

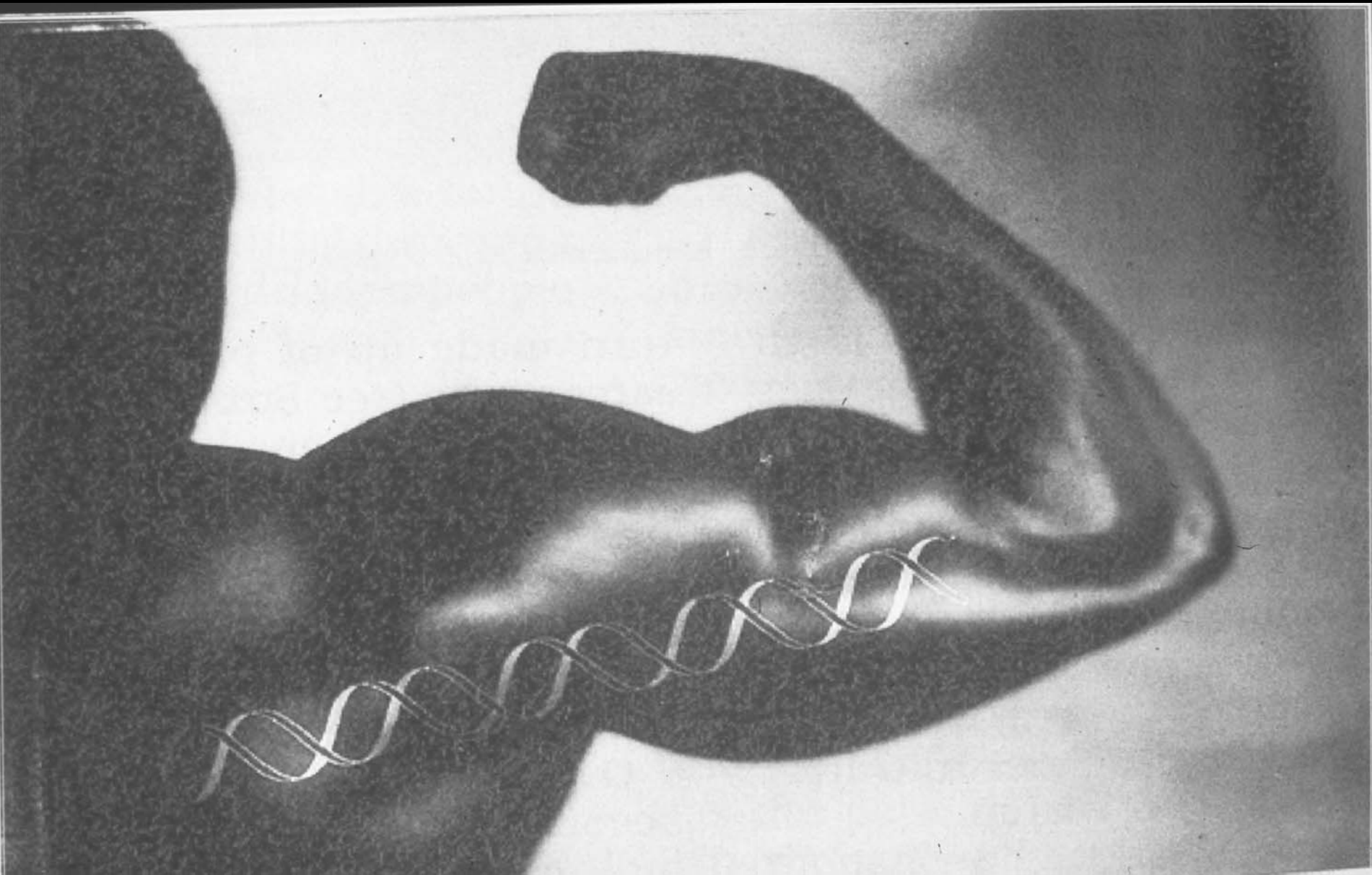
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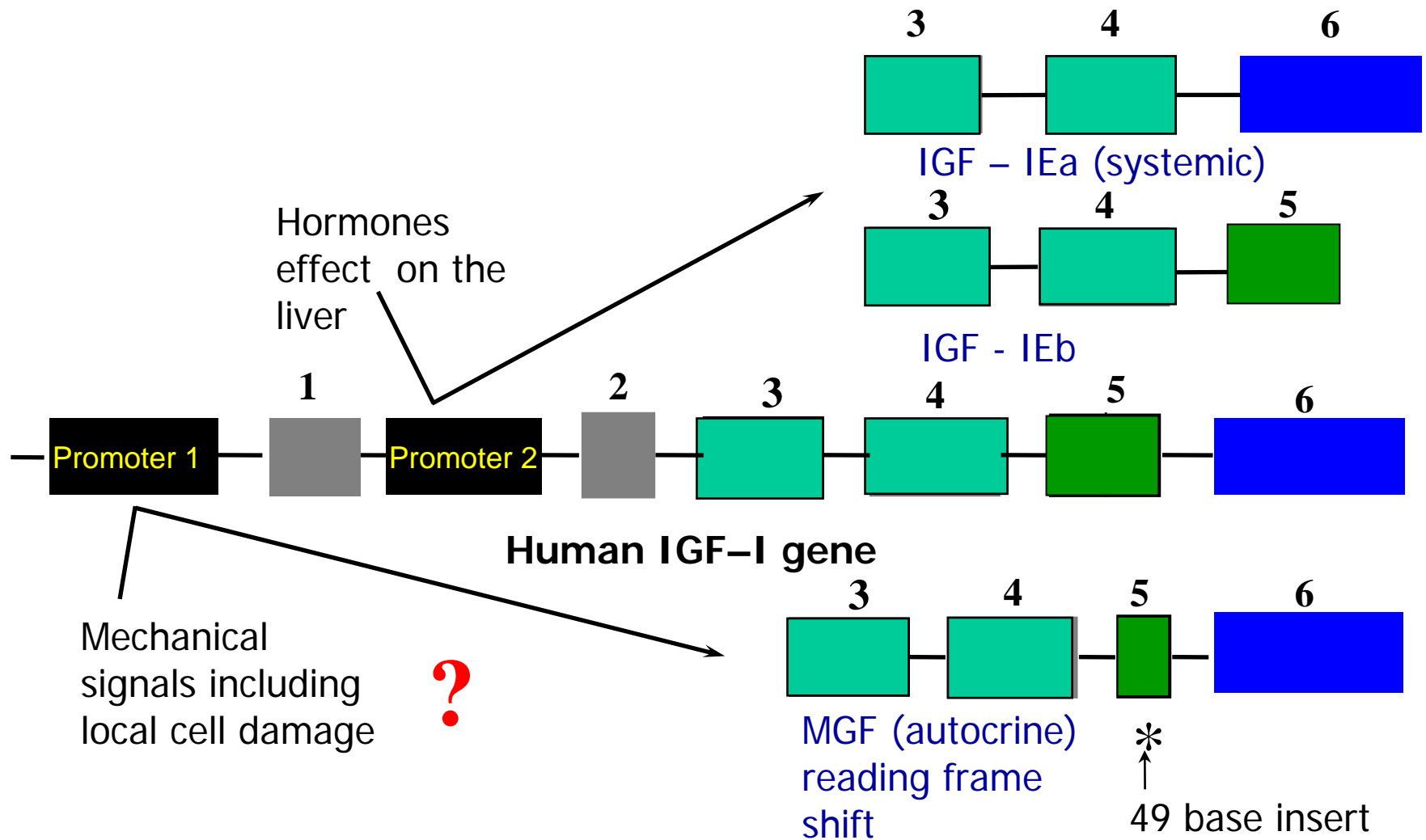
# **Geoff Goldspink**

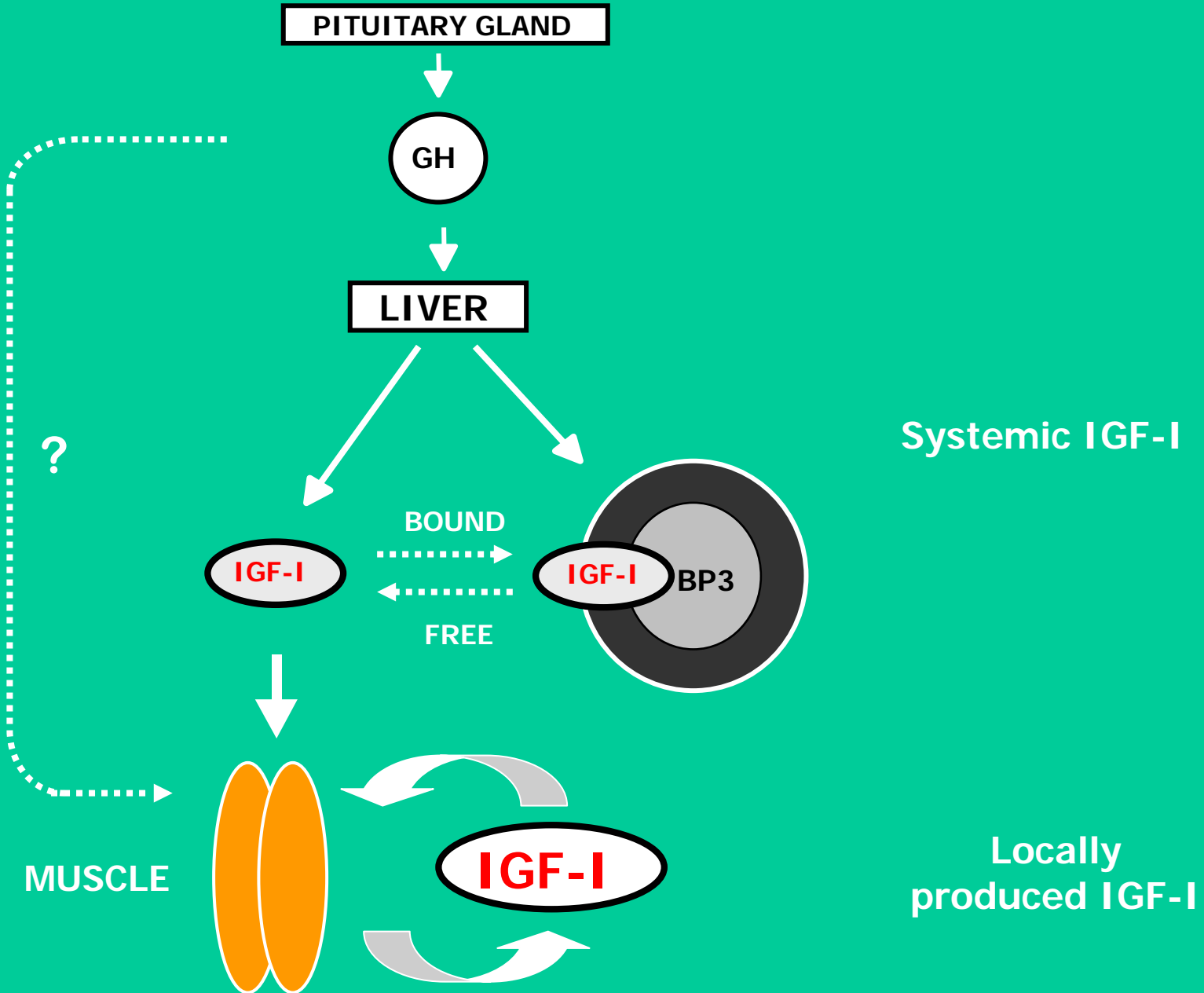
Royal Free & University College Medical  
School, University College, London, UK

Methods of enhancing  
muscle performance  
including gene doping.



# Alternative Splicing of the Human IGF-I Gene





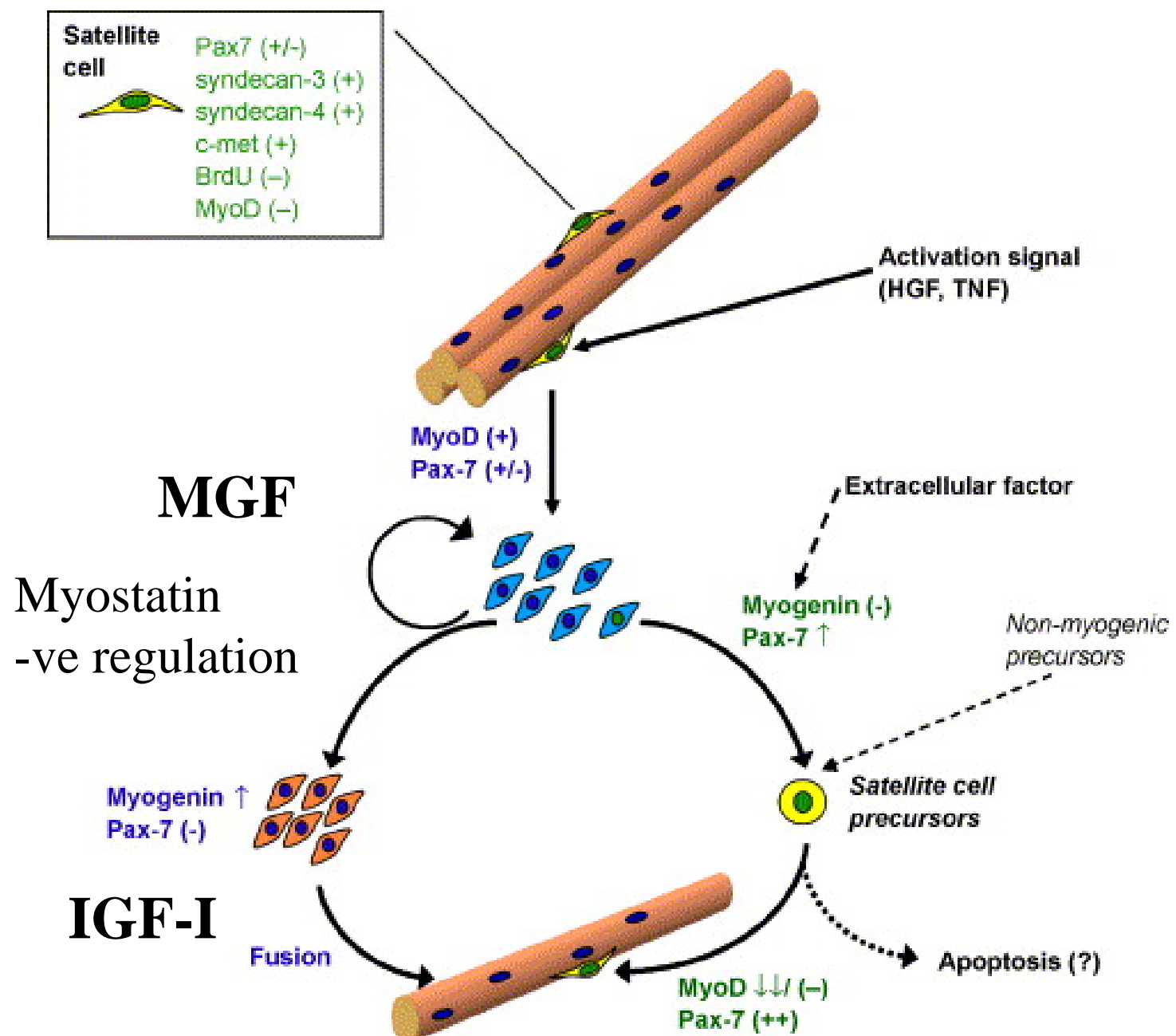
Biological actions of  
GH/IGF-I system  
particularly MGF.

## A. Injection construct

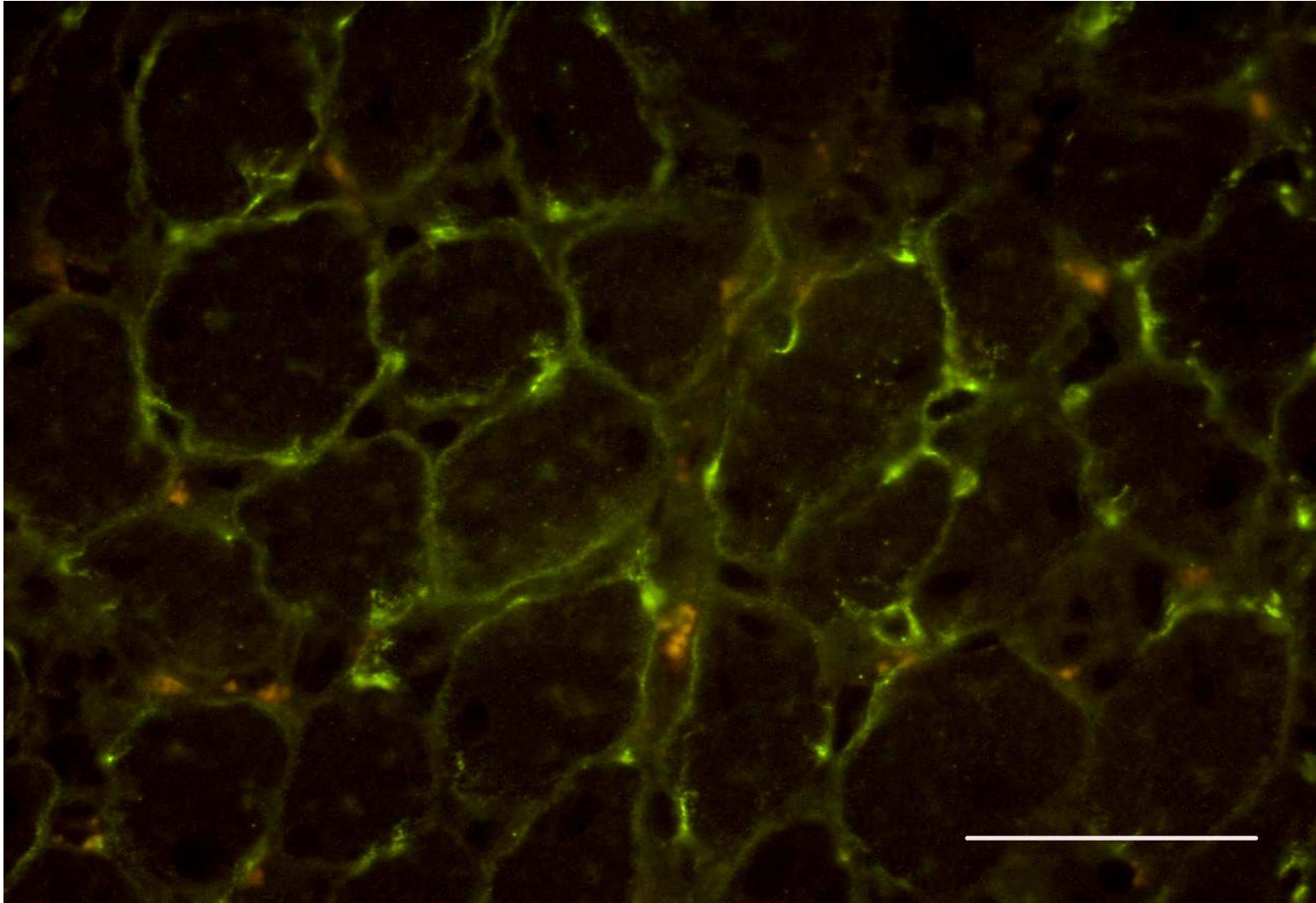


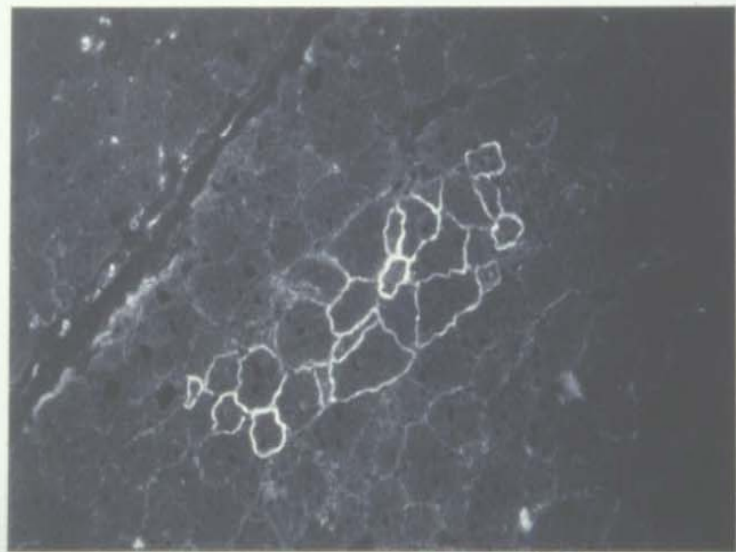
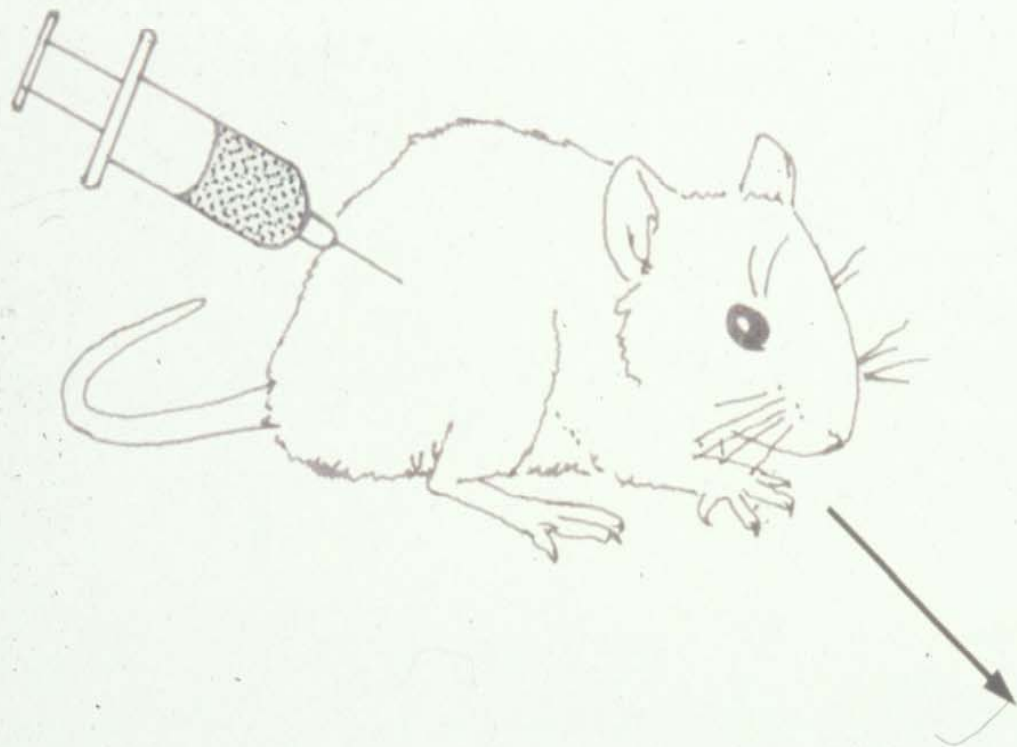
## B. Transfection (expression) construct



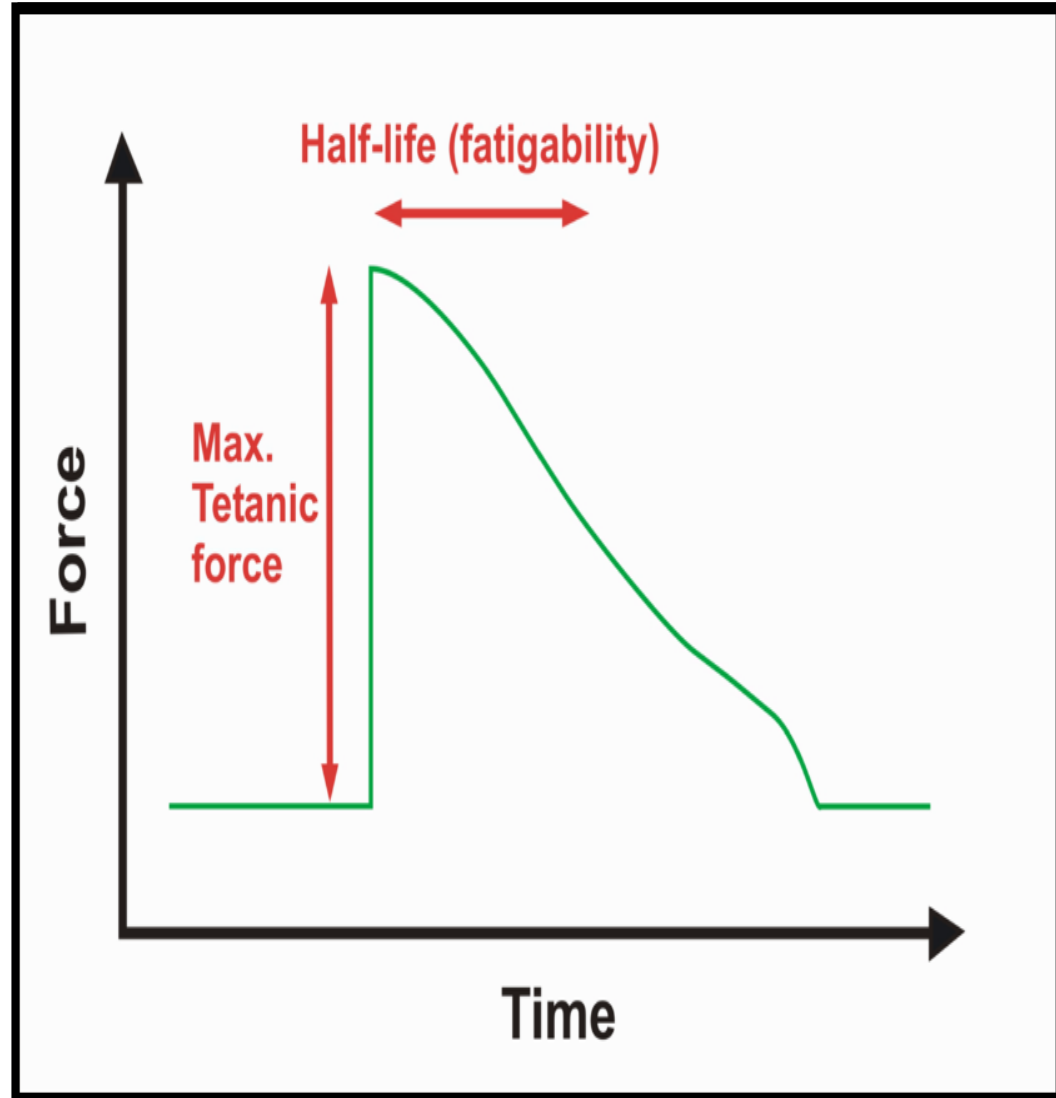
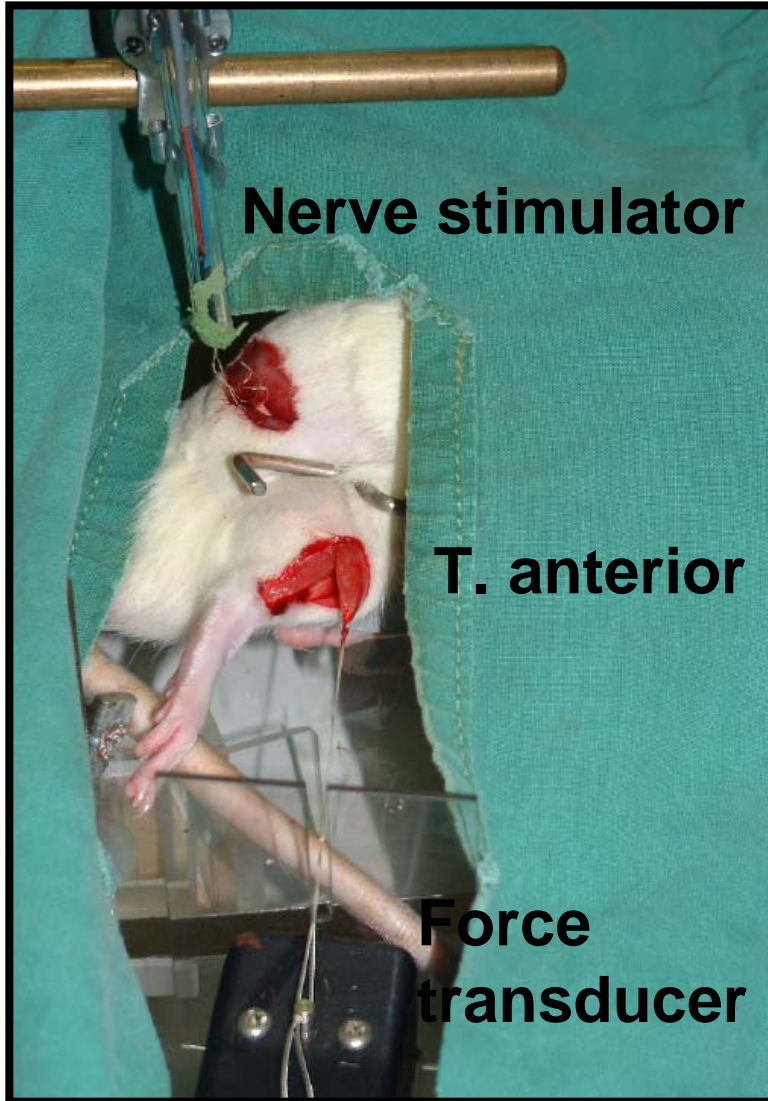


# Damage and M-cad antibody staining

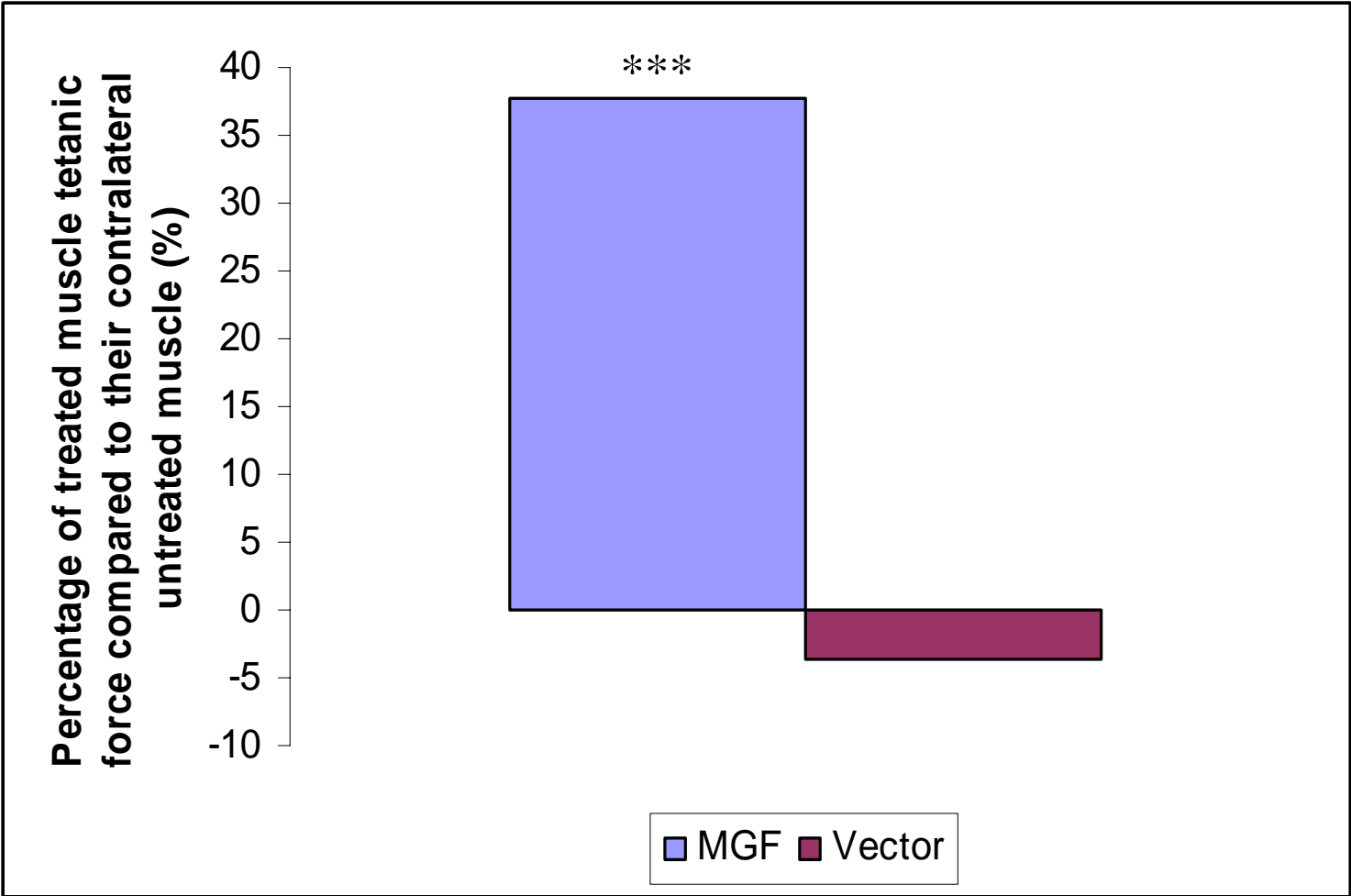


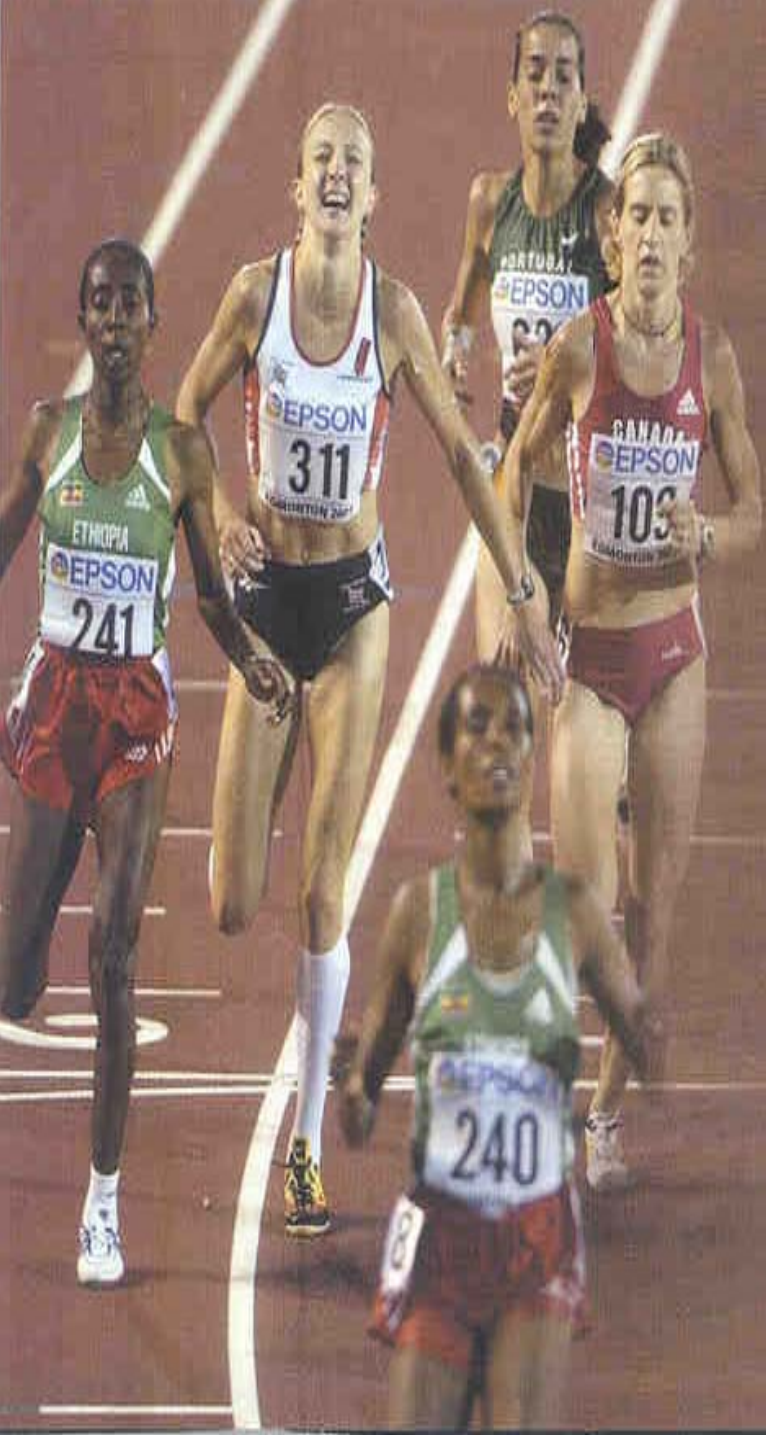


# Muscle myophysiology measurements



**The effect of treating the muscles of mdx dystrophic mice with MGF in a plasmid vector after only 3 weeks on muscle strength**

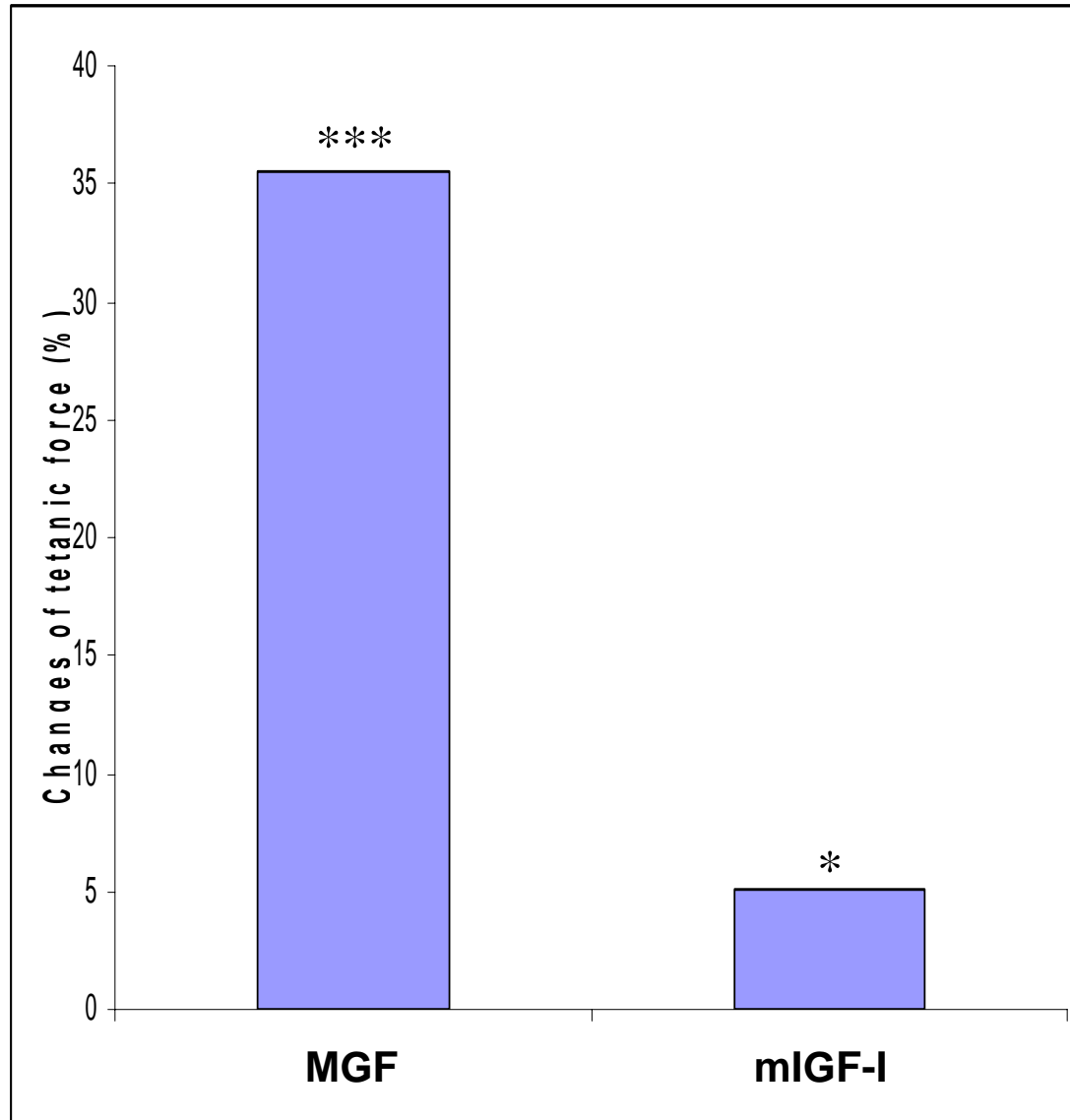




# Playing the gene game

The latest developments in genomics could create the biggest doping problem that the sporting world has ever faced, Fiona Salvage reports

Effect of MGF C terminal and IGF-I receptor domains on strength gain in dystrophic muscle after 3 weeks.





# PHOENIX

PHARMACEUTICALS

THE PEPTIDE ELITE

## ▶ WHAT'S NEW

- **Obestatin** opposes Ghrelin's effect on food intake
- **Peptide Level Determination \$20 / Sample**
- **Ghrelin** level decreased in *H. pylori* infection
- **MGF** strong neuroprotective effect
- **ATX** Possible role in obesity-associated pathologies
- **Retinol Binding Protein 4**  
links obesity to type 2 diabetes
- **Desnutrin** lipase induced by fasting
- **Visfatin** a new insulin mimetic adipokine
- **Relaxin-3 (INSL-7)** stimulates feeding
- **Obesity Biomarker Array**
- **Apelin** a new obesity related adipokine
- **Adiponectin & Adiponectin Receptors**

## ▶ EUROPE BRANCH

*More new products ... ▶▶*

## ▶ OBESITY BIOMARKER / ELISA ARRAY

## ▶ OBESITY / DIABETES PEPTIDE SUMMARIES

## ▶ CARDIOVASCULAR PEPTIDES & KITS

## ▶ LABELED PEPTIDES

## ▶ RIA, EIA, MICROARRAY KITS & PROTOCOLS

## ▶ PEPTIDE ANTIBODIES

## ▶ CATALOG PEPTIDES

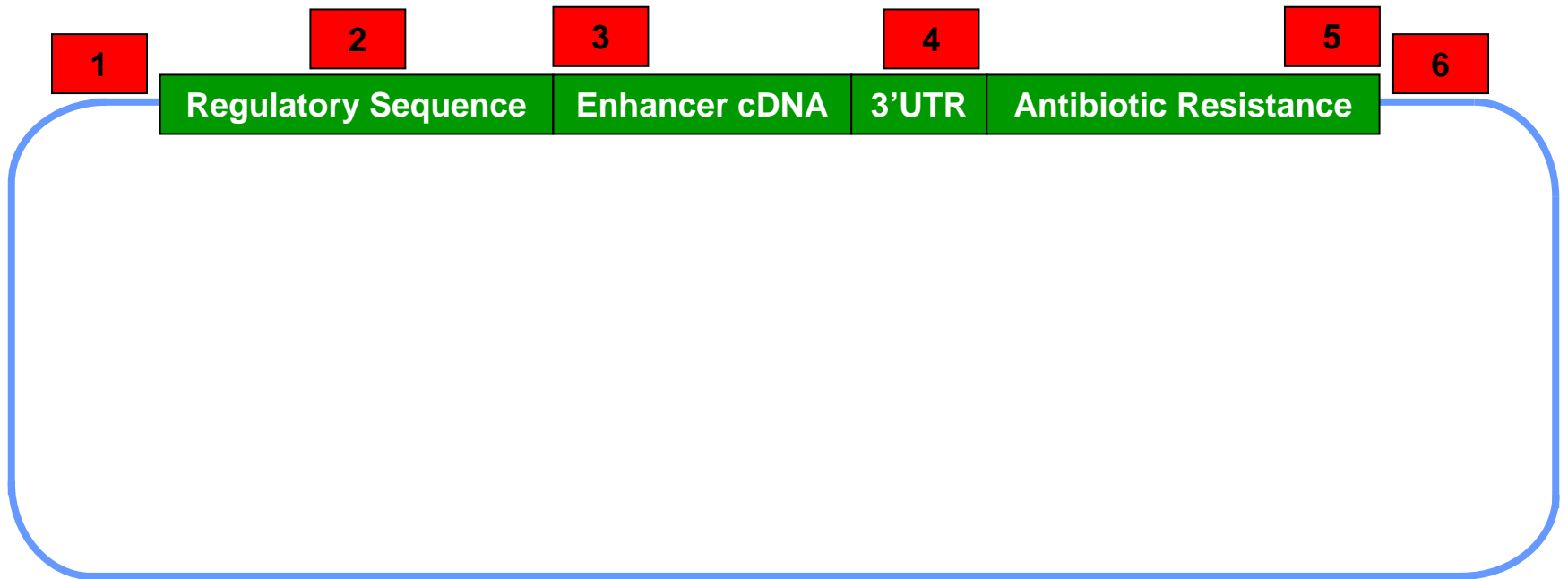
## ▶ PEPTIDE HORMONE LIBRARIES

## ▶ CUSTOM SYNTHESIS & GMP

# Detection Methods

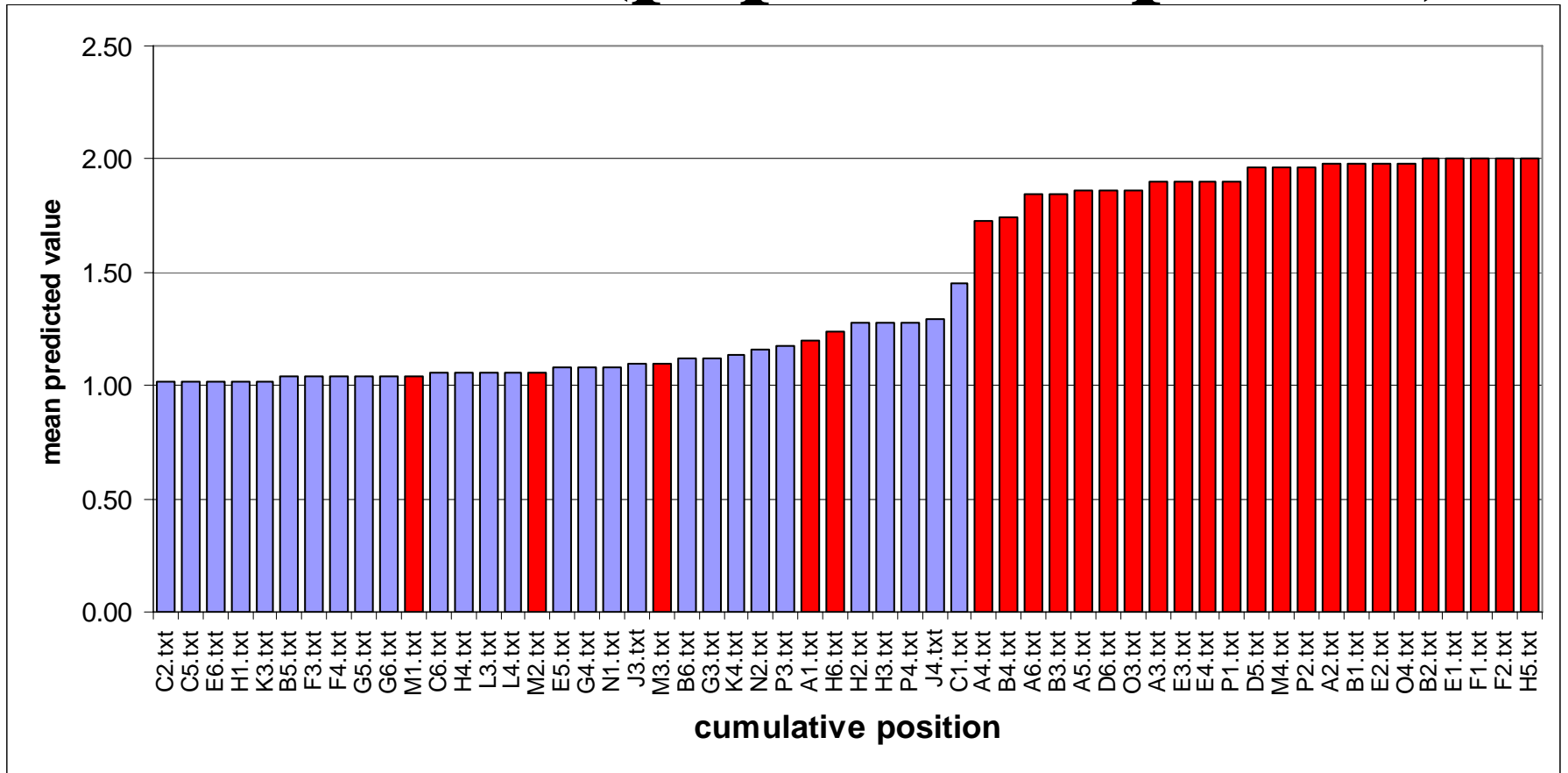
- Rapid Screening using Mass Spec
- Confirmatory tests based on
  - a) antibody methods and/or
  - b) cell signalling using differential gene expression.

Introduced gene with cDNA of enhancer substance:  
Detection using different primer combinations



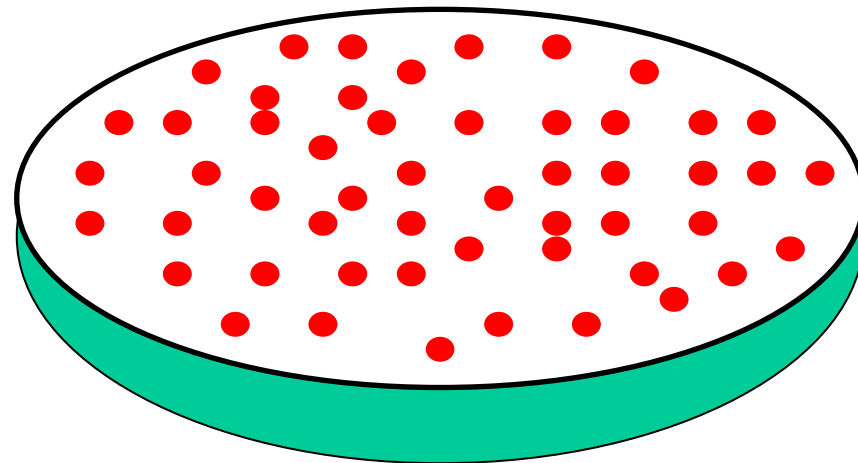
1 to 6, pairs of primers that detect known vectors, cDNAs of enhancer substances and required components of gene constructs.

# Prediction (population profile)



**GH**  
100ng/ml

**IGF-IR3**  
100pg/ml



24h

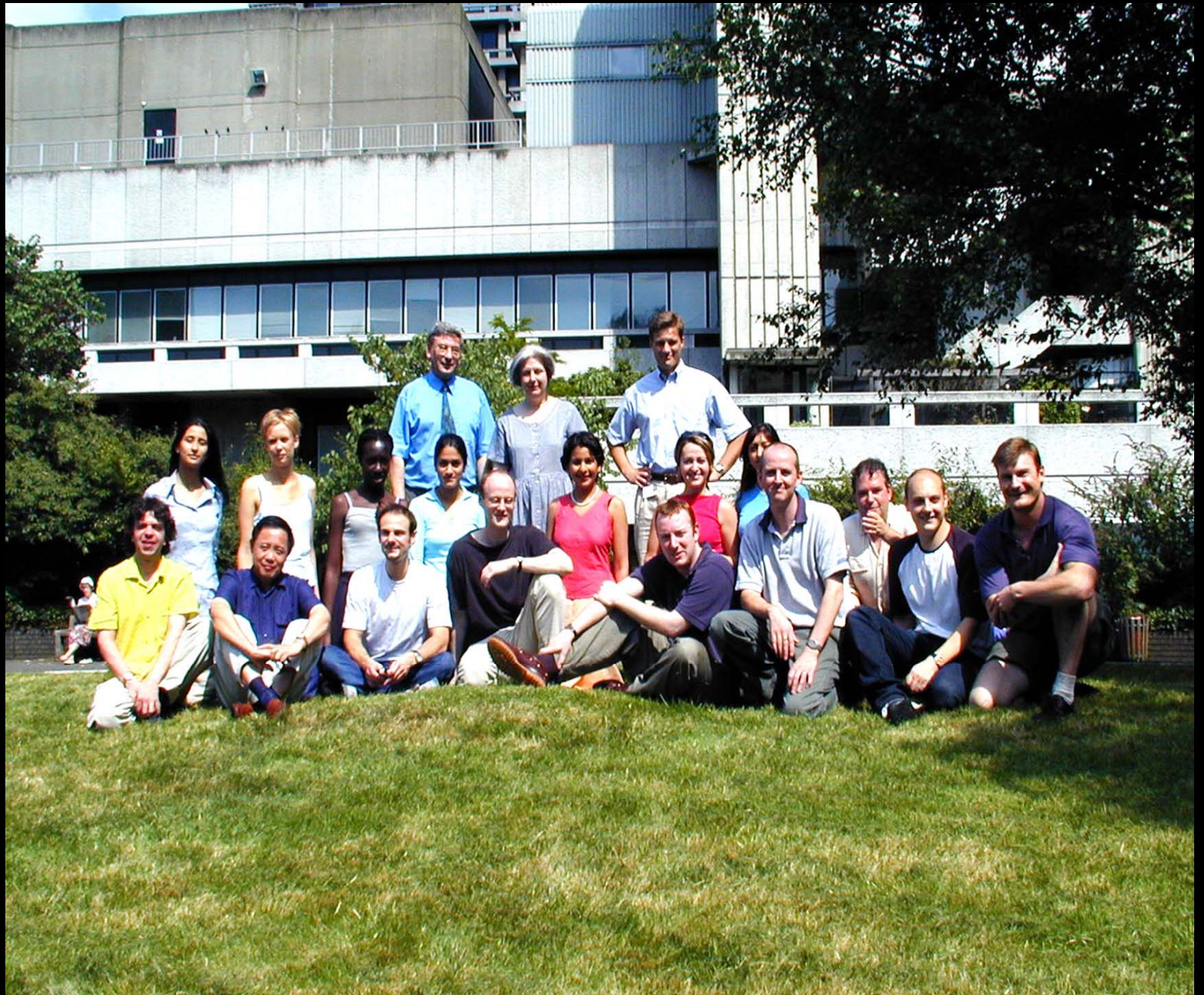
RNA

cDNA

quantitative PCR

# **Present and future challenges in detecting enhancing substances.**

- 1) Synthetic/recombinant analogues.
- 2) Generic substances and signalling pathway initiators (mimetics).
- 3) New methods of administration.
- 4) Gene doping.













# **Geoff Goldspink**

Royal Free & University College Medical  
School, University College, London, UK

## **Research Report**

on studies of GH/IGF-I axis and  
muscle performance including  
gene doping.



# Study Protocol

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## Exercise

- Knee extensor weightlifting exercise
- 3 sessions / week
- 3-5 sets of 8-12 repetitions per session
- (60-70% 1- RM)

## GH administration or placebo

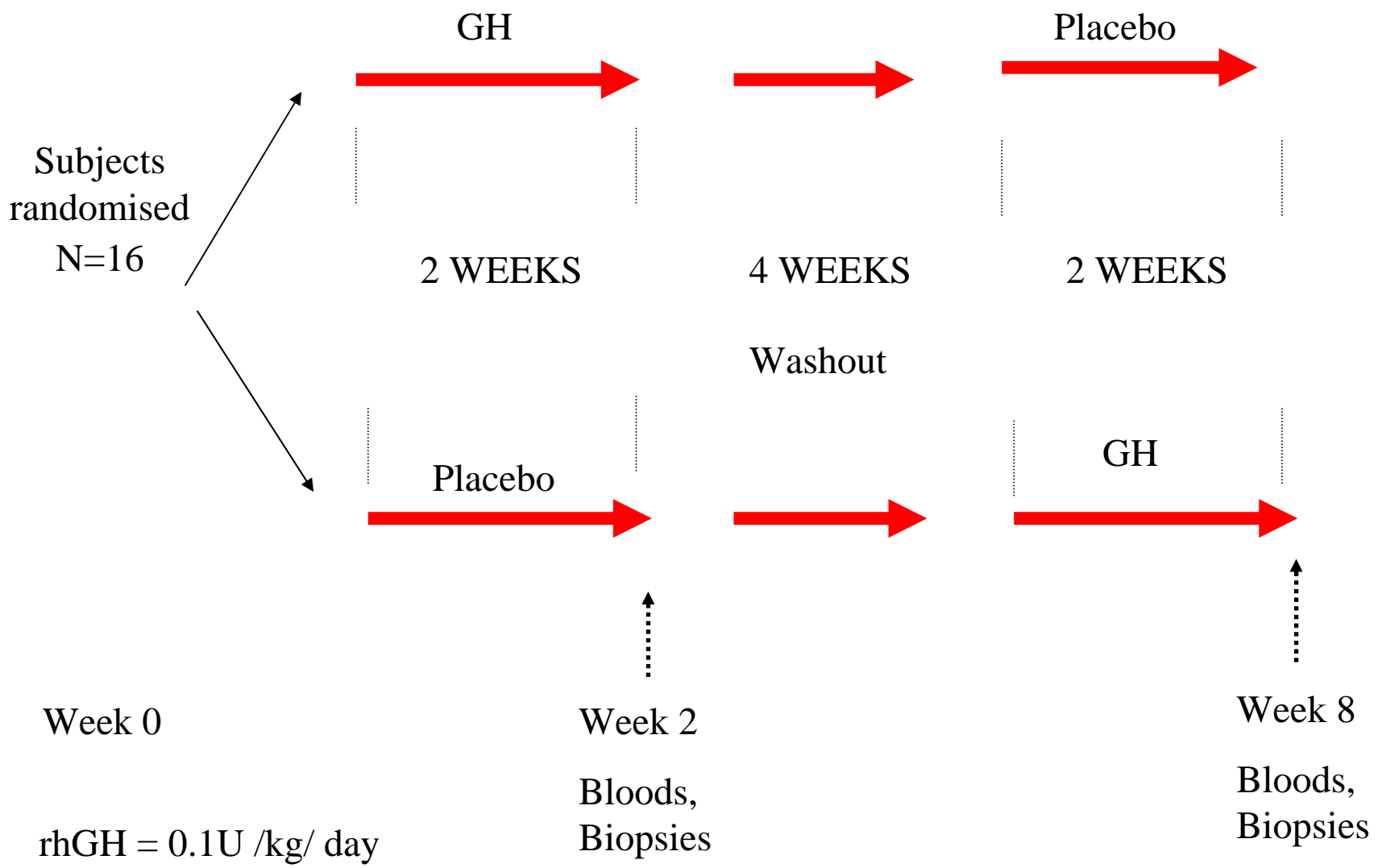
Lange et al 2002

## Muscle Sampling

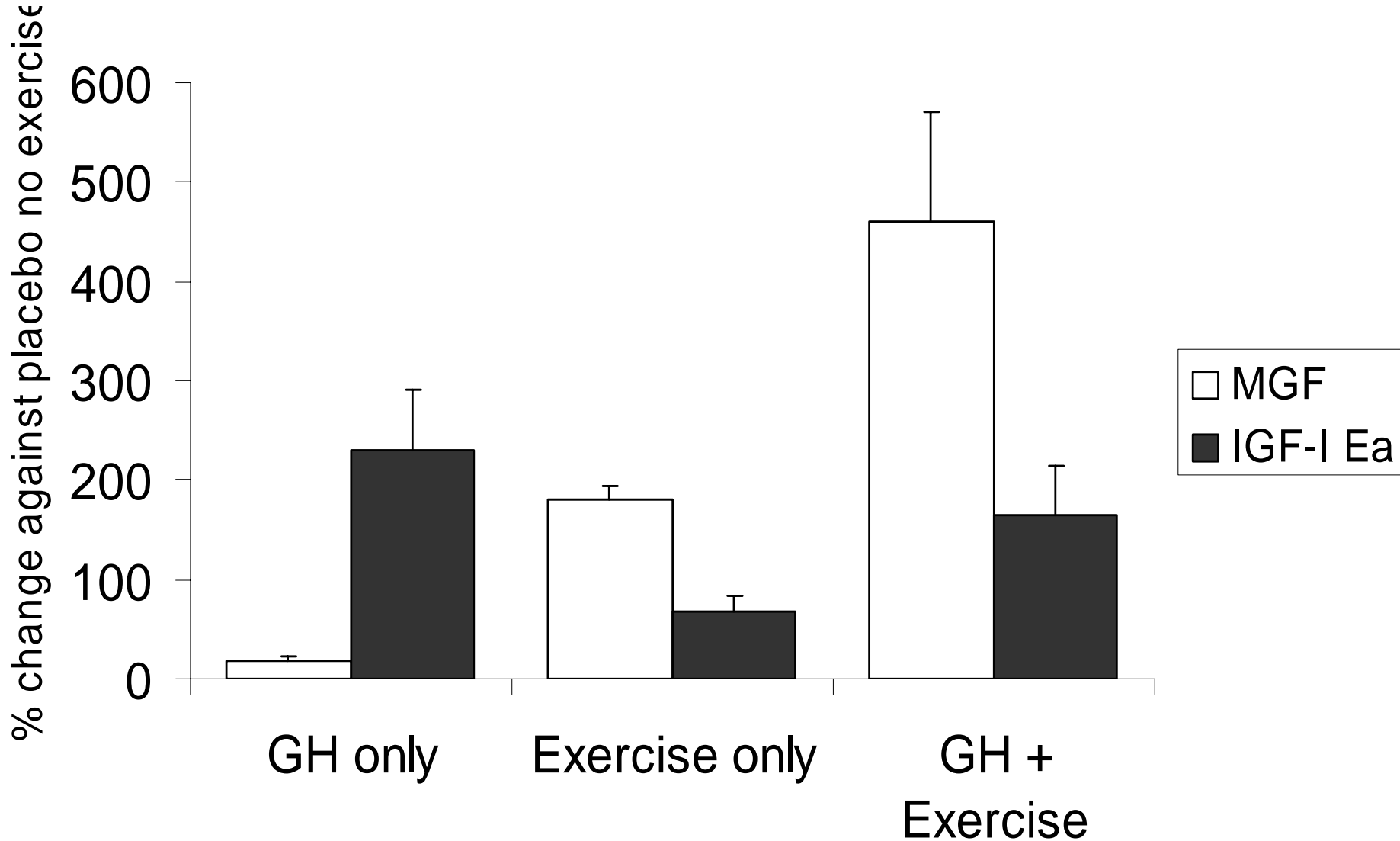
- Muscle biopsies pre, 5 and 12 wks
- 24 hours after last training session



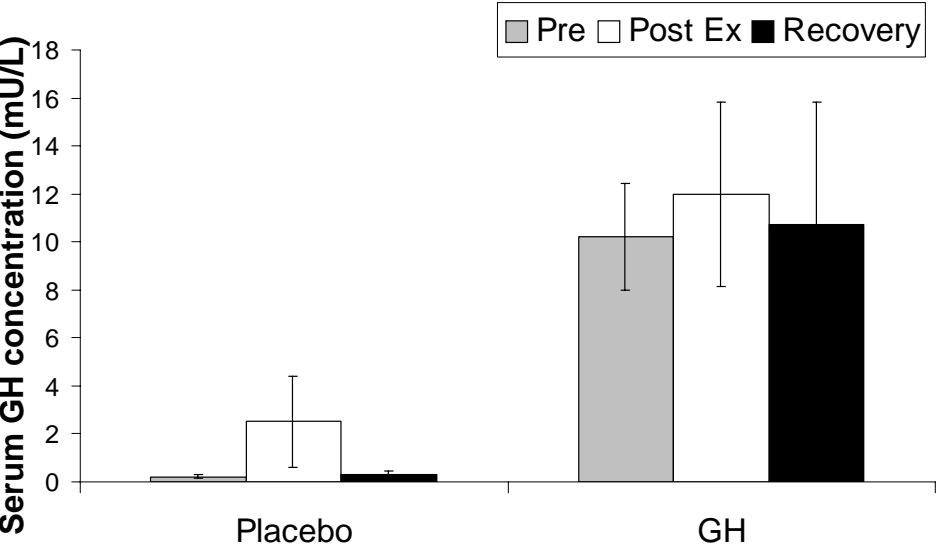
# Study 1-Untrained Subjects Experimental Protocol



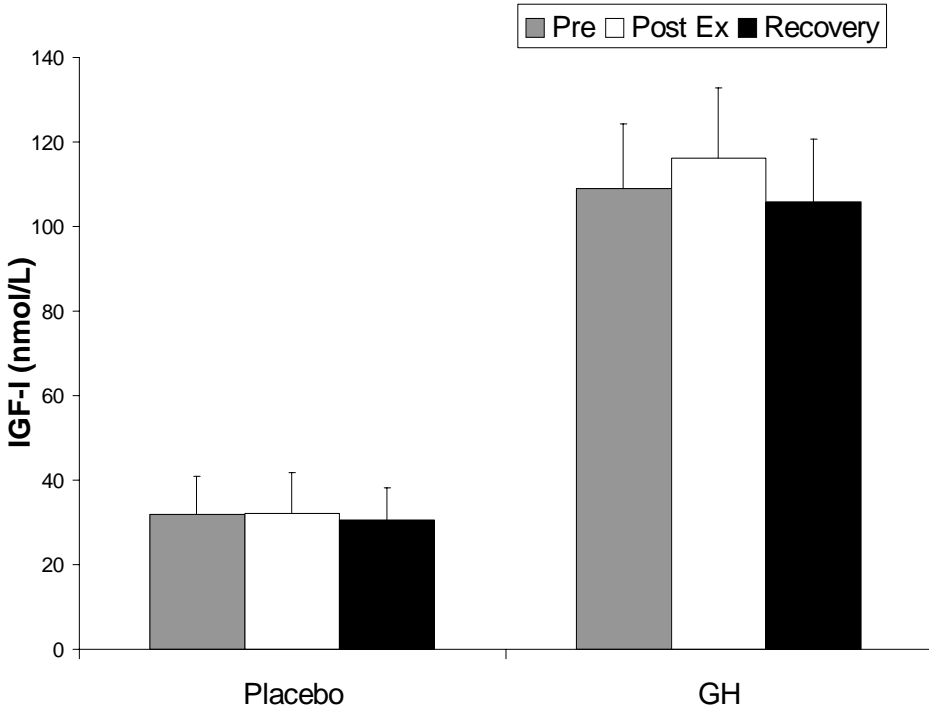
# Growth Hormone (GH) and Exercise on IGF-I Isoforms in Elderly Muscle



# Effect of exogenous GH administration on serum GH concentration

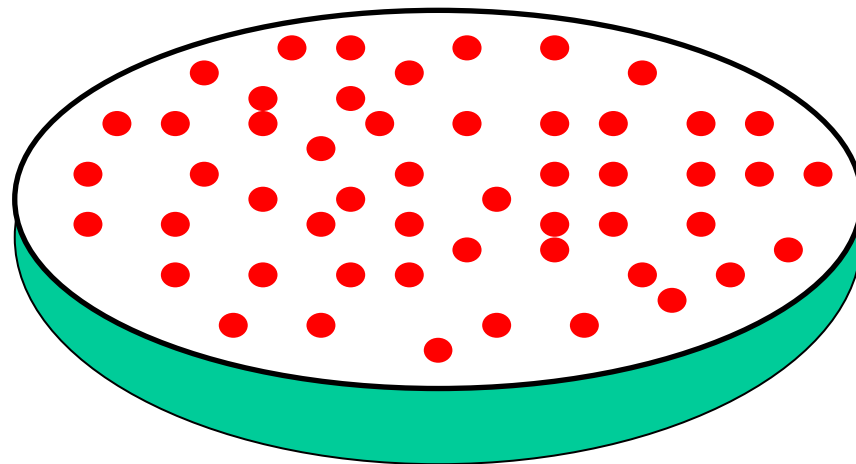


# Effect of exogenous GH administration on serum IGF-I concentration



**GH**  
100ng/ml

**IGF-IR3**  
100pg/ml



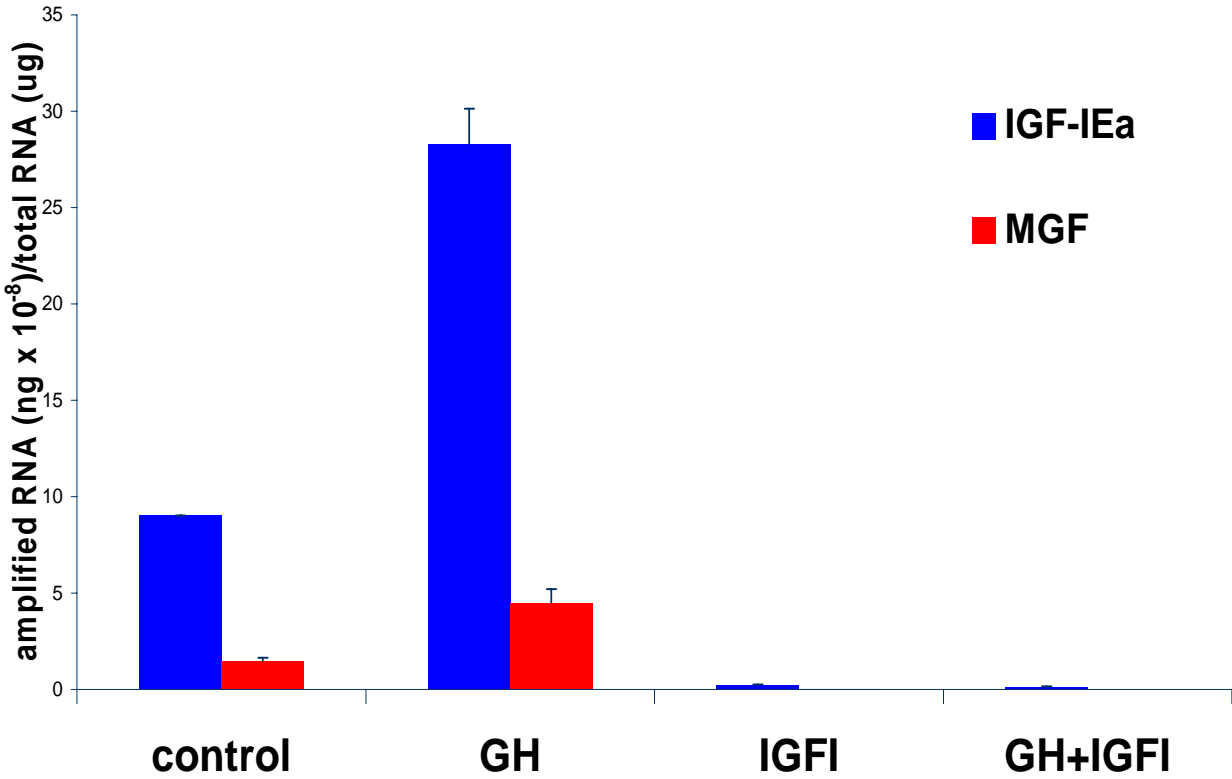
24h

RNA

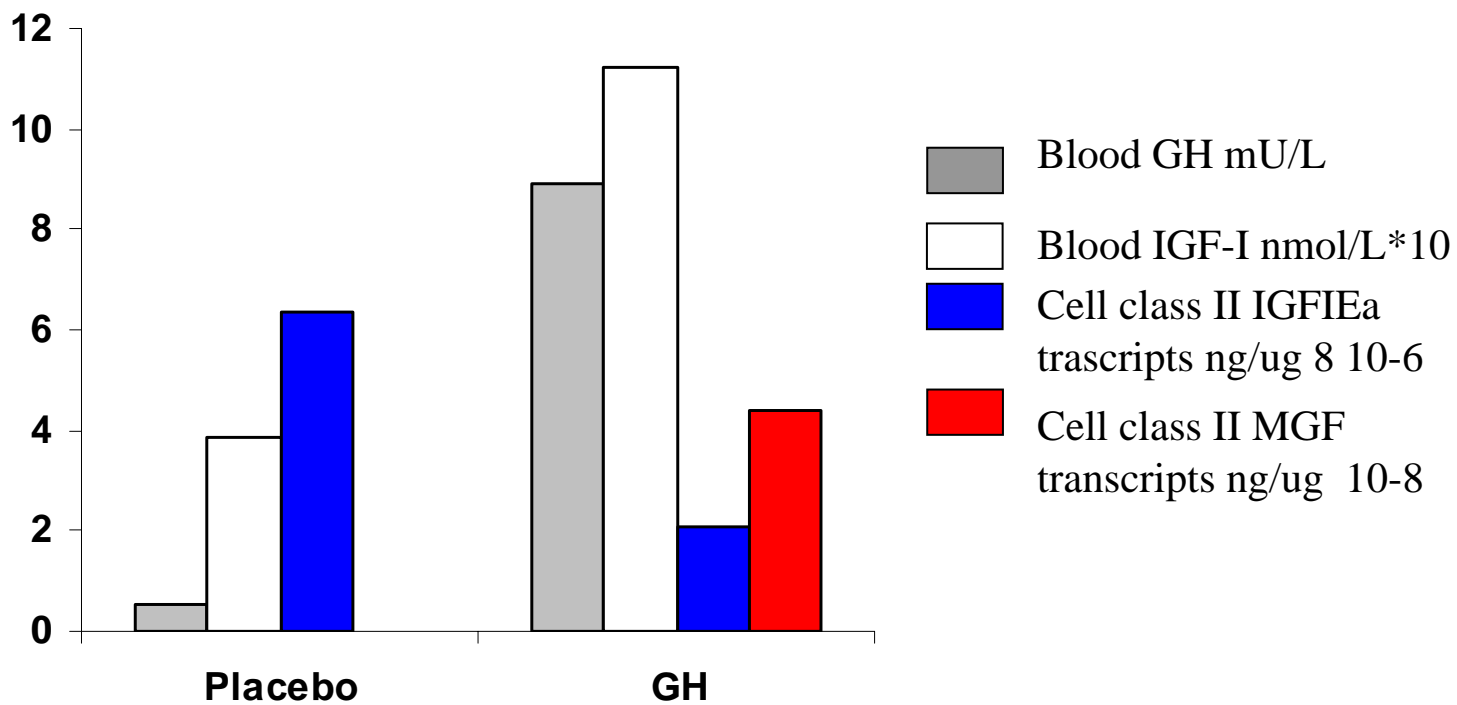
cDNA

quantitative PCR

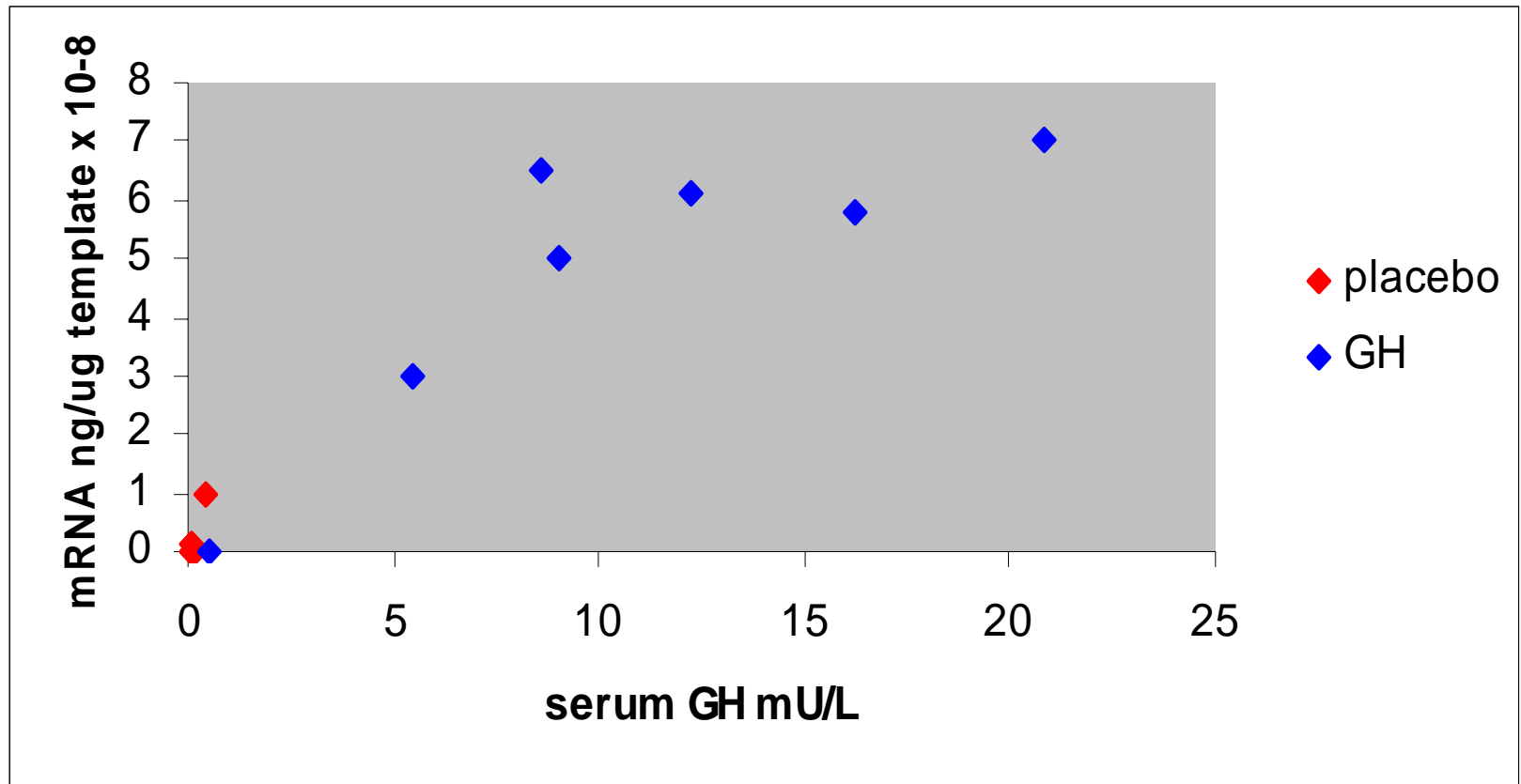
# MGF and IGFI-Ea transcript levels in biosensor cells C2C12 treated with rh GH and/or IGF-I



# Human Blood serum IGF-I and GH levels and class II IGF-I transcripts in C2C12 cells treated with same human serum

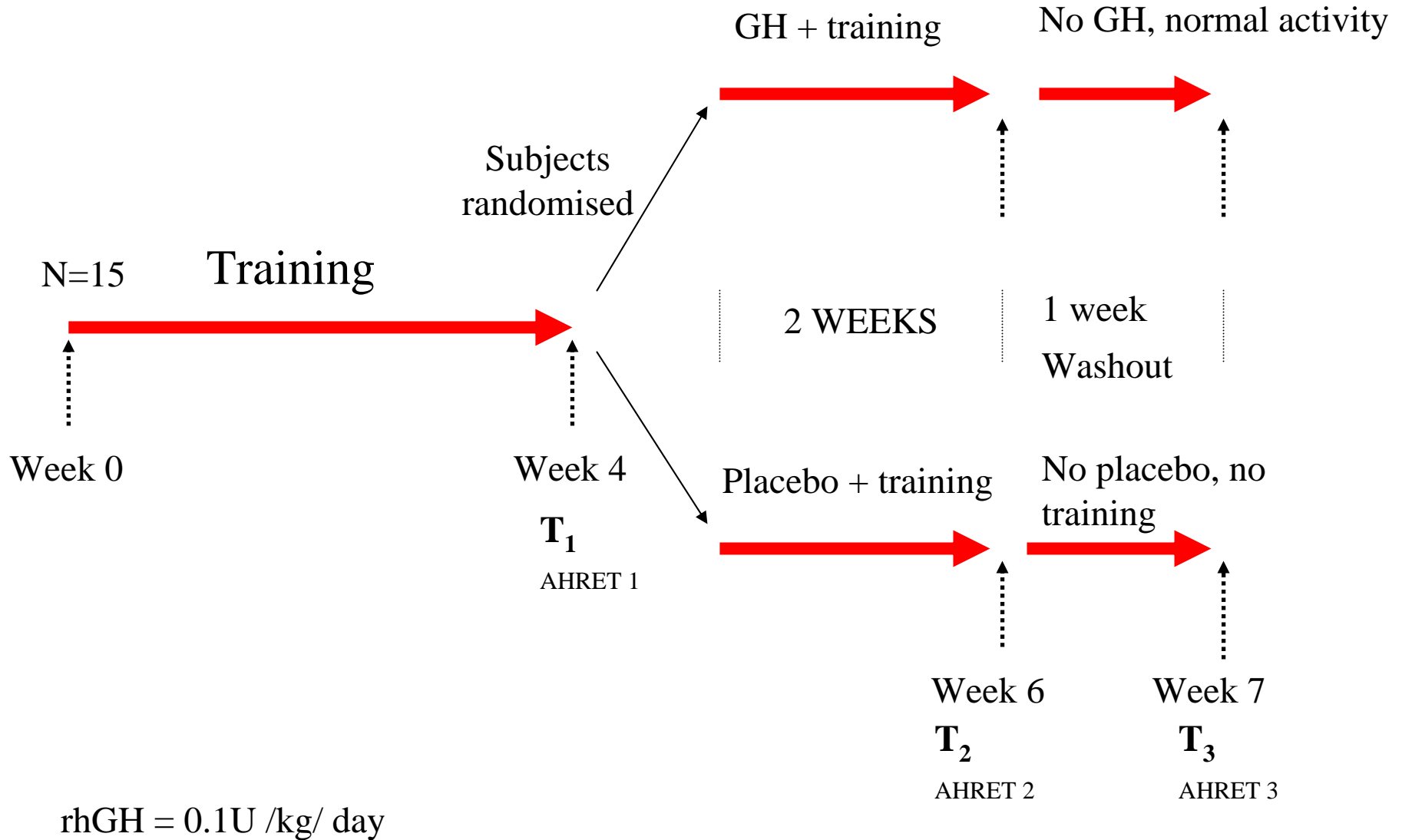


# Class II MGF transcripts in cells treated with Human Serum Samples



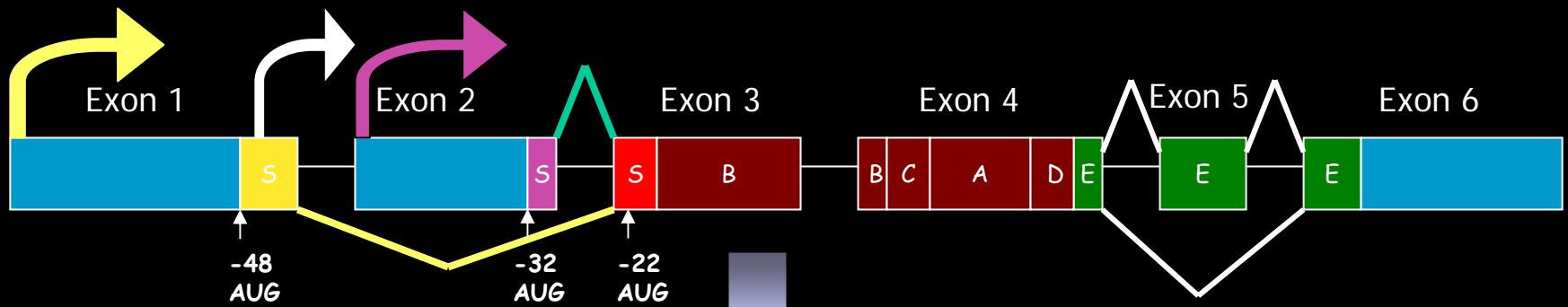
# Study 2- Trained Subjects

## Experimental Protocol



# IGF-I gene transfer in rodents.

Rat IGF-I splicing to produce several isoforms with different functions.



Class 1 IGF-1A



Class 1 IGF-1B



MGF (IGF-I Ec)  
in human muscle

Class 2 IGF-1A



Class 2 IGF-1B



MGF in human muscle  
but not in CNS

MGF C-terminal peptide



**Table: Quantification by real time RT-PCR amplification of total class 1 and class 2 IGF-I splice variant mRNAs from muscle biopsies following RT, GH or RT + GH**

mRNA transcript	Placebo		GH	
	non-ex	ex	non-ex	ex
Class 1 IGF-I	$5.19 \times 10^{-4}$ $\pm 6.35 \times 10^{-5}$	$4.20 \times 10^{-4}$ $\pm 5.12 \times 10^{-5}$	$5.18 \times 10^{-4}$ $\pm 6.23 \times 10^{-5}$	$4.70 \times 10^{-4}$ $\pm 7.48 \times 10^{-5}$
Class 2 IGF-I	$4.51 \times 10^{-4} \pm$ $\pm 8.83 \times 10^{-5}$	$3.82 \times 10^{-4}$ $\pm 8.65 \times 10^{-5}$	$4.65 \times 10^{-4}$ $\pm 1.01 \times 10^{-4}$	$4.00 \times 10^{-4}$ $\pm 9.05 \times 10^{-5}$
Ratio Class 1:2	0.87	0.91	0.90	0.85

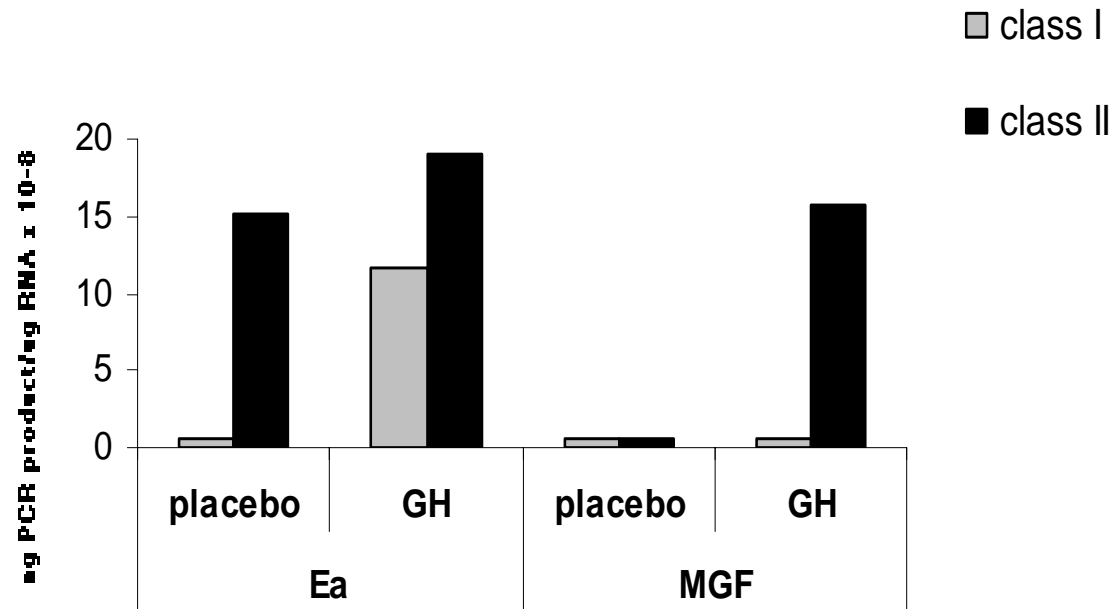
Data for total IGF-I are expressed as ng mRNA ( $\mu\text{g total RNA}$ )<sup>-1</sup>.

Note: Again there was a difference in the splicing of the IGF-I gene with MGF being upregulated by GH combined with exercise.

Results from proteomic profiles  
analyses of serum samples from  
control and hGH treated mice

Lancashire L, Boateng J, Matharu-  
Ball B, Creaser C and Ball, GR

# IGF-I transcript levels in C2C12 cells treated with 10% human serum (endogenous GH level < 0.1mU/L) and 100ng/ml GH



# Current collaborative studies with Nottingham Trent University and HFL Newmarket, UK.

1) Mice receiving hGH delivered using a mini osmotic pump.

*10 hGH plus 10 PBS controls plus 10 normal.*

2) Mice injected hGH gene construct.

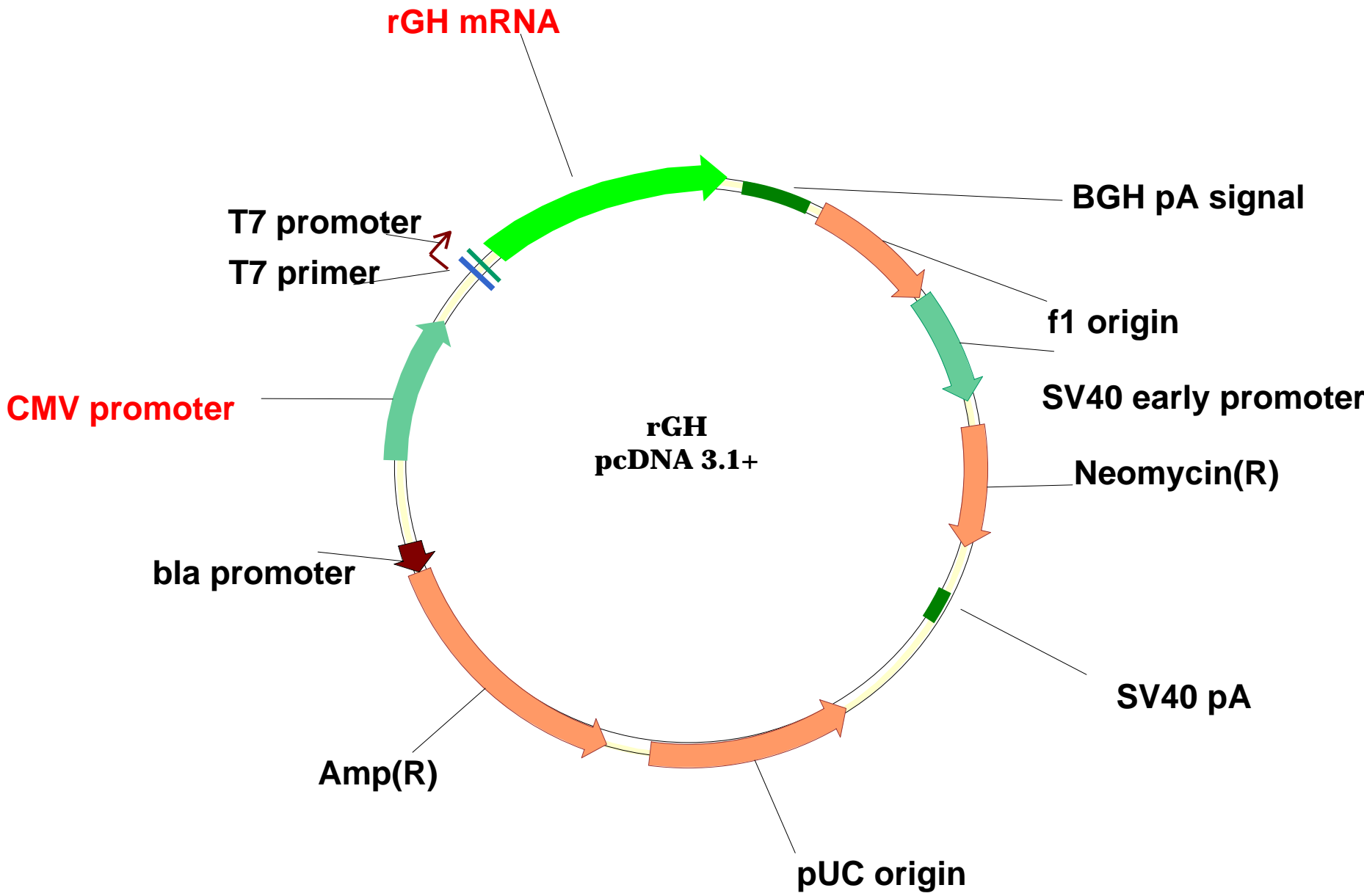
*10 hGH gene injected, 10 with control gene injected plus 10 PBS injected*

3) GH injected none trained human subjects (25y to 30y)

*Cross over study with 8 with 4 blood samples and 2 biopsies.*

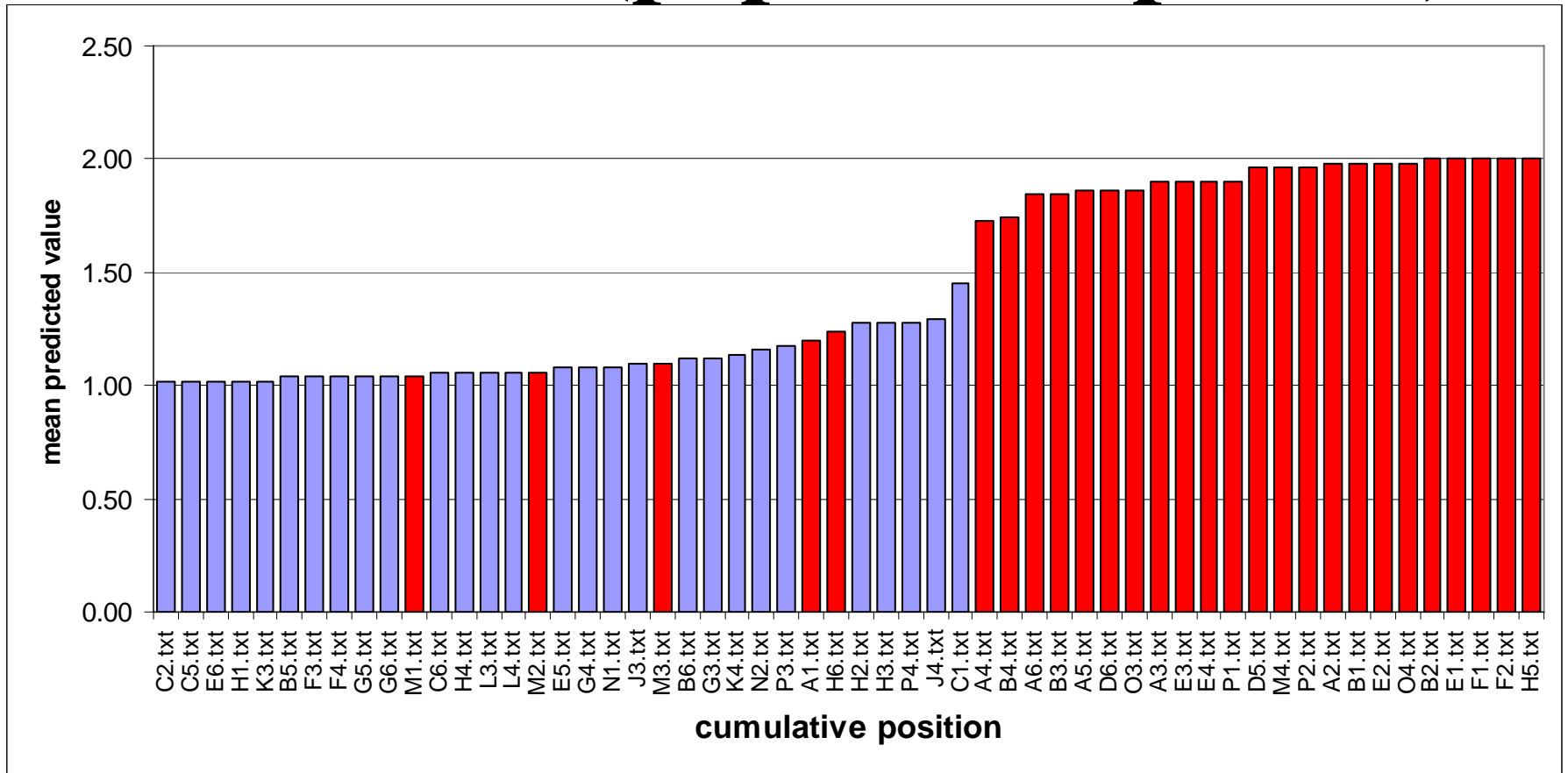
4) GH injected trained human subjects

*Cross over study with 15 volunteers (25y to 30y), from each there are 4 blood samples plus 2 biopsies.*



Mammalian Expression Vector rGH Construct

# Prediction (population profile)



# **Present and future challenges in detecting enhancing substances.**

- 1) Synthetic/recombinant analogues.
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