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**Some physiological effects of breathing singlet oxygen activated air.
An experimental pilot study with ergospirometry**

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Summary

A simple singlet oxygen producing device can be used to strengthen the physical power in athletics. The device activates air oxygen so that even a short term exposure by breathing produces physiological changes in blood chemistry. Singlet oxygen as such is not the exposing agent. This in turn enables better oxygen uptake, and increased muscular power output. Oxygen consumption increase with 7%, energy production with 8%. Both carbon dioxide and lactate production decreased 7%. Energy production seems to become more aerobic. The results are based on statistical ergospirometry data from ten male volunteers. The results are preliminary and should be verified by other studies.

Introduction

Air oxygen is needed for respiration in all aerobic living organisms. Oxygen content of the atmosphere has been fairly stable for millions of years. Living organisms have adapted their metabolism to current oxygen level. The effects of oxygen on physiological parameters and functions have been extensively studied during many decades in western medical sciences. Main features of today's physiology are connected to reductive and oxidative cell processes, oxidative biochemistry.

Anyhow, oxygen biochemistry seems still to be an unexhaustible reservoir to new studies extending from cancer research to basics of space medicine. Still, most oxygen biochemistry of cell metabolism as a dynamic and control variable, is poorly understood. Oxygen is needed in right amounts e.g. in energy production, immunological defense processes, and cell mitogenesis. In some clinical diseases breathing extra oxygen may be health promoting, e.g. in restrictive lung diseases and diver's pressure disease.

Introducing excess oxygen or activated oxygen species, like ozone, into the body, may be hazardous to health. This is known from air pollution periods in large cities or industry areas when near ground level ozone concentrations are high (above approx. 100 $\mu\text{g}/\text{m}^3$). In the medical sciences in some parts of the world excess oxygen therapy has been used for some decades to treat conditions like infectious skin diseases and deep tissue infections of bacterial or viral origins. With excess oxygen has been tried to cure HIV and other treatment resistant states. Results have been equivocal (Carpendale and Freeberg, 1991).

Singlet oxygen is excited but non-radical form of dioxygen (O_2) (see references). Singlet oxygen is a very short lived activated oxygen species. Investigations at the universities of Helsinki and Dusseldorf have confirmed the formation of singlet oxygen in the activating unit, used in the VALKION equipment of this study. The conclusion of these studies was that no singlet oxygen can enter the body when using the device for breathing. As the singlet state of oxygen is deexcited, probably some extra energy is left in the oxygen molecule, e.g. in the form of molecular rotation or vibration. This proposition should be verified in future studies.

Athletics have used oxygen breathing to improve physical fitness, muscle strength and endurance. Our interest has been to understand the effects of the basic mechanisms of activated oxygen (singlet oxygen from room air) on cell metabolism and cell

functions, relevant to sports medicine and some pathologic conditions.

Body's energy metabolism

To produce energy the cells need oxygen and some substrate molecules (food stuff). Food stuff contains energy sources like carbohydrates, fats (lipids), and proteins. These energy source molecules are converted (in cells) into right type of molecules to be utilized in many energy producing processes (in cells). The consumption of oxygen in cells is called aerobic respiration.

Aerobic cell respiration produces ATP-molecules, which in turn can be used as energy source for metabolic processes. ATP-bound chemical energy is transformed to mechanical work in skeletal muscles. ATP, adenosine 5-triphosphate is an energy source for every living cell. Adipose tissue, approx. 15% of the body weight, is the main site of long term energy source. Liver is to balance energy metabolism from various chemical forms (carbohydrates, triglycerides, and proteins) by enzymatic cycles.

Maximal oxygen consumption, i.e. physical performance capacity, i.e. aerobic power is a measure of skeletal muscles to consume oxygen for energy production. Individual sedentary baseline oxygen consumption (basal metabolic rate) is needed for basic metabolism which is regulated e. g. by body mass, many hormones (thyroidea hormones, cortisol, insulin) and enzymes, and environmental conditions (temperature).

Glycogen is one of the most important energy source for muscles activity. Glycogen is produced e.g. from blood glucose (carbohydrate), lactate (carbohydrate), and glycerol (lipid). Glycogen storage and production occur in liver and muscles tissues. Food carbohydrates, lipids, and proteins are converted by complex enzyme processes in cells e. g. to glucose and fatty acids.

Washing blood, in vitro, with singlet oxygen activated air is known to improve the red cell capacity to release oxygen (Varga, 1991). The right-shifting of the oxygen binding curve towards higher oxygen partial pressures is achieved in vivo by enhancement of the 2,3-diphosphoglycerate in red blood cells. Right-shifting means that more oxygen is released from red blood cells in the periphery, e.g. in muscles (Gersonde and Niccolau, 1979).

Materials and Methods

Ten voluntary healthy athletes, randomly from our social environment, aged from 25 to 49 years, participated in this experimental ergospirometry study. Study plan was simple and not blinded. There were two test rounds for every participant, separated by 10 days. During this time everyone breathed daily at a condition hall for 20 minutes singlet oxygen activated room air. Baseline ergospirometry tests were performed at the Fitness Clinic of Helsinki before singlet oxygen treatment. After 10 days activated air breathing ergospirometry tests were repeated using the same test protocol as in the baseline tests. Ergospirometry variables are shown in Table 1.

Ergospirometry contained e.g. measuring the following primary variables: carbon

dioxide and oxygen content (%) in the exhale air and blood lactate concentration from finger tip. To the ergospirometer (Medikro 202) attached gas analyser includes a fast (200 ms) paramagnetic oxygen-analyser and as fast carbon dioxide analyser which works on infrared principle. Analysers are maintenance-free. Both ambient and sample temperature, pressure and humidity are measured for the calculations of O₂-consumption and CO₂-production. Gas concentrations are two-point calibrated by the automated sequence with standard concentrations of oxygen and carbon dioxide in nitrogen gas. Room air is used as zero gas.

Medikro 202 ergospirometry and calibrated Tunturi electronic ergometer 802E were utilized in tests. Singlet oxygen from room air was produced by a Polyvalk equipment. Detailed description of the equipment can be found in the manufacturer's manuals.

Statistics. Measured variable values from ergospirometry between the post- and preexposure were compared pairwise. Every person being his/her own reference. Variable values were measured once in every minute, but in statistics calculations only value at 0, 6, 12, 18, and 24 minutes from the beginning of every ergospirometry test were utilized (corresponding loads of 0, 50, 100, 150, and 200 W each lasting 6 minutes between 50-200 W). Some cases were measured at 30 minutes and 250 W load. Pairwise group comparisons were made by the use of 2-sided t-tests. Bivariate linear regression analysis was used to evaluate the trend of change between the post- and preexposure variable values. The difference between post- and preexposure values was dependent and the cycling time (or load) was the independent variable. So, every test occasion produced 5-6 variable values per variable. Excel-tabulation program produced the regression drawings.

Results

The following results are preliminary. Statistical calculations were performed by one of the authors (E.S.) with PC statistical data package.

Comparisons between the second and first ergospirometry test results reveal the following features. We can see a clear statistically significant increase in the following variables: heart rate, energy production, utilized oxygen. Carbon dioxide production decreased instead significantly. Carbohydrate usage showed borderline significance.

Discussion

The results indicate, that exposure by breathing singlet oxygen activated room air for a relatively short period, produces metabolic changes, especially in the carbohydrate burning cycle. The oxygen dependent metabolism seems to switch to the known Embden-Meyerhof carbohydrate burning cycle. This seems legitimate because the relative carbon dioxide production decreases at the same time as heart rate and energy production increase. This in turn is in harmony with earlier research results: blood exposed to singlet oxygen or ozone increases 2,3-DPG (diphosphoglycerate) content in red blood cells.

Breathing singlet oxygen activated air seems to increase physical power output in demand situations, and almost all subjects indicated lower subjective strain during and

after the second test.

The preliminary results should be verified by other larger studies and preferably with blinded so that sham exposure is included without subject's knowledge.

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Literature

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Tables 1. List of ergospirometric variables.

Time (s)
Flow of expiratory ventilation (l/s)
Temperature of expired gas (deg)
The O ₂ - and CO ₂ -concentration of expired gas (%)
Ambient temperature (deg), atmospheric pressure (mbar) and relative humidity (%)
Sample temperature, pressure and humidity
Temperatures inside the analyser unit
Respiratory rate, FR (1/min)
Tidal volume, TV (l)
Minute ventilation, VE (l/min)
True oxygen, tO ₂ (%)
True carbon dioxide, tCO ₂ (%)
Oxygen consumption, VO ₂ (l/min)
Carbon dioxide production, VCO ₂ (l/min)
Respiratory quotient, VCO ₂ /VO ₂ , RQ
Heart rate, HR (1/min, beats per minute)

Abbreviations:

ATP	Adenisine 5-triphosphate is an energy rich molecule, the energy source for every living cell.
(Di)xygen	Gaseous component of the atmosphere.
Singlet oxygen	An excited form of oxygen, contains more energy than normal oxygen.
True oxygen	Measures the true oxygen content in per cents of expired air.
Atmospheric pressure	The total pressure (in some units, e.g. mbar) of the atmospheric gases temporary and locally at a site depending e.g. weather conditions and height from the mean sea level.
Humidity	A measure of the water content of air, usually relative humidity in per cents of the maximal possible at certain temperature.
SD	A dispersion or variability measure of observation values in statistical analyses.
p	Indicates statistical significance; if low (e.g. 0.01) then a pure chance cannot explain the result, e.g. difference between the means of two comparison group values.
in vitro	In living organism.
in vivo	Outside living organism.

Table 2. Difference between post- and preexposure values in some variables according to paired t-tests. Variables ending with P resp. A mean post- resp. before exposure values. For abbreviations, see Table 1. E=energy production, FAT=fat burning rate, CH= carbohydrate burning rate, LA=lactate concentration after exercise, n=number of observation values, mean=arithmetic mean value of observations, SD= standard deviation of observations. Statistical test significances: $p < 0.05$, almost significant; $p < 0.01$ significant; $p < 0.001$ highly significant.

Variable	n	mean	SD	p
VO2P (oxygen uptake)	58	1.460	0.74	0.014
VO2A	58	1.367	0.66	
tO2P (oxygen cont/exp air)	51	4.21	0.62	0.001
tO2A	51	4.48	0.46	
tCO2P (carb.diox./exp air)	51	3.77	0.64	0.001
tCO2A	51	4.05	0.62	
LAP (lactate conc)	54	2.09	1.28	0.25
LAA	54	2.26	1.20	
EP (energy prod)	57	442.9	223	0.004
EA	57	409.9	201	
CHP (carb hydr burn)	57	84.5	72.1	0.048
CHA	57	76.6	57.7	
FATP (fat burn)	57	11.6	7.6	0.43
FATA	57	11.0	6.8	
HRP (heart rate)	51	111.4	29	0.016
HRA	51	107.8	24	

VALKION SINGLET ENERGY TREATMENT

HELSINKI STUDY

TEN MALE LONG-DISTANCE ATHLETES INHALED SINGLET OXYGEN ACTIVATED AIR FOR 20 MINUTES DURING 10 DAYS (TOTALLY 2 WEEKS)

TESTS WERE PERFORMED BEFORE AND AFTER THE FULL TREATMENT PERIOD.
THE METHOD OF MEASUREMENT WAS ERGOSPIROMETRI.

RESULTS:	n	Before	After	P-Value	Change
Oxygen uptake	58	1,367	1,460	0,014	+ 7 %
Oxygen exhale	51	4,48	4,21	0,001	- 6 %
CO2 exhale	51	4,05	3,77	0,001	- 7 %
Lactate Concentration	54	2,26	2,09	0,001	- 8 %
Energy production	57	409,9	442,9	0,004	+ 8 %
Fat burn	57	11,0	11,6	0,43	+ 5 %
Carbohydrate burn	57	76,6	84,5	0,048	+ 10 %
Heart rate	51	107,8	111,4	0,016	+ 3 %

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