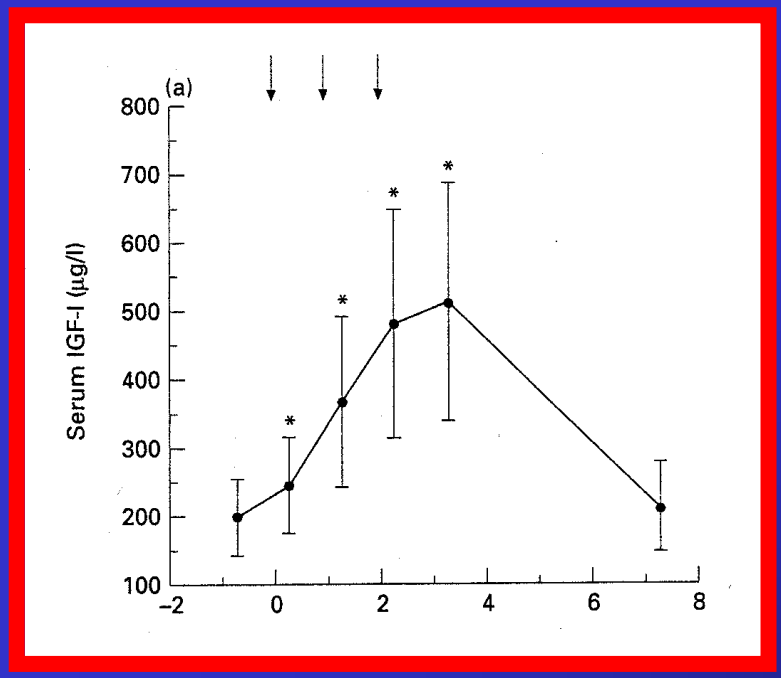
To test the method in a blinded manner, 40 serum samples were obtained from either pharmacodynamic profiles after r-hGH injection or from growth-hormone releasing hormone (GHRH) stimulation tests. The samples were matched for absolute hGH concentrations in Aarhus and sent to the Munich laboratory in random order, number-coded. All 20 samples taken after administration of r-hGH were correctly identified with assay 1/assay 2 ratios between 1·188 and 1·780, while those 20 sera taken after GHRH

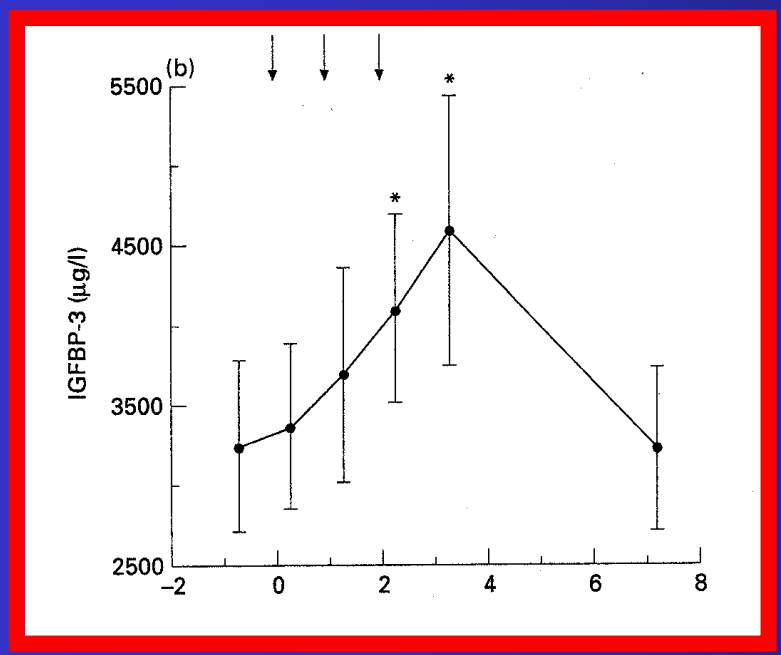
Ratio between assay 1 (22 kD-hGH) and assay 2 (total hGH) in serum samples No overlap was observed between the groups (mean [SD] r-hGH treatment 1·434 [0·212], controls 0·5 [0·115]; Mann-Whitney *U*: p<0·0001). The 90th, 75th, 50th, 25th, and 10th percentile and individual outliers are shown.

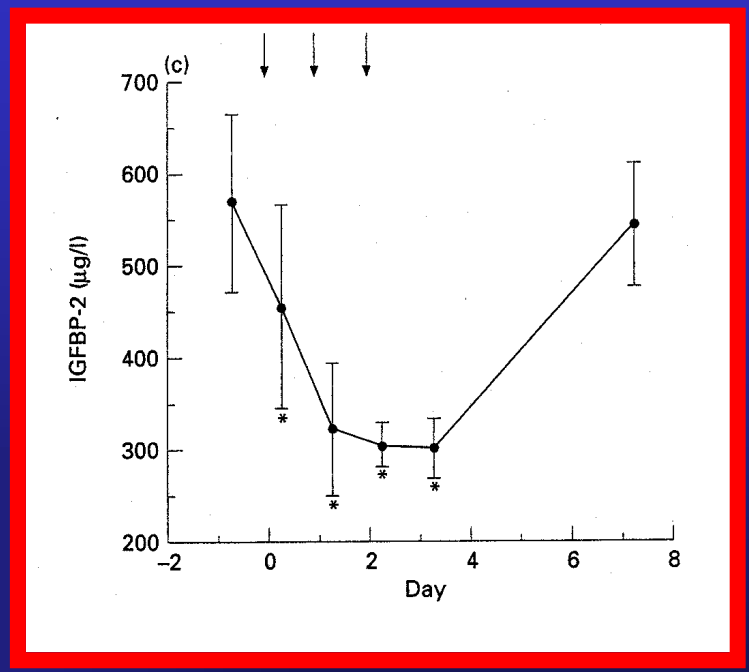
Strasburger et al., 1999



A summary of the potential markers thought to be most useful in developing a test of GH abuse. Pyridinoline (Pyd) and deoxypyridinoline (Dpd) are urinary metabolites of collagen markers.

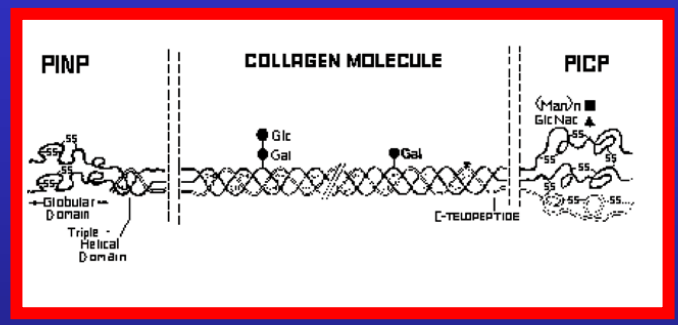






Kicman et al., 1997

GH



- MARCATORI DI FORMAZIONE
- MARCATORI DI RIASSORBIMENTO

MARCATORI DI FORMAZIONE

- Fosfatasi alcalina ossea
- Osteocalcina
- Propeptide C-terminale del procollagene (PICP)
- Propeptide N-terminale del procollagene di tipo III (*soft tissue marker*; PIIIP)

MARCATORI DI RIASSORBIMENTO

- Telopeptide C-term. del collagene I (ICTP)
- Fosfatasi acida tartrato-resistente
- Galattosilidrossilisina
- Telopeptide N-term. del collagene I (NTx)
- Telopeptide C-term. del collagene I (CTx)
- Desossipiridinolina libera
- Piridinoline libere

0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 4
Printed in U.S.A.

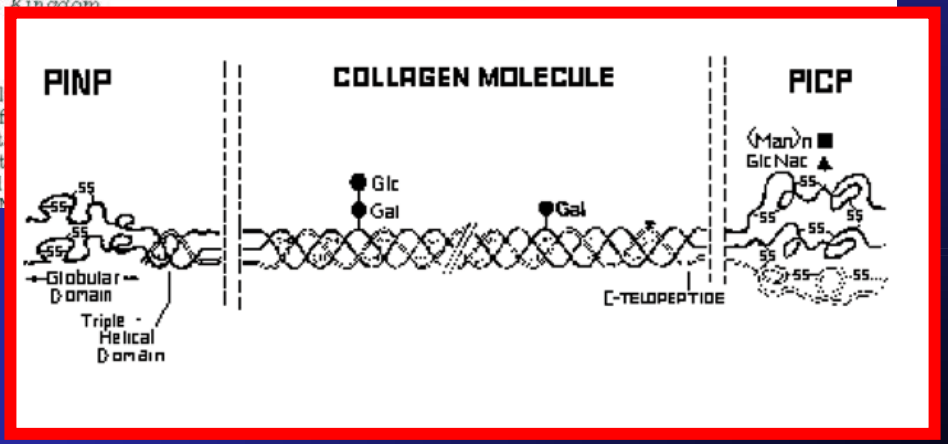
Growth Hormone (GH) Effects on Bone and Collagen Turnover in Healthy Adults and Its Potential as a Marker of GH Abuse in Sports: A Double Blind, Placebo-Controlled Study*

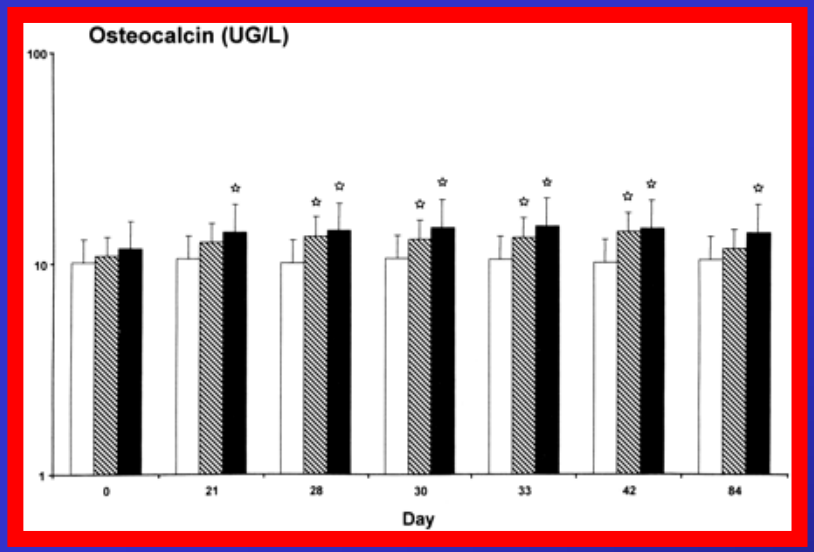
S. LONGOBARDI†, N. KEAY†, C. EHRNBORG, A. CITTADINI, T. ROSÉN, R. DALL, M. A. BOROUJERDI, E. E. BASSETT, M. L. HEALY, C. PENTECOST, J. D. WALLACE, J. POWRIE, J. O. JØRGENSEN, AND L. SACCÀ
ON BEHALF OF THE GH-2000 STUDY GROUP

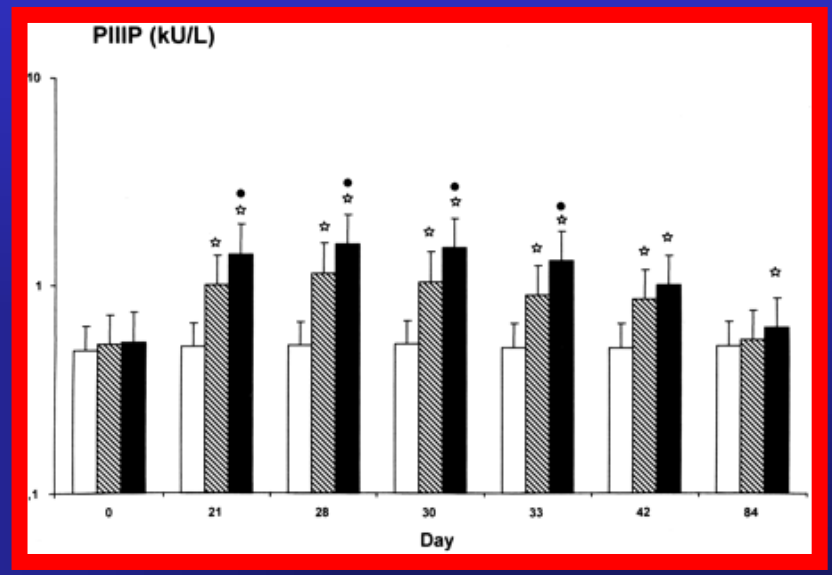
Department of Clinical Medicine and Cardiovascular Sciences, University Federico II (S.L., A.C., L.S.), 80131 Naples, Italy; Department of Endocrinology, St. Thomas's Hospital (N.K., M.A.B., C.P., J.P.), London SE1 7EH, United Kingdom; Research Center for Endocrinology and Metabolism, Sahlgrenska Hospital (C.E., T.R.), S-41345 Göteborg, Sweden; Department of Endocrinology, Aarhus Community Hospital (R.D., J.O.J.), Aarhus, Denmark; and Institute of Mathematics and Statistics, University of Kent (E.E.B.), Canterbury, Kent CT2 7NF, United Kingdom

ABSTRACT

The effects of GH on bone remodeling in healthy adul
been systematically investigated. An analysis of these ef
provide insights into GH physiology and might yield dat
the detection of GH doping in sports. The aim of this st
evaluate the effects of GH administration on biochemical
bone and collagen turnover in healthy volunteers. M

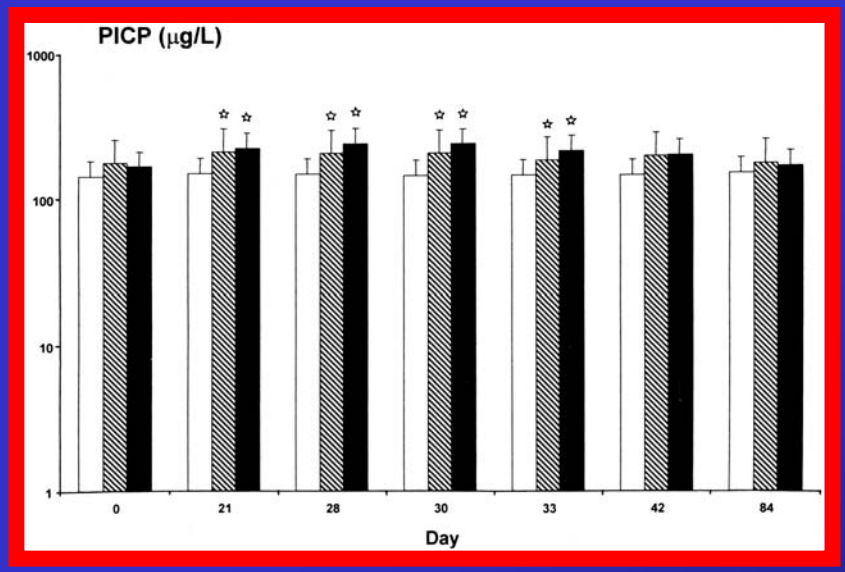


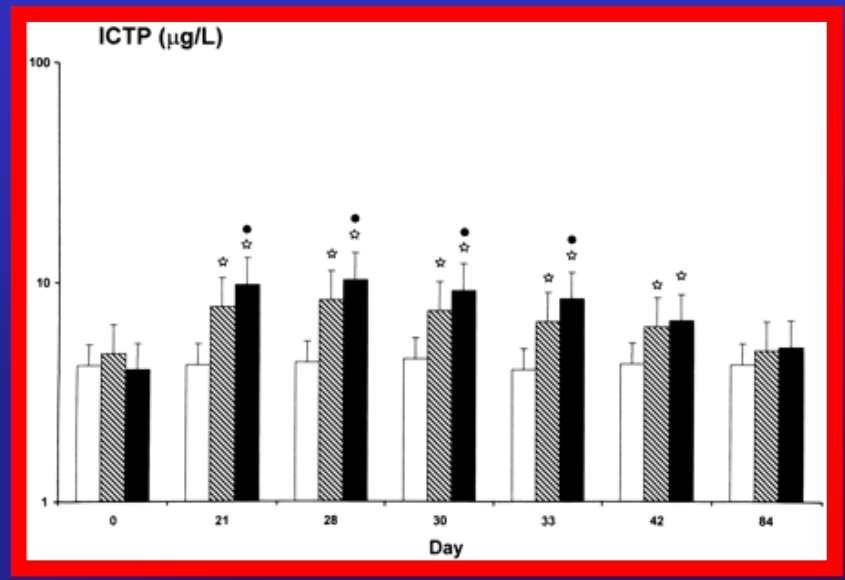




Effect of GH administration (up to day 28) and withdrawal on serum osteocalcin and PIIIP (*procollagene di tipo III*) concentrations in normal subjects. Asterisks indicate significant differences vs. placebo. White, Placebo; gray, low dose group; black, high dose group.

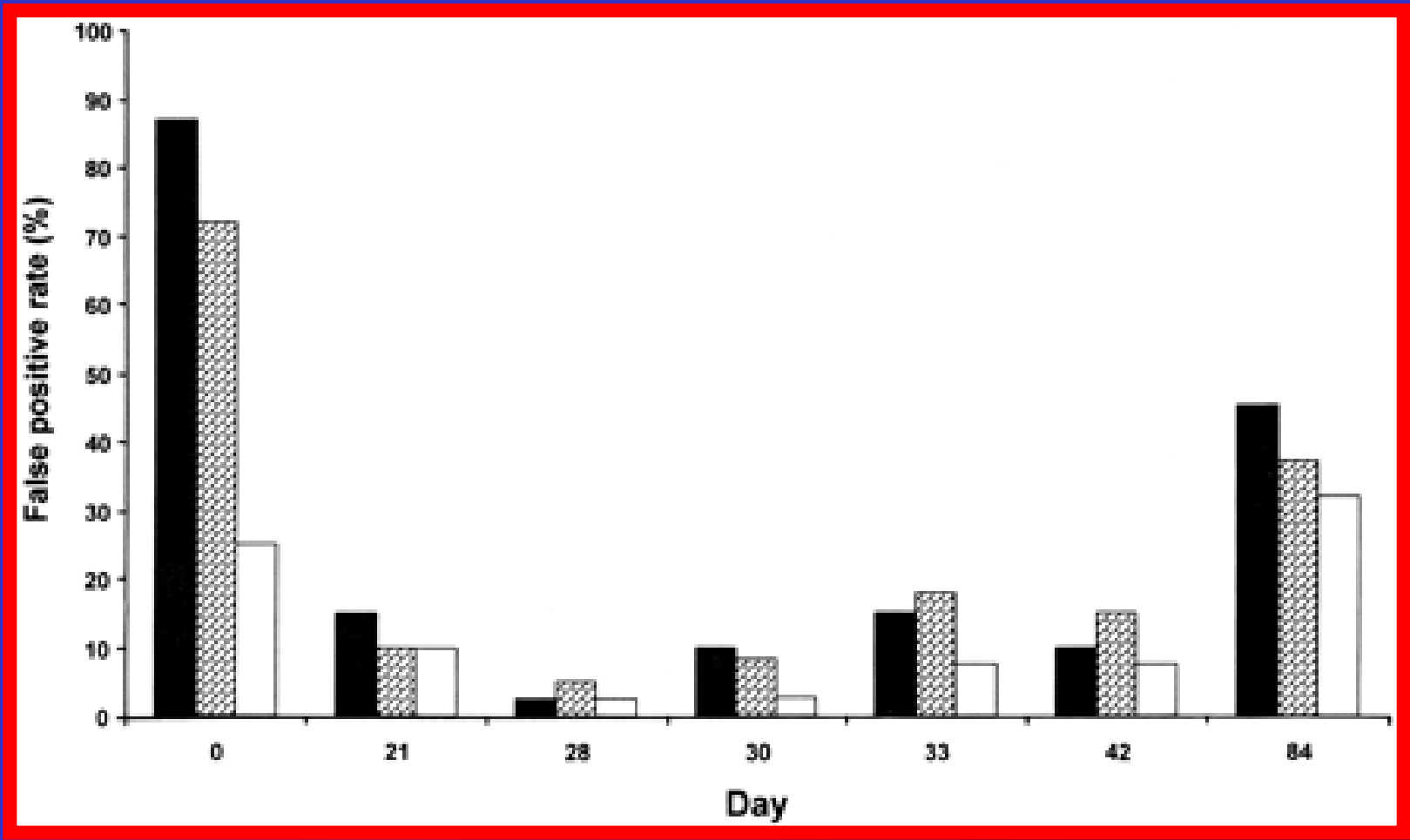
Longobardi et al., 2000





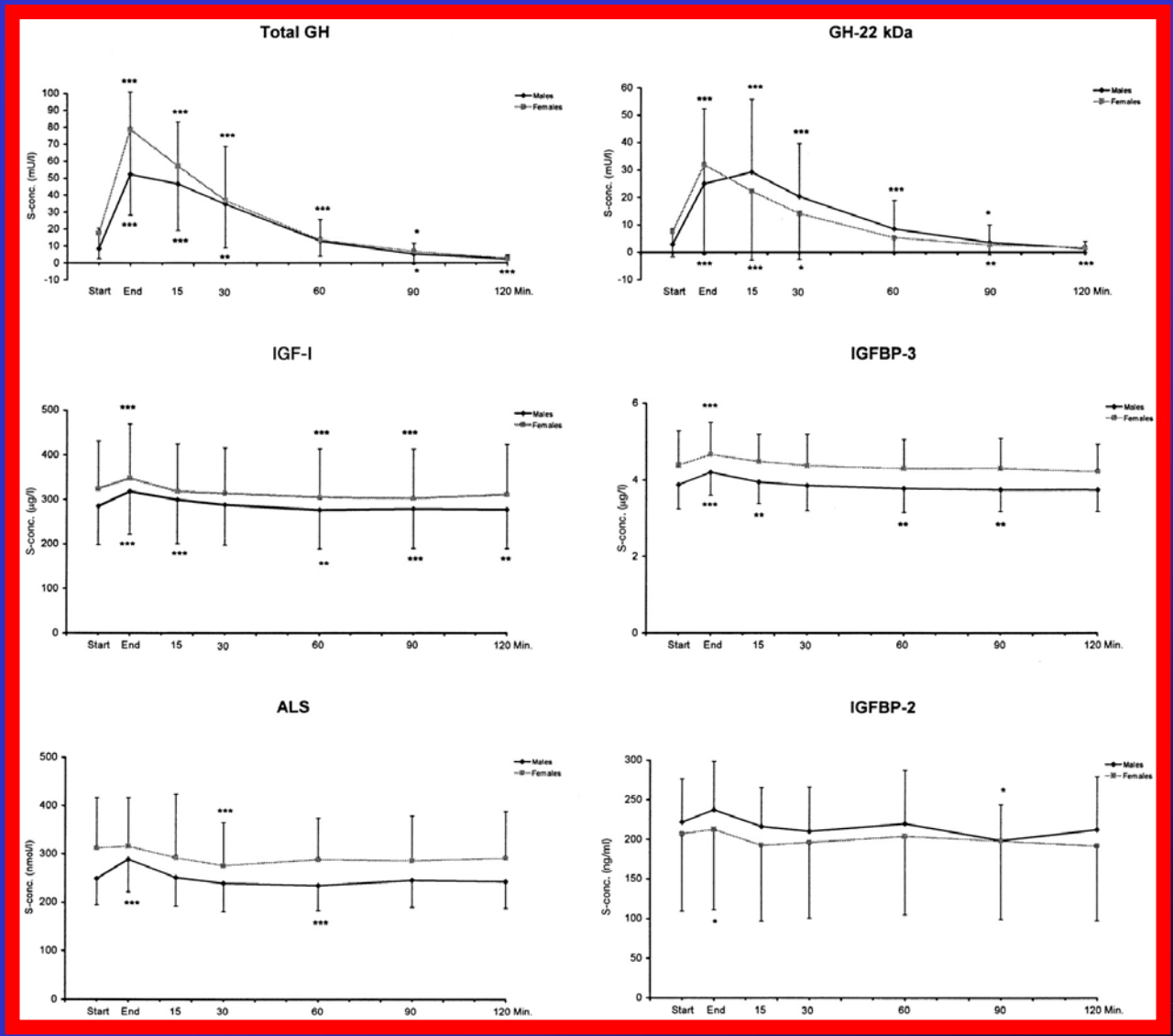
Effect of GH administration (up to day 28) and withdrawal on serum PICP (*propeptide C-terminale del procollagene*) and ICTP (*telopeptide C-term. del collagene I*) concentrations in normal subjects. Asterisks indicate significant differences vs. placebo. White, Placebo; gray, low dose group; black, high dose group.

Longobardi et al., 2000



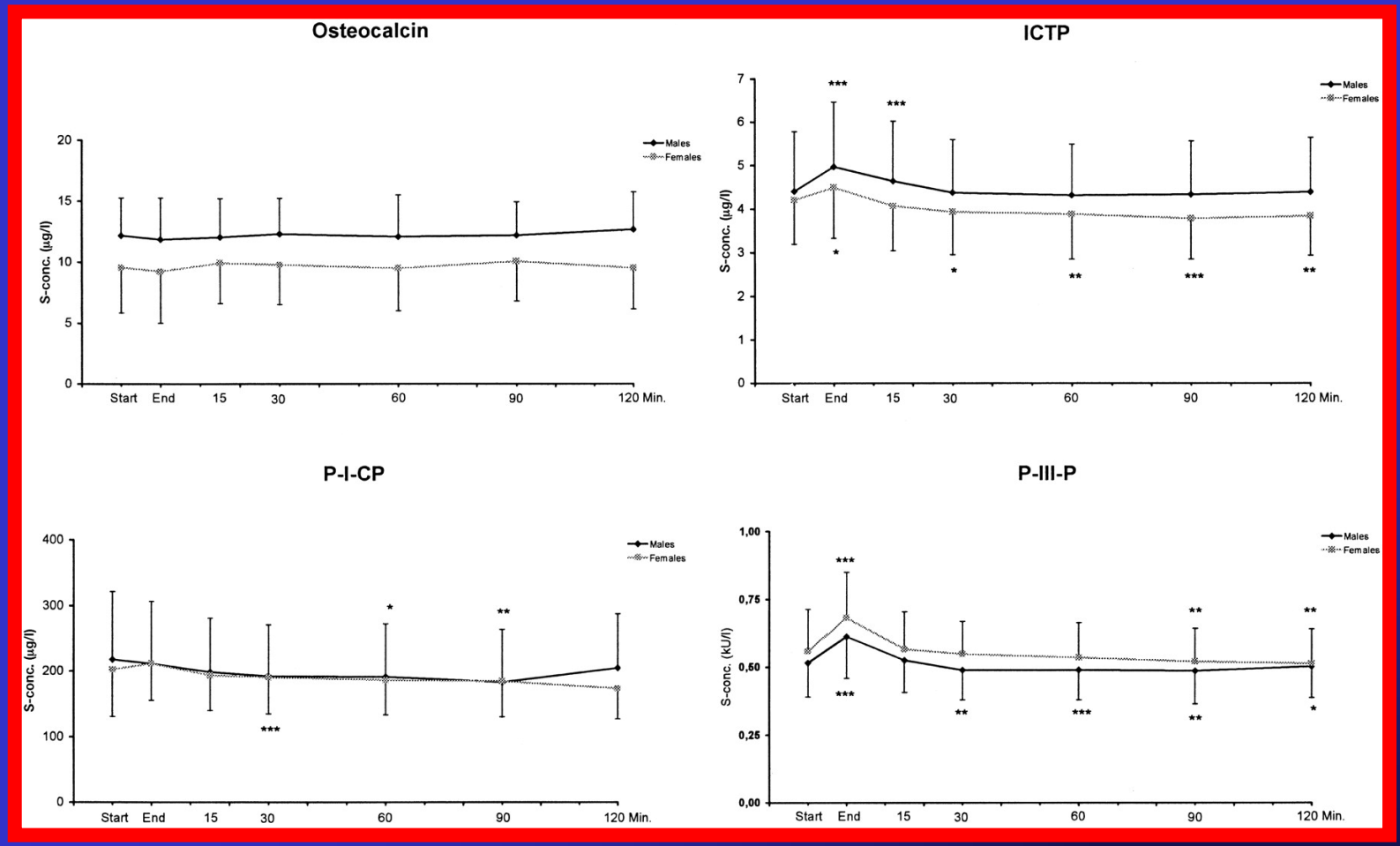
Percentage of subjects erroneously diagnosed as having taken GH by discriminant analysis based on a single marker (PIIIP; black), two markers (ICTP and PIIIP; gray), and four markers (white).

Longobardi et al., 2000



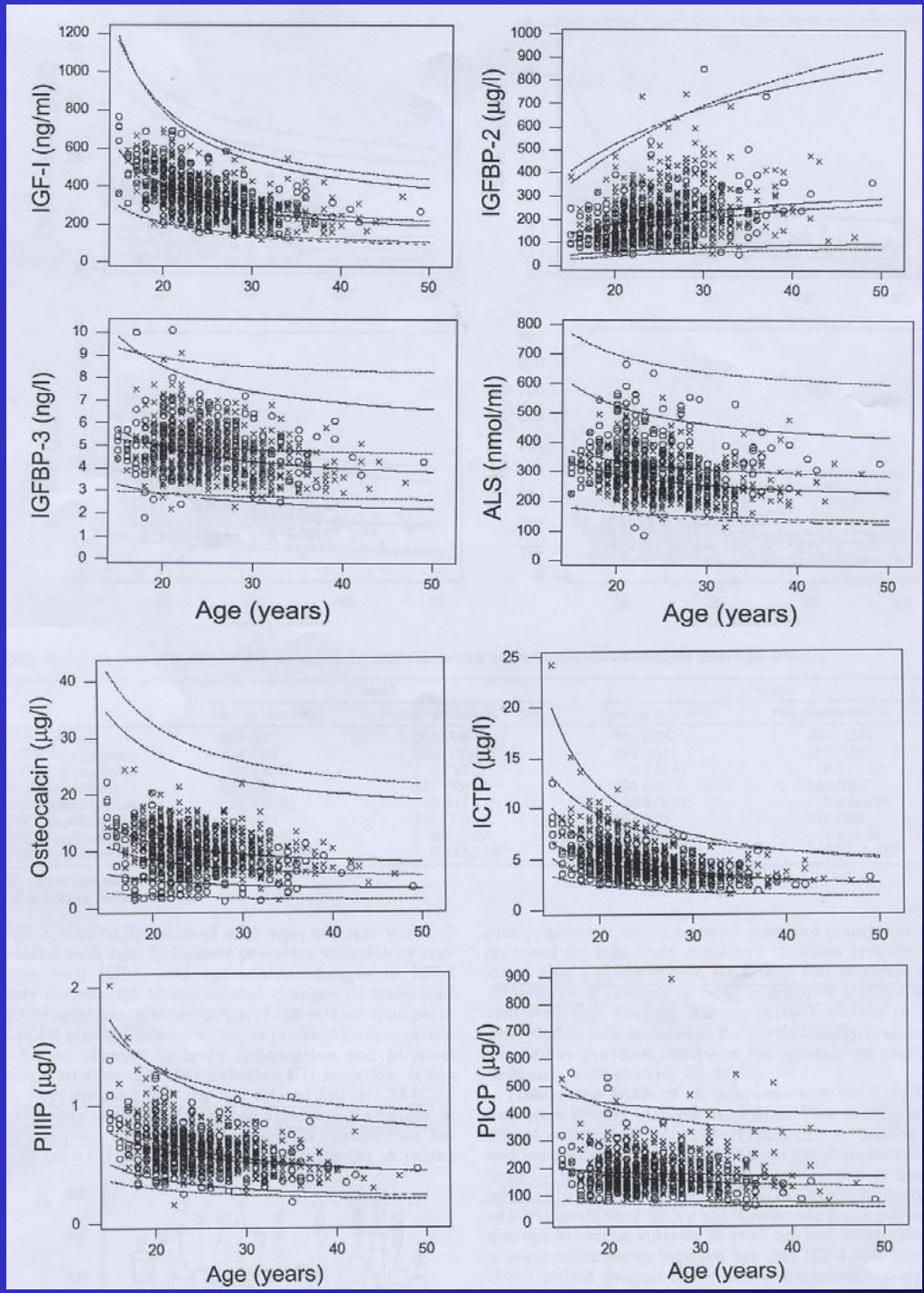
Serum-concentrations (mean ± SD) of components in the GH/IGF-I axis in 84 male and 33 female elite athletes in connection with a maximum exercise test. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$, indicate changes compared with baseline.

Ehrnborg *et al*., 2003



Serum-concentrations (mean ± SD) of components in the bone markers in 84 male and 33 female elite athletes in connection with a maximum exercise test. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$, indicate changes compared with baseline.

Ehrnborg *et al*., 2003


IGF-I (ng/ml)
IGFBP-2 (µg/l)
IGFBP-3 (ng/l)
ALS (nmol/ml)
Age (years)
Age (years)
Osteocalcin (µg/l)
ICTP (µg/l)
PIIIP (µg/l)
PICP (µg/l)
Age (years)
Age (years)

PROSPETTIVE FUTURE…

First level tests

Measurement of 4 parameters. To each first level parameter is assigned a score

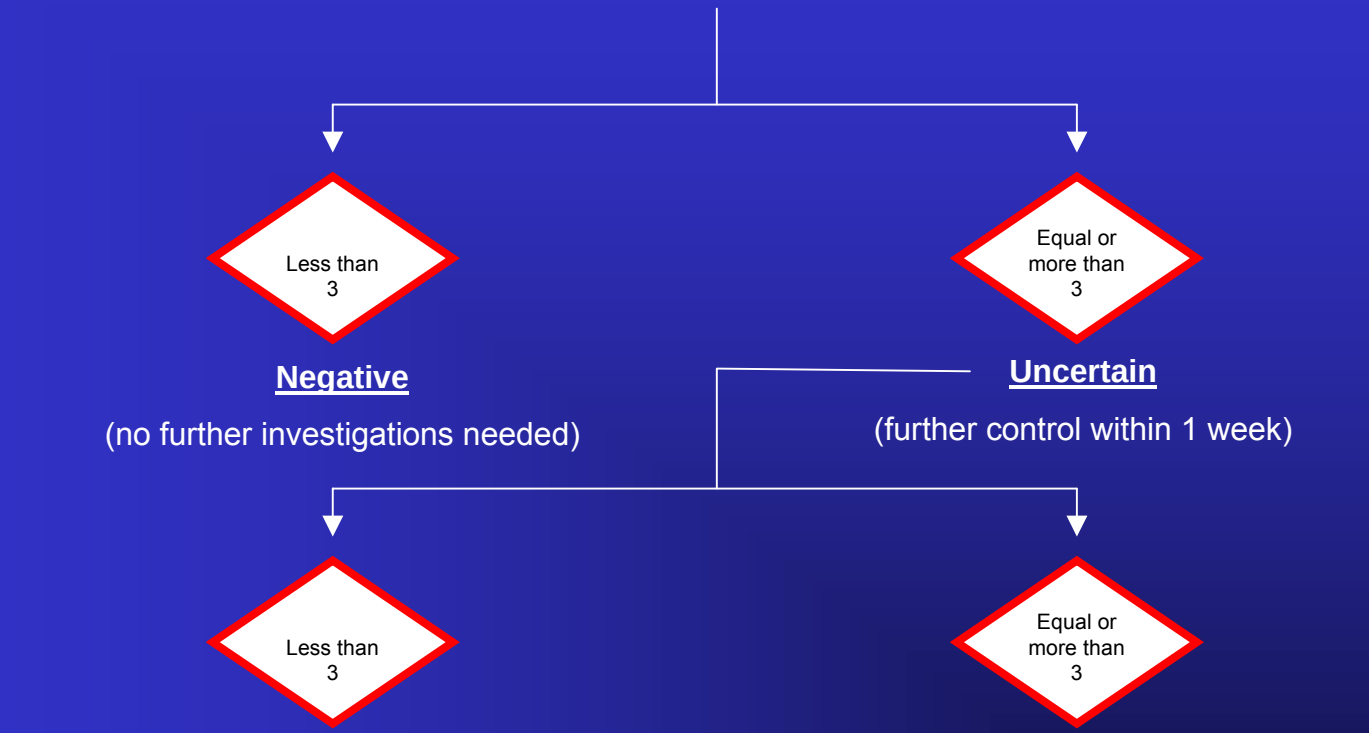
Parameter	*Cut-off value**	*Score*
GH (ng/ml)	< 3.6 ng/ml (males)	0
	> 3.6 ng/ml (males)	1
	< 9.5 ng/ml (females)	0
	> 9.5 ng/ml (females)	1
IGF-1 (ng/ml)	< 450 ng/ml (age < 30 yr)	0
	> 450 ng/ml (age < 30 yr)	2
	< 300 ng/ml (age > 30 yr)	0
	> 300 ng/ml (age > 30 yr)	2
PIIINP (ng/ml)	< 7.1 ng/ml (age ≤ 20 yr)	0
	> 7.1 ng/ml (age ≤ 20 yr)	1.5
	< 6.2 ng/ml (age ≥ 20 yr)	0
	> 6.2 ng/ml (age ≥ 20 yr)	1.5
ICTP (ng/ml)	< 8.5 ng/ml (age ≤ 20 yr)	0
	> 8.5 ng/ml (age ≤ 20 yr)	1.5
	< 6.6 ng/ml (age ≥ 20 yr)	0
	> 6.6 ng/ml (age ≥ 20 yr)	1.5

*The cut-off values were determined by adding 2 SD to the means obtained in a large number of athletes

Second level tests

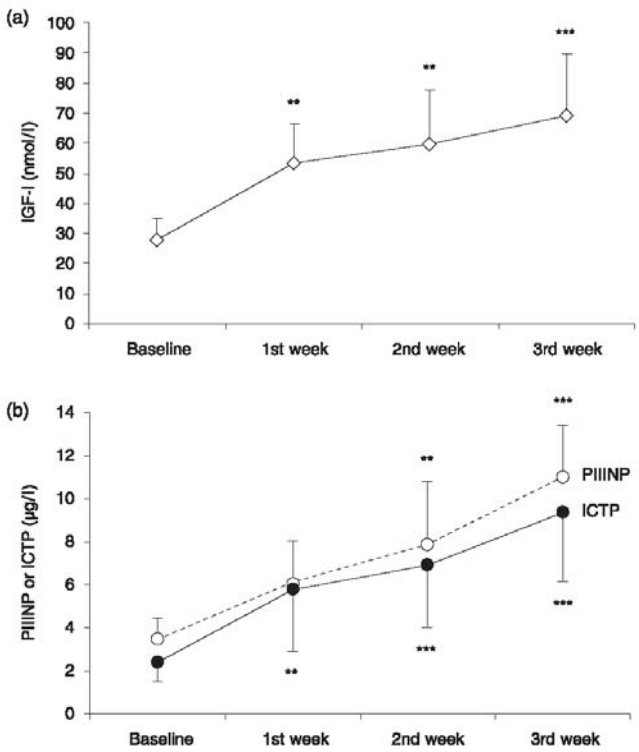
- Assessment of GH secretory profile under resting conditions (positive/negative)
- Stimulatory test with ghrelin (positive/negative)

Sum of scores related to first level tests



Case	Time (weeks)	IGF-I (nmol/l)	PIIINP (μg/l)	ICTP (μg/l)	Total score
28	1st	41·3	5·4	6·7*	1·5
	2nd	38·6	6·0	9·1*	1·5
	3rd	47·6	10·1*	13·0*	3†
29	1st	40·2	3·7	3·3	0
	2nd	41·4	5·1	5·0	0
	3rd	55·7*	7·0*	6·7*	5†
35	1st	47·0	3·8	1·7	0
	2nd	57·9*	4·8	2·7	2
	3rd	59·9*	10·8*	4·7	3·5†
39	1st	55·5*	8·1*	8·6*	5†
	2nd	62·2*	10·7*	8·7*	5†
	3rd	82·5*	11·6*	9·8*	5†
40	1st	74·3*	7·2*	5·3	3·5†
	2nd	85·9*	8·8*	5·7	3·5†
	3rd	102·8*	12·4*	9·5*	5†
41	1st	61·0*	7·9*	9·0*	5†
	2nd	71·9*	11·6*	10·1*	5†
	3rd	66·9*	14·2*	12·4*	5†

*Abnormal values, †positive values.



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Combined evaluation of resting IGF-I, N-terminal propeptide of type III procollagen (PIIINP) and C-terminal cross-linked telopeptide of type I collagen (ICTP) levels might be useful for detecting inappropriate GH administration in athletes: a preliminary report

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RESULTS **Abnormal IGF-I, or PIIINP or ICTP levels were found, respectively, in one, two and four subjects (1·5–6·1%) of the control group (in the younger athletes); only one 19-year-old subject of this group obtained a positive score. Abnormal IGF-I, PIIINP and ICTP levels were found in 61·1–66·7% samples of the treated group. Positive cases were 3/6 at the 1st and 2nd week and 6/6 at the 3rd week. The sensitivity of the screening approach was 50–100% (at the 1st–2nd and 3rd week, respectively) and specificity was 98·5%.**

CONCLUSION **This 'first-level' screening test is safe**

IGF-I (a), PIIINP and ICTP (b) levels in the treated group (n = 6) at baseline and after 1, 2 and 3 weeks of rGH administration. All values are expressed as the mean ± SD. **P < 0·01, ***P < 0·001, compared with baseline (repeated measures anova followed by Tukey posthoc). There were no significant changes in GH levels from the baseline values at any points.

Growth Hormone

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Abuse of Recombinant Human Growth Hormone: Studies in Two Different Dog Models

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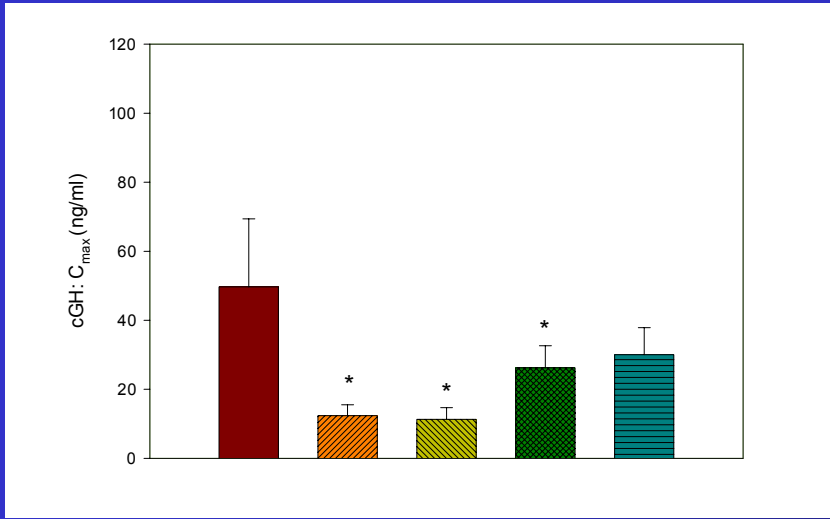
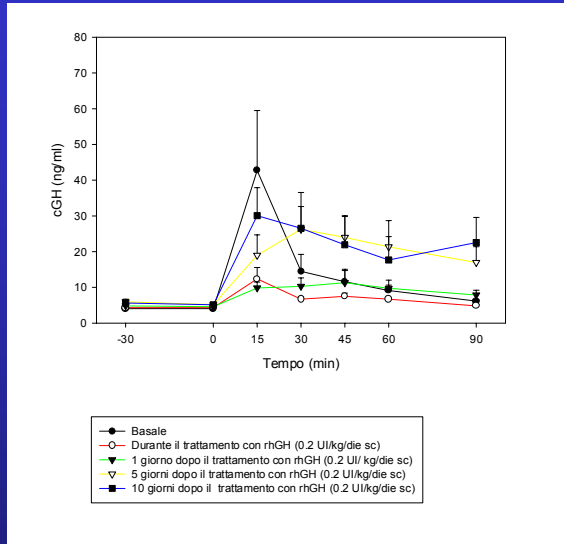
Key Words
Growth hormone · Growth hormone secretagogues · Growth hormone releasing hormone · Somatostatin

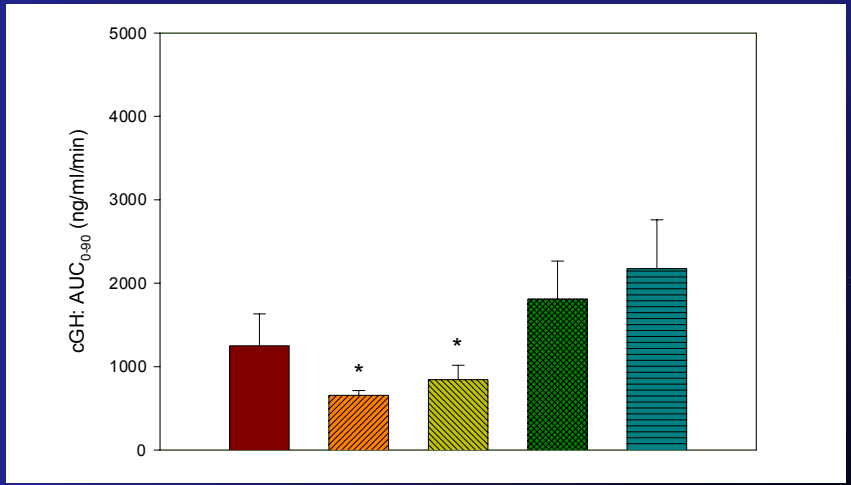
rise elicited by SSIW was completely abrogated. In the set of experiments with a GHRP challenge, 13 dogs of either gender (2–12 years old) received the following

rhGH (0.2 UI/kg sc) per 12 giorni





Basale
Durante il trattamento con rhGH (0.2 UI/kg/die sc)
1 giorno dopo il trattamento con rhGH (0.2 UI/kg/die sc)
5 giorni dopo il trattamento con rhGH (0.2 UI/kg/die sc)
10 giorni dopo il trattamento con rhGH (0.2 UI/kg/die sc)



*: P < 0.05 vs. basale

Rigamonti *et al.*, 2004