Erythropoietin, Erythropoiesis and Chronic Mountain Sickness (CMS)

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  Oxygen sensing mechanisms

- Methods

- Human studies on EPO, sTfR
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  **Hypobaric-Hypoxic Environment**
  Short, intermittent, and long-term exposure

  **Chronic Mountain Sickness (CMS)**

- Summary and Conclusions
Erythropoietin, Erythropoiesis and CMS

- **Introduction**

- **Exercise Physiology**
  - Marathon running
  - Survival training

- **Tropics/Desert**
  - Gold Mining

- **Humans in Extreme Environments**
  - Palaeophysiology
  - Evolutionary Anthropology
  - Comparative Physiology

- **Exercise Physiology**
  - Palaeophysiology
  - Evolutionary Anthropology
  - Comparative Physiology

- **History of Applied Science**
  - Nathan Zuntz (1847 – 1920)
  - Operation „Paperclip“

- **High Altitude**
  - Moderate Altitude (<2,300 m)
  - High Altitude (>3,000 m)

- **Micro-G (Space)**
  - Simulated Micro-g
  - Real Micro-g
  - Space (near Earth orbit)

- **Survival training**

- **Micro-G (Space)**
  - Bed rest
  - HDT
  - Isolation
  - Immersion

- **Real Micro-g**
  - Space (near Earth orbit)
The relationship of hominids to modern humans and African apes.

Robust (R) and gracile (G) lineages are indicated.
Rift Valley, Human Evolution, and Altitude
Hochachka PW, Gunga HC & Kirsch K.  
Our ancestral physiological phenotype: An adaptation for hypoxia tolerance and for endurance performance?  
Erythrocytes in different Species

Human red blood cells (7.5 μm; PCV 44% ~ 3.200 m²; Blood volume in mammals 6-8% - diving mammals > 8% of body mass)

Withers PC. Comparative animal physiology. Saunders 1992
Signal Transduction Pathway - Hypoxia Inducible Factor (HIF-1 alpha)
Erythropoietin

- **Erythropoietin (EPO)** is a glycoprotein hormone.

- **EPO** is produced by specialized cells in the kidneys (90 %) and the liver (10 %).
Erythropoietin and Erythropoiesis

- **Stammzellen-pool**
- **Proliferations-Kompartment**
  - CFU-GM
  - CFU-MK

- **Reifungs-Kompartment**
  - Lymphozyten-Reihe
  - Granulozyten / Monozyten-Reihe
  - Thrombozyten-Reihe

- **Erythrozyten-Reihe**
  - BFU-E
  - CFU-E
  - Erythroblast
  - Retikulozyt
  - Erythrozyt

- **CFU = Kolonie bildende Einheit**
- **CFU-GEMM = Kolonie bildende Einheit der Granulozyten-, Erythrozyten-, Monozyten-, Megakaryozyten-Reihe**
- **GM = Granulozyten-Monozyten-Reihe**
- **MK = Megakaryozyten-Reihe**
- **IL-3 = Interleukin 3**
- **IL-6 = Interleukin 6**
- **Multi-CSF = multipotentiel Kolonie bildender Faktor**
- **GM-CSF = Granulozyten, Makrophagen Kolonie bildender Faktor**
- **E = Erythrozyten-Reihe**
- **BFU = "burst" bildende Einheit**
- **Erythropoietin**
Physiological variables in the etiology of CMS

CMS/EE:
Severe Hypoxemia
Pulmonary hypertension
Erythrocytosis
Consensus Symptoms....
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- Summary and Conclusions
ELISA Tests

• **EPO (IBL)**
  Median: 12 ml U/ml    Range: 4 - 36 ml U/ml

• **sTfR (R&D)**
  Caucasians (living <300 m above sea-level)
  Median: 18.4 nmol/l    Range: 8.7 – 28.1 nmol/l
Transferrin and Transferrin-receptor

- **Transferrin** is a transport protein for iron (Fe).

- The diferric (2*Fe) transferrin is binding to TfR.

- During the phase of cellular proliferation iron is incorporated into the interior of the erythroblast.

- This is important for the **synthesis of hemoglobin**.
Transferrin Receptor

- Transferrin Receptor (TfR) is a transmembranal protein, expressed mainly on the surface of erythroid cells.
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  - **Chronic Mountain Sickness (CMS)**

- **Summary and Conclusions**
Circadian Variations of Serum Erythropoietin

Erythropoietin under real and simulated microgravity conditions in humans.
Erythropoietin during Isolation and Confinement

Erythropoietin under real and simulated microgravity conditions in humans.
Annual variations of serum erythropoietin concentrations

Erythropoietin before, during and after moderate hypobaric-hypoxic exposure (2,315 m)

Erythropoietin, Erythropoiesis and CMS

Erythropoietin and intermittent hypobaric–hypoxic exposure (3,600 m)

Shift working in the Chilean Andes (3,600 m) and its influence on erythropoietin and the low-pressure system.
The distribution of hematocrit values at sea level, Cerro de Pasco, and Morococha

Winslow and Monge, 1987
Gender differences in four common high altitude medical conditions as reported in the literature by Hultgren (1997)
Erythropoietin, Erythropoiesis and CMS

**Age distribution**

N = 105

**Females (N = 34)**
63.5 ± 9.4, min 37.0, max. 80 yrs

**Males (N = 71)**
62.1 ± 11.0, min 35.0, max 89.0 yrs

**Inclusion criteria:**
- Whole life at high altitude: (3,600 - 4,100)
- Excessive erythrocytosis: [Hct > 60 %]
Differences between females and males for Hct ($p=0.011$) and Hb ($p=0.011$)
No differences between females and males for age, EPO and sTfR
No correlation between the hematological parameters (Hct, Hb) and EPO
No correlation between the hematological parameters (Hct, Hb) and sTfR

[Graph showing scatter plots comparing sTf-R levels with Hct and Hb for males and females.]
No correlation between age, EPO, and sTfR
No correlation between EPO and sTfR in the majority of the subjects with high sTfR
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- Summary and Conclusions
• **Males** are more frequently affected by EE than **females**

• **Males** with EE show higher **Hct** and **[Hb]**

• **High [sTfR]** in the majority of the subjects with EE but no correlation to **[EPO]**

• Effects of **blood letting** on circulating **[EPO]** have to be clarified in patients with EE

• Effects of **iron status/metabolism** on hematopoiesis in patients with EE are unclear
Physiological variables in the etiology of CMS

Altitude

Decreased HVR

Sleep desaturation

Hypoxia

Impaired $O_2$ supply

Pulmonary hypertension

Polycythemia

Impaired lung function

Increased systemic resistance

RV failure

LV failure

Decreased cardiac output
Changes induced by decreasing the hematocrit in eight patients with CMS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Posthemodilution</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>67%</td>
<td>49%</td>
<td>-27%</td>
</tr>
<tr>
<td>Maximum work</td>
<td>475 Kpm</td>
<td>638 Kpm</td>
<td>+ 34%</td>
</tr>
<tr>
<td>Maximum heart rate</td>
<td>138/min</td>
<td>162/min</td>
<td>+ 17%</td>
</tr>
<tr>
<td>Maximum ventilation</td>
<td>53 L/min</td>
<td>52 L/min</td>
<td>no change</td>
</tr>
<tr>
<td>PA pressure</td>
<td>40/30 mm Hg</td>
<td>23/15 mm Hg</td>
<td>-43/50%</td>
</tr>
<tr>
<td>Ventilatory threshold</td>
<td>0.793 L/min</td>
<td>1.005 L/min</td>
<td>+27%</td>
</tr>
<tr>
<td>O₂ saturation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rest</td>
<td>80%</td>
<td>85%</td>
<td>+ 6%</td>
</tr>
<tr>
<td>exercise</td>
<td>71%</td>
<td>70%</td>
<td>no change</td>
</tr>
<tr>
<td>Cardiac output</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rest</td>
<td>4.1 L/min</td>
<td>5.4 L/min</td>
<td>+32%</td>
</tr>
<tr>
<td>exercise</td>
<td>4.6 L/min</td>
<td>9.6 L/min</td>
<td>+109%</td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rest</td>
<td>93/min</td>
<td>109/min</td>
<td>+17%</td>
</tr>
<tr>
<td>exercise</td>
<td>106/min</td>
<td>132/min</td>
<td>+25%</td>
</tr>
<tr>
<td>A-V difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rest</td>
<td>15.0 ml/100 ml</td>
<td>9.0 ml/100 ml</td>
<td>-40%</td>
</tr>
<tr>
<td>exercise</td>
<td>17.9 ml/100 ml</td>
<td>11.3 ml/100 ml</td>
<td>-37%</td>
</tr>
</tbody>
</table>

Winslow, Statham and Gibson, 1979
Arterial oxygen saturation ($\text{SaO}_2$) in long-term high altitude residents while awake (A) and during stages of sleep (1-REM) at 3,100 m

During sleep hypoxemia occurs in polycythemics but not in normals. Ear oximeter data.

Kryger and Grover, 1983
Arterial PCO$_2$ in normal high altitude residents (N=159) and patients with CMS (N=22)

Differences in the height of the curves represent different numbers of subjects and patients studied.

Cruz and Recavarren, 1982
Mean nocturnal $O_2$ saturation (A) and apnea-hypopnea index (B) in groups of patients before and after treatment with 250 (D250) or 500 (D500) mg of Acetazolamide compared with a CMS placebo-treated group (Placebo) and normal control subjects (Nb = number of events)
Erythropoietin during long-term simulated micro-g conditions (Head-down tilt –6°)

Erythropoietin under real and simulated microgravity conditions in humans. 
*Journal of Applied Physiology 81: 761-773 (1996)*
Modulation of Erythropoietin Regulation in Dogs

Ehmke J, Just A, Eckhardt KU, Persson PB, Bauer C & Kirchheim HR.
Modulation of erythropoietin formation by changes in blood volume in conscious dogs.
Fig. 3. Serum EPO in relation to blood hemoglobin (Hb) levels in nonanemic persons. Hb was 138 g/L (± 11) and EPO 8.6 U/L (± 5.2) in females (n= 19) and 158 g/L (± 13) and 8.5 U/L (± 3.9) in males (n = 23, 1 outlier disregarded; mean ± SD). EPO was measured by ELISA as described (138).
### Table 2. Hematocrit (%) and Hemoglobin (g/dL Whole Blood) Values by Region, Sex, and Altitude (Mean ± Standard Deviation, SD) and Sample Size (N).

<table>
<thead>
<tr>
<th>Region</th>
<th>Altitude, m</th>
<th>Reference</th>
<th>N</th>
<th>Hematocrit, +2SD</th>
<th>Hemoglobin, +2SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>South American Males</td>
<td>3700 Ch</td>
<td>Winslow et al. (1989)</td>
<td>29</td>
<td>52.2 ± 4.6</td>
<td>18.0 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>3800-4065 Bo</td>
<td>Beall et al. (1998)</td>
<td>91</td>
<td>19.1 ± 0.2</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>4000 Bo</td>
<td>This study</td>
<td>1086</td>
<td>52.7 ± 3.9</td>
<td>17.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>4200 Peru</td>
<td>Garruto and Dutli (1983)</td>
<td>45</td>
<td>51.4 ± 3.9</td>
<td>17.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>52.1 ± 60.3</td>
<td>17.9 ± 20.7</td>
</tr>
<tr>
<td>Females</td>
<td>3800-4065 Bo</td>
<td>Beall et al. (1998)</td>
<td>83</td>
<td>48.3 ± 3.7</td>
<td>15.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>4000 Bo</td>
<td>This study</td>
<td>848</td>
<td>47.4 ± 4.1</td>
<td>15.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>4300 Peru</td>
<td>Leon-Velarde et al. (1997)</td>
<td>112</td>
<td>47.4 ± 4.1</td>
<td>15.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>47.8 ± 55.6</td>
<td>16.8 ± 18.8</td>
</tr>
</tbody>
</table>

### Himalayas

| Males          | 3000 Tib     | Moore, unpub.                  | 18  | 50.3             | 15.2             |
|                | 3560 Nep     | Beall and Reichman (1984)      | 126 | 54.4             | 17.4             |
|                | 3700 Tib     | Moore, unpub.                  | 22  | 48.4 ± 4.5       | 16.9 ± 1.2       |
|                | 3700 Nep     | Winslow et al. (1989)          | 30  | 57.4             | 15.6 ± 0.2       |
|                | 3800-4065 Tib| Beall et al. (1998)            | 75  | 55.9             | 17.3             |
|                | 4500 Tib     | Moore, unpub.                  | 47  | 55.9             | 17.3             |
|                | 4850-5350 Tib| Beall (1997)                   |     | 52.2 ± 18.9      | 16.7 ± 18.9      |
| Average        |              |                                |     | 52.2 ± 16.7      | 18.9             |

| Females        | 3000 Tib     | Moore, unpub.                  | 11  | 46.2             | 14.0             |
|                | 3560 Nep     | Beall (1984)                   | 100 | 45.3             | 13.9             |
|                | 3700 Tib     | Moore, unpub.                  | 24  | 54.0             | 16.5             |
|                | 3800-4065 Tib| Beall et al. (1998)            | 61  | 14.2 ± 0.1       | 14.4             |
|                | 4500 Tib     | Moore, unpub.                  | 10  | 16.5             | 16.5             |
|                | 4850-5350 Tib| Beall (1997)                   | 56  | 16.7 ± 1.5       | 19.7             |
| Average        |              |                                |     | 48.5 ± 15.0      | 17.1             |

Abbreviations: Ch, Chile; Bo, Bolivia; Tib, Tibet; Nep, Nepal.

The morphology of red blood cells at high altitudes

(Winslow and Monge, unpublished data.)
### Red-cell indexes in subjects at low and high altitudes

<table>
<thead>
<tr>
<th></th>
<th>Sea-level Caucasian</th>
<th>Sea-level Indian</th>
<th>La Oroya Indian (3,700m)</th>
<th>Morococha Indian (4,500m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 175</td>
<td>N = 25</td>
<td>N = 40</td>
<td>N = 32</td>
</tr>
<tr>
<td>Rbc (10^6/mm)</td>
<td>5.14 ± .34</td>
<td>5.00 ± .32</td>
<td>5.67 ± .39</td>
<td>6.15 ± .57</td>
</tr>
<tr>
<td>Retic (%)</td>
<td>.5 ± .3</td>
<td>---</td>
<td>.8 ± .5</td>
<td>1.5 ± .6</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>46.8 ± 2.3</td>
<td>44.0 ± .3</td>
<td>54.1 ± 3.9</td>
<td>59.9 ± 5.6</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>16.0 ± .8</td>
<td>14.7 ± 1.0</td>
<td>18.8 ± 1.5</td>
<td>20.8 ± 1.7</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>91.3 ± 4.5</td>
<td>88.0 ± 4.6</td>
<td>95.2 ± 5.5</td>
<td>97.5 ± 6.3</td>
</tr>
<tr>
<td>MCH (pg/cell)</td>
<td>31.2 ± 1.9</td>
<td>29.6 ± 2.2</td>
<td>33.0 ± 2.4</td>
<td>33.9 ± 2.5</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.1 ± 1.4</td>
<td>33.4 ± 1.2</td>
<td>34.8 ± .9</td>
<td>34.7 ± 1.0</td>
</tr>
</tbody>
</table>

*Source*: Hurtado, Merino, and Delgado 1945.

*Note*: rbc = red blood cells, Retic = reticulocytes, MVC = mean cell volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration.
Two main Groups: “normal” [EPO] (< 36 U·l⁻¹) and with very high [EPO] (> 100 U·l⁻¹). What about the “intermediates” (36 ≤ [EPO] ≤ 100)
At least two groups with excessive erythrocytosis (EE):

- "normal" [EPO] (< 36 U·l⁻¹)
- very high [EPO] (> 100 U·l⁻¹)

No studies before evaluated “normal” reference levels for [EPO] and [sTfR] in altitude residents

→ can the published cut-off values be adopted?
Effect of high altitude (4,300 m) on hematocrit levels of men and woman

Hannon et al. 1966
Normal hematocrit values at sea level as a function of age for 7,426 normal males and 7,704 normal females in the United States

(Fulwood 1982)
Histograms of showing frequency (counts) for hematocrit values and the mean and 2 standard deviation (SD) values observed in 1934, 15- to 29 healthy male and female residents in Potosi, Bolivia (4,000 m)

Vásquez R., and Villena M.
Normal Hematological Values for Healthy Persons Living at 400 Meters in Bolivia.