Genetic Signatures: Application to EPO detection

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Epo anti-doping tests

The different approaches

Direct approach
Limited detection window (36-48h)
Cut-off limits (e.g. 55% Hct)
False positive
False negative

Indirect approach
Blood passport
Improves specificity and sensitivity
Despite more than 250,000 tests being carried out in the last year [2010], only 36 came back as positive for EPO.

“We are catching the dopey dopers, but not the sophisticated ones”.

David Howman
director general of WADA
Epo anti-doping tests

The different approaches

**Direct approach**

**Indirect approach**

Limited detection window (36-48h)

**Systems Biology based approach**

Improves specificity and sensitivity?

Current markers of the Athlete Blood Passport do not flag microdose EPO doping

Michael Ashenden · Clare E. Gough · Andrew Garnham · Christopher J. Gore · Ken Sharpe

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The Omics Cascade ≠ Systems Biology

What CAN happen

GENOMICS

What APPEARS to happen

TRANSCRIPTOMICS

What MAKES it happen

PROTEOME

What HAS happened

METABOLOME

PHENOTYPE
World Anti-Doping Agency (WADA)  
Health, Medical, and Research Committee  

Application Form for Scientific Research Grants – 2008 Competition  

Date: 

Project category:  
√ Compounds and/or methods enhancing oxygen delivery  
☐ Non-steroidal compounds or methods enhancing growth  
☐ Projects relating to the Prohibited List: classical methodologies  
☐ Projects relating to the Prohibited List: novel methodologies  
√ Identification and/or detection of substances with suspected doping potential  

Gmeiner Günter, Head of WADA-accredited Doping Control Laboratory, Austrian Research Centres GmbH – ARC, Doping Control Laboratory, A-2444, Seibersdorf, Austria
Research hypotheses and objectives

1. To measure blood parameters and gene-expression profiles in sea-level and altitude-adapted trained athletes after r-HuEpo administration;

2. Determine the effects of ethnicity on haematological parameters and gene-expression profiles; and

3. Formulate revised methods with improved discriminatory power relative to standard haemoglobin and haematocrit detection protocols.
<table>
<thead>
<tr>
<th>Cohort</th>
<th>No of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epo trial east African runners (n=20) x 19 serial blood samples</td>
<td>380</td>
</tr>
<tr>
<td>Epo trial Caucasian runners (n=20) x 19 serial blood samples</td>
<td>380</td>
</tr>
<tr>
<td>Total samples possible</td>
<td>760</td>
</tr>
</tbody>
</table>
Study design

Endurance trained (Caucasian, sea level, n = 20)
Endurance trained (East Africans, altitude, n = 20)
Demographic characteristics of elite Kenyan endurance runners

VINCENT O. ONYWERA¹, ROBERT A. SCOTT², MICHAEL K. BOIT¹, & YANNIS P. PITSILADIS¹,²

¹Department of Exercise and Sports Science, Kenyatta University, Nairobi, Kenya, and ²International Centre for East African Running Science, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK

Figure 1. Districts of Kenya reflecting distribution of participants in the present study. “Other” covers Coast, North-Eastern and Western provinces.
Maximum aerobic capacity (VO$_2$max)

WADA supported Human Performance Lab, Glasgow, Scotland.

WADA supported Human Performance Lab, Eldoret, Kenya.
VO$_2$max
VO2max

![Graph showing relative changes in VO2max (%) over baseline, end of rHuEpo, and end of study for SCO and KEN groups.](image-url)
3km time trial running performance

Indoor athletic track (200 m) at Kelvin Hall International Sports Arena, Glasgow, Scotland.

Erythropoietin doping in cycling: lack of evidence for efficacy and a negative risk–benefit

Jules A. A. C. Heuberger,¹ Joost M. Cohen Tervaert,¹ Femke M. L. Schepers,¹ Adriaan D. B. Vliegenthart,¹ Joris I. Rotmans,² Johannes M. A. Daniels,³ Jacobus Burggraaf⁴ & Adam F. Cohen⁵

¹Biopharmaceutical Sciences, Leiden University, Leiden. ²Department of Nephrology, Leiden University Medical Centre, Leiden. ³Department of Pulmonary Diseases, VU University Medical Centre, Amsterdam. ⁴Leiden Amsterdam Centre for Drug Research, Leiden and ⁵Leiden University Medical Centre, Leiden, The Netherlands.

Outdoor athletic track (400 m) at Eldoret, Kenya.
Time trial
Time trial

![Graph showing relative changes in time trial (%).](image-url)

- **Baseline**
- **End of rHuEpo**
- **End of study**

**Axes:**
- Relative changes in time trial (%)
- Time points: Baseline, End of rHuEpo, End of study

**Legend:**
- SCO
- KEN
Standard methods of analysis

Sysmex XT-2000i
Haematocrit

A. Haematocrit (%)

Time (Weeks)
Haemoglobin concentration
Reticulocyte
"Omics" workflow

1. Tempus® Blood RNA tubes
2. MagMAX™ for stabilized blood tubes RNA isolation kit
3. Cragen® RNA Saliva Kit
4. Total RNA (80 µl)
5. Nano Drop (1 µl)
6. Agilent Bioanalyzer (4 µl)
7. Total RNA aliquots (3 x 20 µl + remaining)

Gene expression microarray

- 500 ng Total RNA
- Illumina® Total Prep™ Reverse Transcription
- cDNA
- Illumina® Total Prep™ in vitro Transcription
- Biotin labeled cRNA
- Hybridization
- Illumina® BeadArray Reader

miRNA expression profiling

- 400 ng Total RNA
- Megaplex™ Reverse Transcription
- cDNA
- Quantitative real time PCR

Nano Drop
Agilent Bioanalyzer
Microarray experiment

8/20 time points:

2 Baseline, 3 during and 3 post r-HuEpo

= sample collection  = rHuEpo injection
5 % FDR significant and ≥ 1.5FC: During rHuEpo

SCO (n=18)

2 days
41 (41 ↑ 0↓)

2 weeks
811 (736 ↑ 75↓)

4 weeks
394 (390 ↑ 4↓)
5 % FDR significant and ≥ 1.5FC: During rHuEpo

**SCO (n=18)**

2 days
41 (41↑ 0↓)
811 (736↑ 75↓)

2 weeks
0
0
419

4 weeks
394 (390 ↑ 4↓)

**KEN (n=18) (n=6)**

2 days
63 (63↑ 0↓)
57 (54↑ 3↓)

2 weeks
0
0
384

4 weeks
901 (718↑ 183↓)
527 (520↑ 7↓)
536 (533↑ 3↓)
379 (377↑ 2↓)
5% FDR significant and ≥ 1.5FC: Post rHuEpo

SCO (n=18)

Post 2 weeks
249 (7↑ 242↓)

Post 4 weeks
139 (0↑ 139↓)

114

135

4

= sample collection
↑ = rHuEpo injection
5% FDR significant and ≥ 1.5FC: Post rHuEpo

**SCO (n=18)**

- **Post 2 weeks**
  - 249 (7↑ 242↓)

- **Post 4 weeks**
  - 139 (0↑ 139↓)

- 114
- 135
- 4

**KEN (n=18)**

- **Post 2 weeks**
  - 187 (4↑ 183↓)
  - 168 (44↑ 124↓)

- **Post 4 weeks**
  - 31 (0↑ 31↓)

- 156
- 31
- 0
- 151
- 17
- 12

- 135 (↓)
- 31 (↓)
- 17
Future applicability?

The Athlete Biological Passport?
Athlete **biological passport?**

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**A**

**HGB**

- seq HGB: prob = 73%

**OFFS**

- seq OFFS: prob = 78%

**ABPS**

- seq ABPS: prob = 10%

**RET%**

- seq RET%: prob = 64%
Athlete biological passport?
Conclusions

- The first “molecular signature” of rHuEpo doping has been discovered.

- These results provide the strongest evidence to date that “OMICS” technologies such as gene expression have the potential to add a new dimension to currently applied ABP in terms of specificity and sensitivity for drug detection, not confined to rHuEpo.

- These very encouraging results serve to strongly reinforce the feasibility and need for this complex, expensive and technically demanding approach involving leading industry partners for the development of greatly improved detection methods.
“To those who devote their life to science, nothing can give more happiness than increasing the number of discoveries, but .. the cup of joy is full when the results of these studies immediately find practical applications”

Dr Louis Pasteur (1822-1895)
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