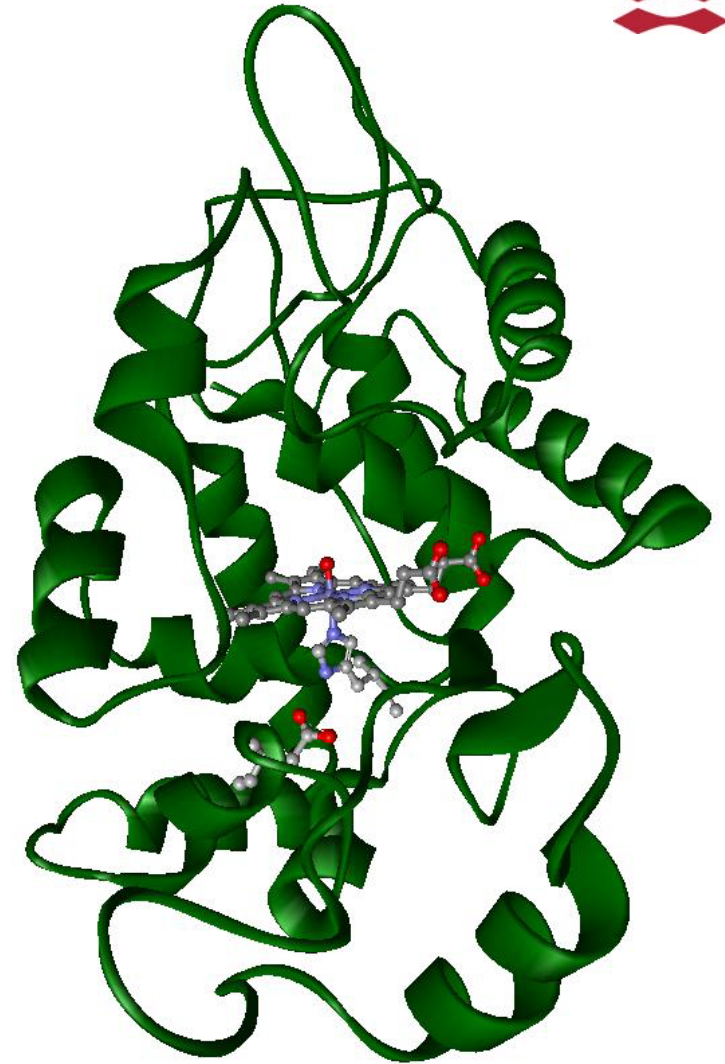


Oxygen varieties and delivery

Dr. Kasper P. Jensen

Technical University of Denmark, Chemistry – DTU

DK-2800 Kongens Lyngby, Denmark. E-mail: kpj@kemi.dtu.dk



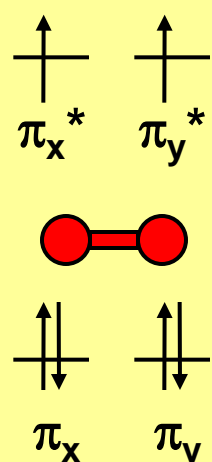
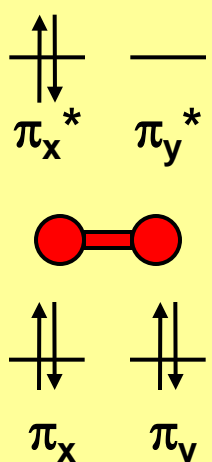
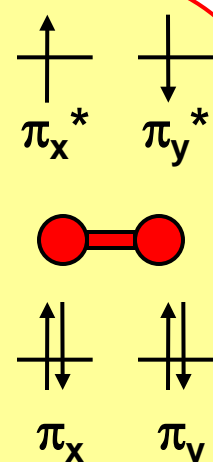
Purpose of talk

**To provide insight into O₂-varieties, O₂-transport, and O₂-activation
relating to performance enhancement in sports**

Overview

- **Oxygen forms – Triplet and singlet oxygen**
- **Oxidative metabolism and activation of O₂**
- **Binding of O₂ to hemoglobin and myoglobin**
- **Reactive Oxygen Species (ROS)**
- **Manipulation of O₂-delivery to cells (blood doping & beyond)**
- **AIRENERGY[®] and similar devices**
- **Ozonisation**
- **Conclusions**

Oxygen forms – Triplet and singlet oxygen

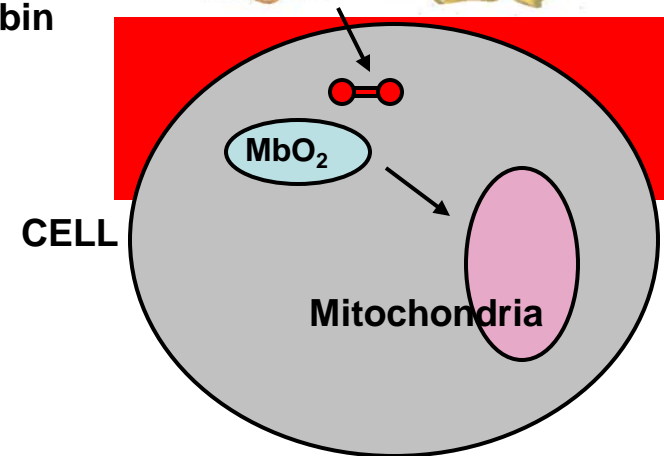
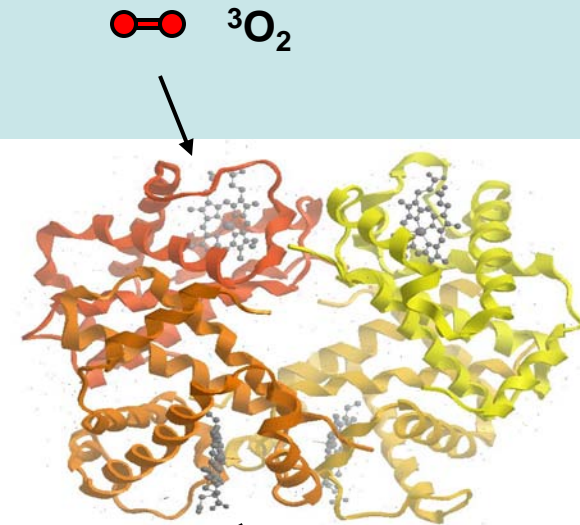
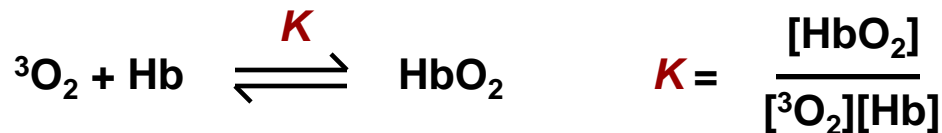
<p>ELECTRONIC STRUCTURE</p>			
<p>NAME</p>	<p>TRIPLET OXYGEN</p>	<p>SINGLET OXYGEN</p>	
<p>SYMBOLS {</p>	<p>${}^3\text{O}_2$</p> <p>${}^3\Sigma_g^-$</p>	<p>${}^1\text{O}_2$</p> <p>${}^1\Delta_g$</p>	<p>${}^1\text{O}_2'$</p> <p>${}^1\Sigma_g^+$</p>

Oxidative metabolism and activation of O₂

MOLECULAR OXYGEN = ³O₂

21% OF ATMOSPHERIC GAS BY WEIGHT

- When ³O₂ is inhaled into the lungs, it passes into small blood vessels (*capillaries in the alveoli*) and binds to **Hemoglobin** in blood.
- Hemoglobin circulates O₂ to the working cells where [³O₂] is low.
- Most of the ³O₂ is taken up in target cells by **Myoglobin**. Myoglobin transports O₂ to the mitochondria where [³O₂] is even lower.^[1]



^[1] Wittenberg, J. B.; Wittenberg, B. A. *J. Exp. Biol.* **2003**, *206*, 2011-2020.

Oxidative metabolism and activation of O₂

- O₂ diffuses across mitochondrial membrane.
- The tricarboxylic acid cycle produces ATP necessary to perform muscular activity

Improvement in quantity (constant K)

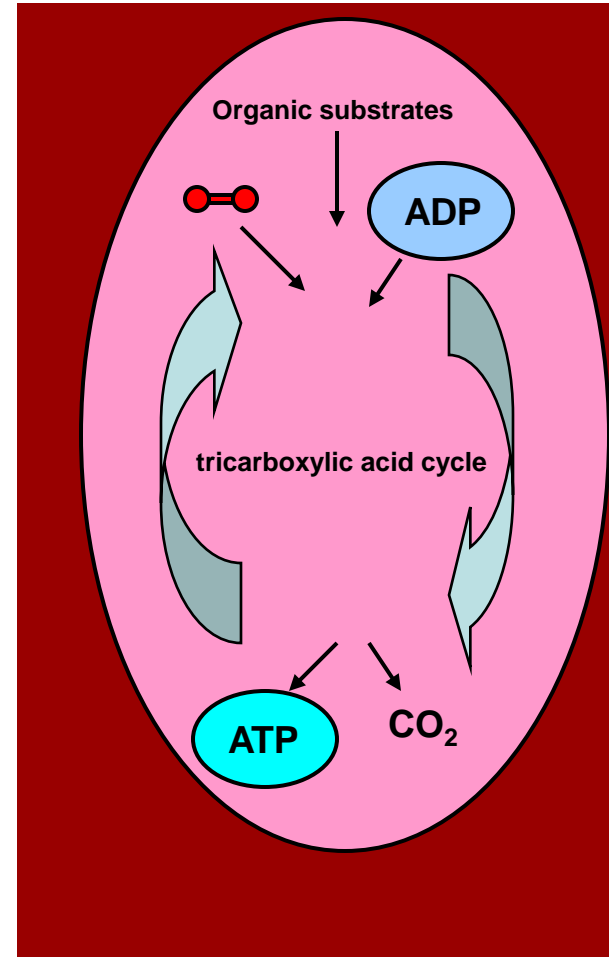
Increase [³O₂] or [Hb] or [Mb], thereby increasing [MbO₂]

Improvement in quality (changing K)

More efficient transport. Changing K, changing conditions (pH), or changing chemical nature of O₂ or carrier.

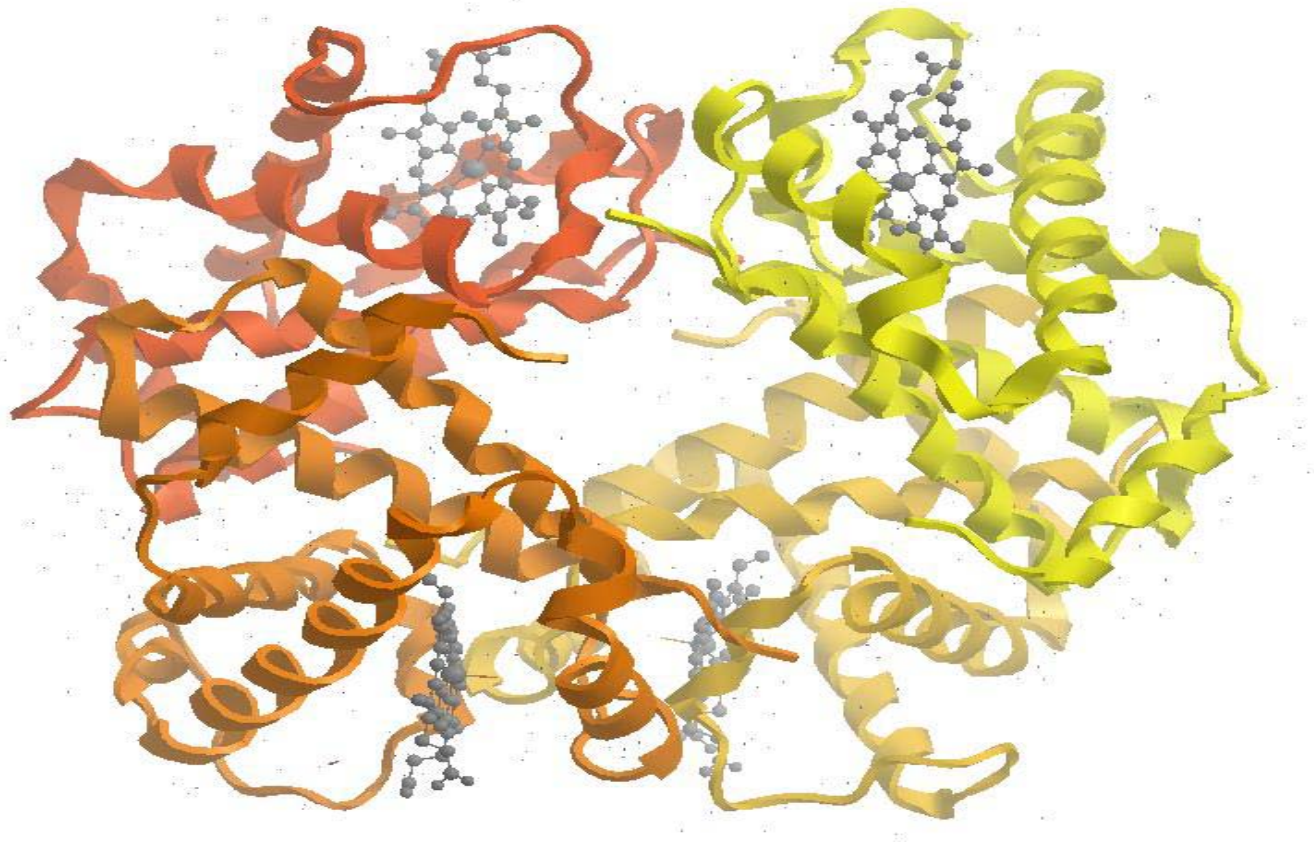
(The 'active oxygen' concept)

Mitochondria



Binding of O₂ to hemoglobin and myoglobin

Hemoglobin constitutes most of the dry weight of red blood cells and contains four identical subunits that work together to take up ³O₂^[1].

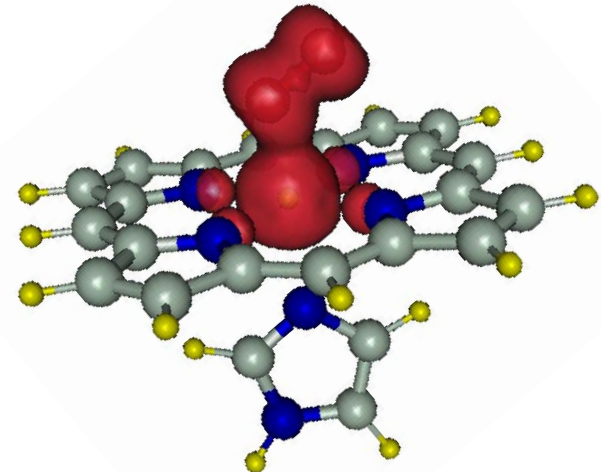
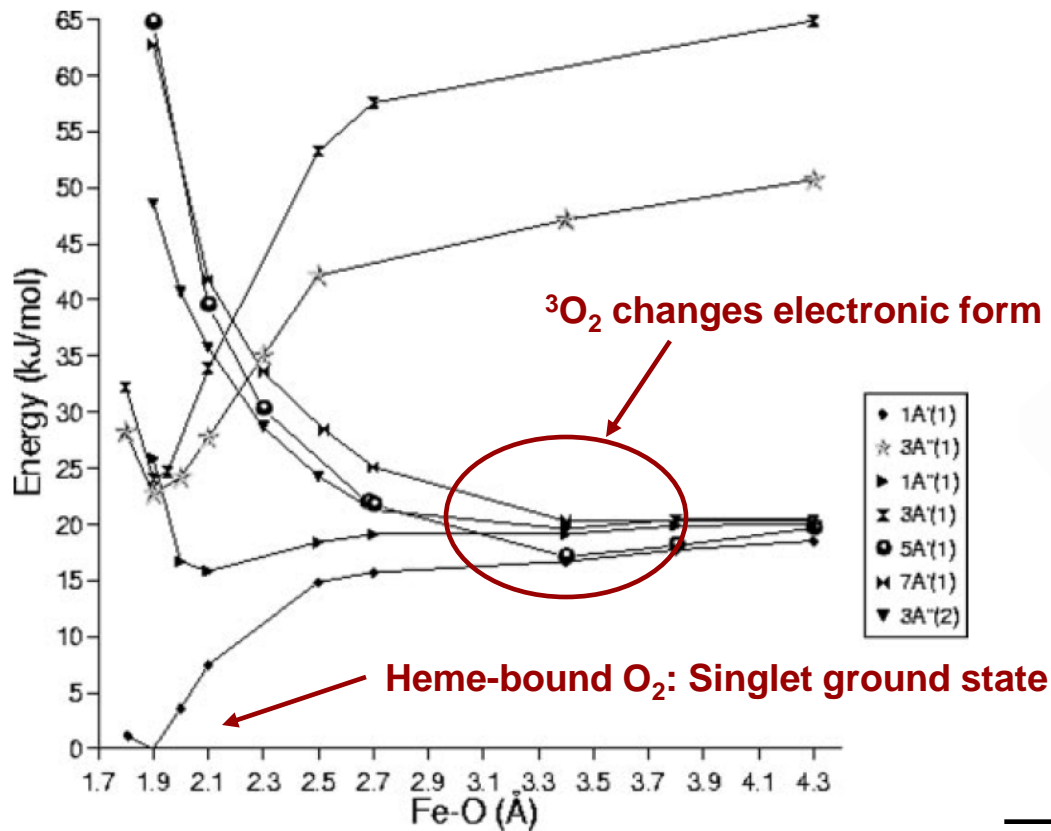


^[1] Baldwin, J.; Chothia, C. *J. Mol. Biol.* **1979**, *129*, 175-220.

Binding of O₂ to hemoglobin and myoglobin

Heme “circumvents” the Pauli Principle by bringing spin states close together.

³O₂ is only 10 kJ/mol higher than ¹O₂ in heme. The states convert at physiological time scales.



Binding of O₂ to hemoglobin and myoglobin

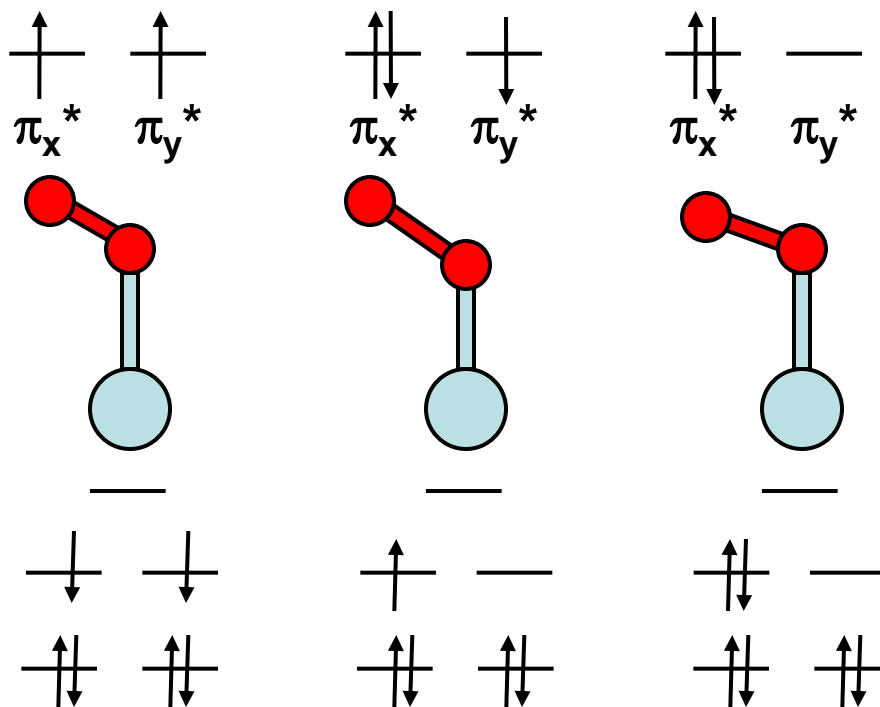
Three electronic forms explain the diamagnetic nature of heme-bound O₂:

The **McClure state**: $^3\text{Fe}^{\text{II}} - ^3\text{O}_2$ [1][2]

the **Weiss state**: $^2\text{Fe}^{\text{III}} - ^2\text{O}_2^-$ [3]

the **Pauling state**: $^1\text{Fe}^{\text{II}} - ^1\text{O}_2$ [4][5]

The most correct description
is a mixture of Weiss and Pauling [6][7]



Fe d-orbitals

[1] McClure, D. S. *Radiat. Res. Suppl.* **1960**, 2, 218-242.

[2] Olafson, B. D.; Goddard, W. A. *Proc. Natl. Acad. Sci. USA* **1977**, 74, 1315-1319.

[3] Weiss, J. J. *Nature* **1964**, 202, 83-84.

[4] Pauling, L.; Coryell, C. D. *Proc. Natl. Acad. Sci. USA* **1936**, 22, 210-216.

[5] Pauling, L. *Nature* **1964**, 203, 182-183.

[6] Jensen, K. P.; Ryde, U. *J. Biol. Chem.* **2004**, 279, 14561-14569.

[7] Jensen, K. P.; Roos, B. O.; Ryde, U. *J. Inorg. Biochem.* **2005**, 99, 45-54.

Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are toxic by-products of oxidative metabolism.

Examples include:

- singlet oxygen $^1\text{O}_2$
- superoxide O_2^-
- hydrogen peroxide H_2O_2
- hydroxyl radical OH^\bullet
- ozone O_3

All of these are converted to $^3\text{O}_2$ and water by a variety of oxidative stress enzymes.

The life time of $^1\text{O}_2$ is up to ca. 3-4 microseconds ($4 \times 10^{-6} \text{ s}$)^{[1][2][3]} – it may diffuse 200 nm.

^[1] He, Y. Y.; Council, S. E.; Feng, L.; Bonini, M. G.; Chignell, C. F. *Photochem Photobiol.* **2008**, *84*, 69–74.

^[2] Hatz, S.; Lambert, J. D. C.; Ogilby, P. R. *Photochem. Photobiol. Sci.* **2007**, *6*, 1106-1116.

^[3] Redmond, R. W.; Kochevar, I. E. *Photochem. Photobiol.* **2006**, *82*, 1178-1186.

Manipulation of O₂-delivery to cells (blood doping & beyond)

From the WADA list of prohibited methods:

M1. ENHANCEMENT OF OXYGEN TRANSFER

1. Blood doping
2. Artificially enhancing the uptake, transport or delivery of oxygen

AIRNERGY® and similar devices

CLAIMS IN THE LITERATURE

- **Airnergy® can utilize the energy of singlet oxygen to increase performance.**^{[1],[2],[3]}
- **Airnergy® works ‘similar to photosynthesis’, and $^1\text{O}_2$ is the ‘physiological active form of oxygen’.**^[1]
- **Users do not inhale $^1\text{O}_2$ produced in the device, ‘but only its energy’.**^[1]
- **Photosensitization in the device causes formation of $^1\text{O}_2$, which relaxes within milliseconds in contact with water, forming $^3\text{O}_2$ again and transferring excess energy to water.**^[1]
- **Activated water molecules (not activated oxygen states) are inhaled.**^[1]
- **‘These highly energetic water molecules are a form of energy known to the organism, which, consequently, it can utilise optimally.’**^[1]
- **Exhaled $^3\text{O}_2$ in 19 test persons decreased by 9.9% after use of Airnergy® for 20 minutes.**^[1]
- **Breathing and pulse frequencies reduced by 12.9 and 6.5%.**^[1]
- **CO_2 exhalation simultaneously decreased ”significantly”.**^[3] (not cited in later material)

^[1] Schöllmann, C. *Ärztezeitschrift für Naturheilverfahren* **2004**, 45, 2-7.

^[2] Hulten, L. M.; Holmström, M.; Soussi, B. *Free Rad. Biol. Med.* **1999**, 27, 1203-1207.

^[3] Rauhala, E. ‘Some physiological effects of breathing singlet oxygen activated air. An experimental pilot study with ergospirometry’, Fitness Clinic of Helsinki, Finland, **1995**.

AIRNERGY® and similar devices

FURTHER CLAIMS IN SALES MATERIAL AND OTHERWHERE

- **‘Metabolism is optimised and regeneration processes are triggered’.**^[1]
- **‘The oxygen in the air is converted into a form that is more “suitable for the body” and can be better utilised by the organism.’**^[1]
- **‘It is still not completely clear how the energy of the water molecules is transformed into metabolic energy. Many findings would suggest that the energy-rich water molecules carry their energy/information to points in the body where the organism activates oxygen itself’.**^[1]
- **The positive effects of Airnergy can be used for many different chronic illnesses such as:**^[1]

Circulatory problems - Heart and vascular illnesses – Exhaustion - Chronic tiredness - Diabetes mellitus types I and II - Multiple Chemical Syndrome (MCS) - Burnout syndrome - High cholesterol - Bronchitis, lung diseases - Chronic fibromyalgia - Rheumatic complaints - Visual problems - Acute and chronic pain - Metabolic disorders of the liver - Skin diseases - Sleeping problems - Lack of concentration - High blood pressure – Allergies - Age-related illnesses (Parkinson’s, Alzheimer’s) - Tinnitus

- **Airnergy increases ATP production, regenerates oxygen utilisation, increases anti-oxidative capacity. ‘Airnergy is also ideal [...] to generate an anti-aging effect’.**^[1]

^[1] Schöllmann, C. “New Air Therapy Sets Benchmarks”. Not dated.

AIRNERGY® and similar devices

FURTHER CLAIMS IN SALES MATERIAL AND OTHERWISE

- **‘The water-molecules in the humidity of the air transfer the energy produced in the catalysers into the lungs.’^[1]**
- **12 of 15 patients with sleep apnoea (pauses in breathing during sleep) subject to Airnergy reported improved conditions after use of Airnergy, by questionnaire.**

No placebo control was performed.^[2]

^[1] Klemm, J. “The Basics to Understand the Idea of Airnergy-Therapy and Technology”, communique, **2006**.

^[2] “Burmam-Urbaneck, M.; Straube, H. “Airnergy Oxygen Therapy is Tested”, Das Schlafmagazin, **2004**, 3.

AIRNERGY[®] and similar devices

CRITICISM

- **Oxygen content in inhaled humid air from Airnergy[®] device was not measured.**
- **Controls for placebo were not been achieved.**
- **Several statements are misleading.**
- **Many statements are not backed by scientific investigations.**
- **The lower exhalation of CO₂ is not cited, whereas other data are cited, in sales material.**
- **Lower exhalation of CO₂ is not consistent with better O₂-utilisation only, but implies lower respiratory activity**
- **Heated or energy-rich water molecules claimed to carry the Airnergy effect, do not in any known way affect oxygen utilisation.**
- **Lowered production of ROS is not consistent with better utilisation of O₂ at same-level O₂ metabolism, but implies reduced O₂-metabolism.**

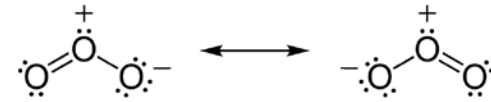
AIRNERGY® and similar devices

ALTERNATIVE EXPLANATIONS

- **Singlet oxygen relaxes to triplet oxygen and gives off rotational, translational, and vibrational energy to water molecules in the device, i.e. heating of the humid air.**
- **Absorption of O₂ in H₂O decreases at higher temperature:^[1] 8.4 at T = 25°C, 6.4 at T = 40°C.**
- **Oxygen content is smaller in hot, humid air compared to room-temperature, dry air.**
- **Humid air leading to lower respiratory activity can explain the data, including:**
 - **the 10%-decrease in exhaled O₂**
 - **the “significant” reduction of exhaled CO₂**
 - **the reduced breathing and pulse frequencies**
 - **claims of lowered production of ROS**
- **If Airnergy® has beneficial effects, it is most likely due to:**
 - **lowered intake of O₂ for extended periods of time, and/or**
 - **placebo effects**

^[1] See for example: water.usgs.gov/owq/FieldManual/Chapter6/table6.2_6.pdf

Ozonisation



- It has been suggested^[1] that ozone, O₃, may also be formed as a ROS in organisms; this suggestion has been questioned.^{[1],[2]}
- It has been suggested that O₃ has performance-enhancing and medically beneficial effects.^[3] Most likely, this is due to controlled oxidative stress triggering regulative mechanisms.^[3]
- It is established that O₃ damages tissue and constitutes a substantial health effect,^[4] causing destructive oxidation of hemoglobin^[5] and vitamin E depletion.^[6]
- Lack of consensus and substantial controversy persist.
- Any device claiming to utilise ozonisation of blood for enhancement of performance can be distinguished according to whether the device is in fact 'innocent', as Airnergy[®], or involves *de facto* addition of activated oxygen in some form to the blood.

^[1] Wentworth Jr., P. et al. *Science* **2003**, *302*, 1053-1056.

^[2] Sies, H. *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 3514-3515.

^[3] Bocci, V.; Borrelli, E.; Travagli, V.; Zanardi, I. *Med. Res. Rev.* **2009**, *29*, 646-682.

^[4] Pryor, W. A.; Squadrito, G. L.; Friedman, M. *Free Rad. Biol. Med.* **1995**, *19*, 935-941.

^[5] Fukunaga, K.; Nakazono, N.; Suzuki, T.; Takama, K. *IUBMB Life* **2008**, *48*, 631-634.

^[6] Thiele, J. J.; Traber, M. G.; Polefka, T. G.; Cross, C. E.; Packer, L. J. *Invest. Dermatol.* **1997**, *108*, 753-757.

Conclusions

- $^1\text{O}_2$ (singlet oxygen) and O_3 (ozone) are reactive oxygen species with serious health risks.
- Enhanced O_2 -delivery to mitochondria can occur by increasing $[\text{O}_2]$, [carrier], or K
- There are no indications that an 'active' form of oxygen can enhance performance by O_2 -delivery to the mitochondria.
- A device such as Airnergy[®] does not chemically affect the body and is unlikely to have any performance-enhancing effect beyond placebo.
- It is thus suggested, based on the material available, that there is no basis for specifically incorporating Airnergy[®] and similar devices into the prohibited list.
- Ozone therapy or other direct infusion of ROS cause oxidative stress in the body.
- Events occur at gene-regulation level, which may be very damaging or possibly beneficial to the organism, depending on therapeutic window. As yet, no consensus exists.
- In such cases, the infused ROS is *not* delivered to the mitochondria.