

Report 30414

**Breathing of singlet oxygen activated air and the effect on
blood parameters in healthy individuals .**

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Introduction

A pilot study in persons with fibromyalgia has been conducted where they were given treatments with activated air from a Valkion equipment (Polyvalk AB, Gothenburg). In this, ambient air is passed through an activation chamber whereby singlet oxygen is produced . For more detail instrumentation data , see manual from Polyvalk . The result of the study , showed positive responses like less pain , better sleep and better working ability (1) . Another study in male athletes could also detect some interesting physiological changes when the participating persons included activated oxygen breathing with their regular training (2) .

Based on these observations it could be interesting to see what effect the breathing of activated air might have on some common blood parameters in clinically healthy adult volunteers .

Material

A total of 10 persons were studied , 4 females and 6 males. The females were 34 to 48 years and their bodyweight ranged 58 to 66 kg . Corresponding data for the males were 32 to 74 years and the bodyweight 74 to 92 kg. They were all non - smokers . All of them had a normal diet and in some cases just a minor and fairly irregular supplement of vitamins and minerals . Seven persons are working in a dental office and 3 in a laboratory .

Methods

The study went on for 4 weeks. Blood samples were withdrawn initially , 2 weeks and 4 weeks after the start of the program. All sampling was done after a 10 hours fasting . The blood was analyzed according to 32 different parameters . A standard bloodserum test was performed by Calab (Multitest) including cholesterol, triglycerides , glucose, fructoseamines , ureate , urea , creatinine , albumin , haptoglobin , CRP, iron, TIBC, transferrin , LD, ASAT,ALAT,GT, ALP, phosphate, calcium,potassium,sodium , HDL and LDL.

Total Antioxidant Status (TAS) i serum was performed using a commercial kit from Randox , Ireland (cat.no NX 2332). Briefly, ABTS[®] is incubated with a peroxidase and hydrogen peroxide. A blue-green radical ABTS⁰⁺ is formed which easily can be detected by a UV-spectrophotometer . The formation of this radical is prevented proportional to the amount of antioxidants in ser

Enzyme activity of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) was analyzed in whole blood samples.

For GSH-Px Randox test RANSEL cat. no RS 505 was used. This kit is based on the method by Paglia and Valentine. GSH-Px catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidised glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm is measured by an UV-spectrophotometer.

For SOD the Randox test RANSOD cat. no SD 125 was used. SOD converts the superoxide radical to hydrogen peroxide and molecular oxygen. This kit employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye.

The superoxide dismutase activity is then measured spectrophotometrically by the degree of inhibition of this reaction.

After the first blood sampling the exposure for activated air started with a 12 minutes "treatment" for the first week. The breathing was done Monday, Wednesday and Friday.

The following weeks a 20 minutes treatment was performed.

Experience has shown that healthy individuals usually can breathe 15 - 20 minutes without any negative effects. To exclude any possible reactions the first week's treatments were carried out with a slightly reduced time.

Results and Comments

General

No one reported any negative effects during the test period. On the contrary, some mentioned a weak positive experience, mainly decreased tiredness.

When testing the influence of certain treatments on healthy individuals there is always a risk that some parameters cannot be improved because the individuals have already an optimal health in respect of that parameter.

Such a parameter is the Total Antioxidant Status (TAS) of blood serum. Values above 1.4 are considered good and above 1.6 rarely seen basically.

According to the present knowledge there should always be a certain level of free radicals available to cope with sudden problems arising in the human body. The human cells are even producing hydrogen peroxide to generate free radicals.

Of the 10 individuals tested 6 showed initial TAS values below 1.4 $\mu\text{mol/l}$.

It is consequently interesting to have a separate evaluation even at the other parameters for these 6 persons compared to the other four.

Total Antioxidant Status (TAS)

The TAS for all 10 individuals as a group increased from a mean of 1.27 to 1.37 after 2 weeks of treatment and remained the same throughout the test period (table 1).

The subgroup with a starting TAS < 1.4 had an initial mean of 1.07 increasing to 1.27 after 2 weeks remaining at that level during the rest of the treatment (1.26 after 4 weeks).

The increase of some 20 % is highly significant , indicative for the treatment and confirmed by in vitro experiments on human plasma (3 , 4) (Fig.1).

Serum Multi Test

The serum multi test includes 23 parameters. Most of these revealed no differences throughout the test period , not for the group as a whole nor taking starting TAS values into consideration. However , some parameters did change in an interesting and significant way.

Total Serum Cholesterol

All ten volunteers showed a significant decrease from an average of 6.8 mmol/l after 2 weeks of treatment to 6.3 (table 2). A minor further decrease was observed after 4 weeks of treatment. Considering the low TAS group the total serum cholesterol value decreased from a mean of 6.5 to 5.8 after two weeks and to 5.7 after four weeks of treatment. The decrease of some 10 % reached already after 2 weeks of treatment is significant .

All but three individuals had a starting value of cholesterol higher than the reference norm of 6.4 mmol/l . After the treatment period just 4 remained above that level.

The LDL to HDL ratio , measured as the ratio of Apolipoprotein B and Apolipoprotein A1 did not change during the test period . However , the ratio was considerably below the established limit of 1.4 for all individuals (Fig 2) .

Serum Triglycerides

All 10 volunteers showed a decrease of about 25 % from an average of 2.2 to 1.6 mmol/l after just 2 weeks of treatment (table 3) . No further change could be noted after 4 weeks .

It should be pointed out that one of the individuals had a very high starting value of 7.7 mmol/l having a great impact on the mean . His triglycerides then decreased to 4.3 and 4.1 mmol/l after 2 and 4 weeks respectively .

The mean for the subgroup , not containing that person , started on 1.57 mmol/l and went down to 1.28 after two weeks and 1.27 after four weeks .

Three persons had a starting value higher than accepted limit of 2.2 mmol/l . They all decreased after 2 weeks , one to 4.3 (see above) and the other two had 2.3 and 2.2 mmol/l (Fig 3) .

Serum urate / uric acid

Of all the 23 parameters investigated in the bloodserum Multi Test the serum urate was the last parameter showing a considerable change over the test period .

For all the 10 individuals a decrease from an average starting value of 374 μ mol/l a mean of 352 μ mol/l could be observed after 2 weeks and a further decrease to 329 μ mol/l after 4 weeks of treatment (table 4) .

There was no difference between the subgroup of 6 and the rest after 4 weeks of treatment. However, it could be noted that the subgroup had a bigger decrease the first 2 weeks and the other 4 individuals the biggest difference the last 2 weeks.

Only 2 individuals had starting values above the reference range of 450 μ mol/l. After 2 weeks everyone was below that level (Fig 4) .

Conclusions

The number of subjects in this study is too small to give more precise conclusions about the effect from singlet oxygen when inhaled on a regular basis in healthy persons. However, the results found in the 4 week period are encouraging and further studies should be set up. The construction of the Valkion equipment gives also an unique advantage for a blind study. The person inhaling the air passed through the Valkion can be completely unknowing whether the activation chamber is on or off. This should give a true answer to what effect the singlet oxygen breathing has on man.

References

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Methods used;
Whitehead T.P. , Thorpe G.M.G , Maxwell S.R.J. Analytica Chimica Acta , 266 (1992),265-277
Backa S, Jansbo,K, Reithager , T. Detection of Hydroxyl Radicals by Chemiluminescence Method. Royal Institute of Technology , Stockholm 1993.
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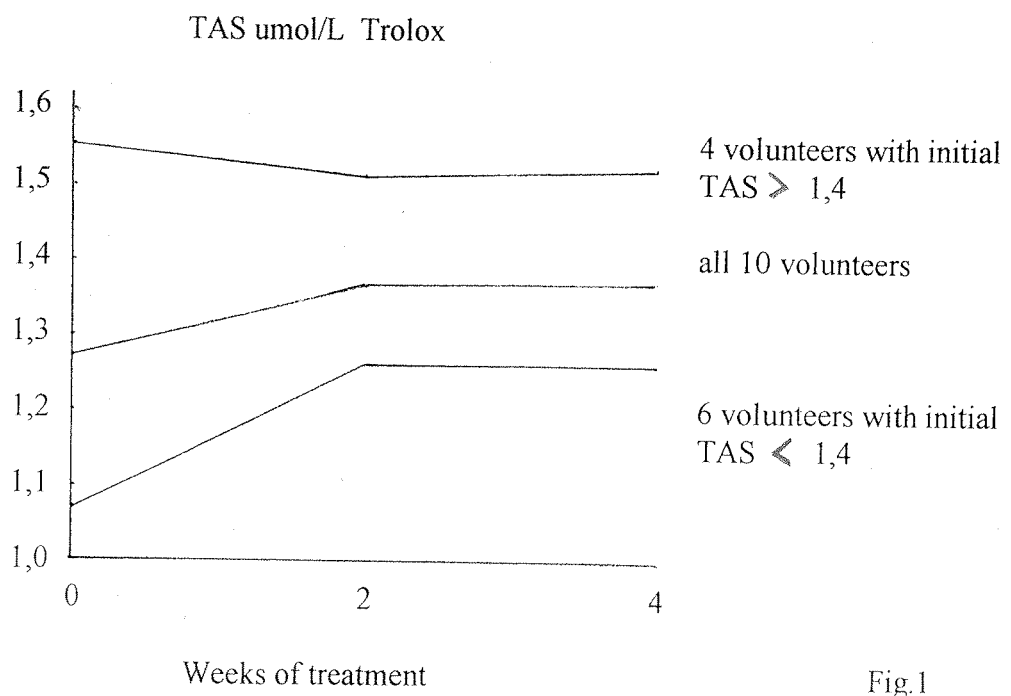


Fig.1

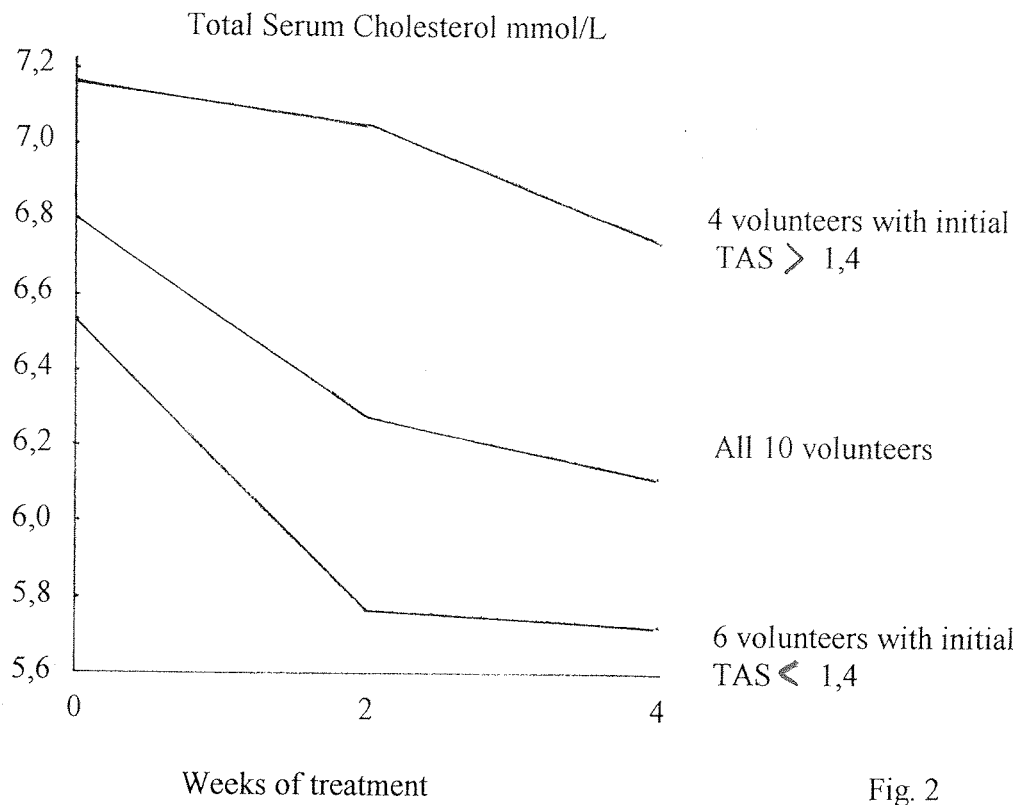


Fig. 2

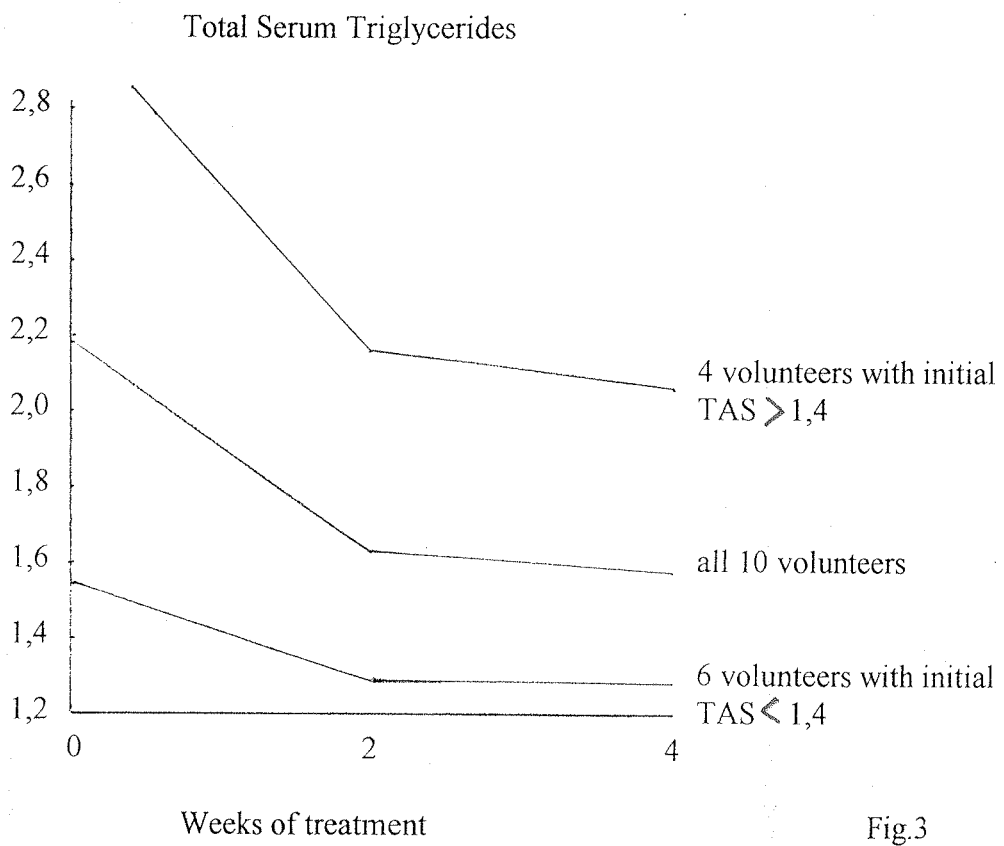


Fig.3

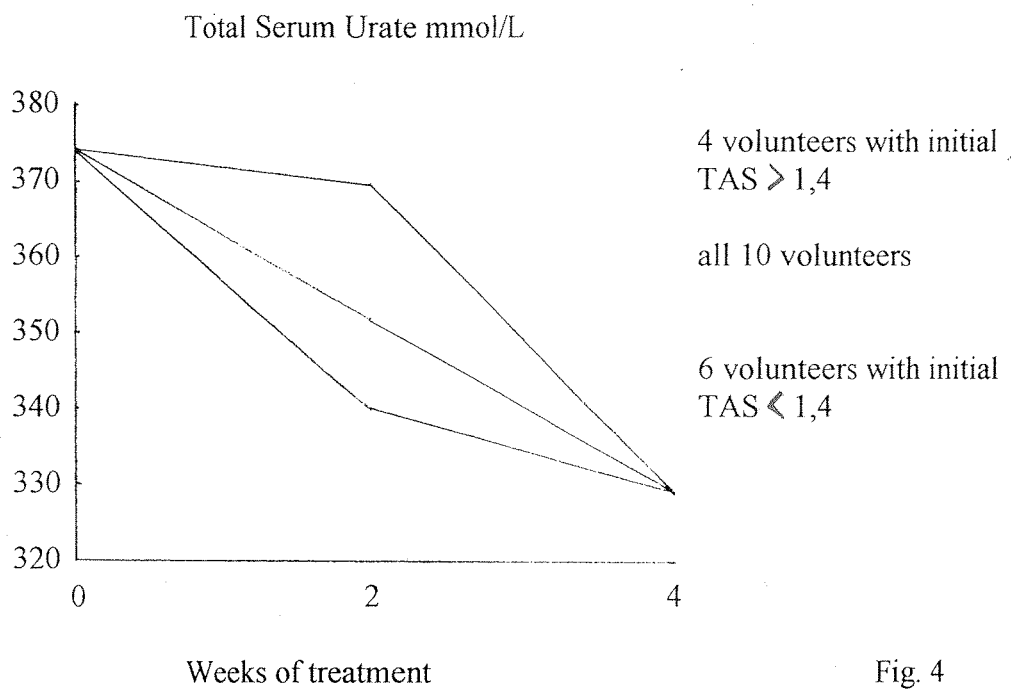


Fig. 4

Table 1.

Total Antioxidant Status of healthy volunteers over a 4 week period of treatment with singlet oxygen activated air. All values are given in $\mu\text{mol/l}$ Trolox units (Randox).

Person no.	Sampling			Difference		
	Initial I	2 week II	4 week III	II - I	III - I	III - II
1	0.95	1.27	1.19	0.32	0.24	-0.08
2	1.57	1.43	1.53	-0.14	-0.04	0.10
3	1.17	1.23	1.25	0.06	0.08	0.02
4	1.58	1.58	1.56	0.00	-0.02	-0.02
5	1.03	1.23	1.25	0.20	0.22	0.02
6	0.82	1.17	1.21	0.35	0.39	0.04
7	1.49	1.50	1.47	0.01	-0.02	-0.03
8	1.39	1.52	1.46	0.13	0.07	-0.06
9	1.05	1.23	1.21	0.18	0.16	-0.02
10	1.61	1.54	1.58	-0.07	-0.03	0.04
Mean	1.27	1.37	1.37	0.104	0.105	0.00
S.D.	0.30	0.16	0.16	0.161	0.145	0.069
t-value				2.04	2.30	0.0
p-value					< 0,05	

Table 2.

Total serum cholesterol in healthy volunteers over a 4 week period of treatment with singlet oxygen activated air . All values are given in mmol/l .

Individual no	Sampling			Difference		
	Initial I	2 week II	4 week III	II - I	III - I	III - II
1	7.3	6.6	6.1	-0.7	-1.2	-0.5
2	5.6	6.5	6.8	0.9	1.2	0.3
3	4.7	4.8	3.9	0.1	-0.8	-0.9
4	8.3	7.1	6.1	-0.8	-1.2	-1.0
5	8.7	7.1	7.2	-1.6	-1.5	0.1
6	6.5	4.8	6.2	-1.7	-0.3	1.4
7	7.4	7.7	7.3	0.3	-0.1	-0.4
8	5.2	5.1	4.9	-0.1	-0.3	-0.2
9	6.8	6.2	6.0	-0.6	-0.8	-0.2
10	7.4	6.9	6.7	-0.5	-0.7	-0.2
Mean	6.79	6.28	6.12	-0.47	-0.57	-0.16
S.D.	1.31	1.04	1.04	0.81	0.77	0.68
t-value				0.81	2.35	0.75
p-value					< 0,01	

Table 3.

Total serum triglycerides in healthy volunteers over a 4 week period of treatment with singlet oxygen activated air . All values are given in mmol/l .

Individual no	Sampling			Difference		
	Initial I	2 week II	4 week III	II - I	III - I	III - II
1	1.4	0.7	1.0	-0.7	-0.4	0.3
2	1.3	1.2	1.1	-0.1	-0.2	-0.1
3	0.9	0.7	0.7	-0.2	-0.2	0.0
4	7.7	4.3	4.1	-2.6	-3.3	-0.2
5	2.5	2.3	2.4	-0.2	-0.1	0.1
6	0.7	1.0	0.7	0.3	0.0	-0.3
7	1.5	1.5	1.3	0.0	-0.2	-0.2
8	0.8	0.8	0.9	0.0	0.1	-0.1
9	3.1	2.2	1.9	-0.9	-1.2	-0.3
10	2.0	1.7	1.6	-0.3	-0.4	-0.1
Mean	2.19	1.64	1.57	-0.47	-0.59	-0.09
S.D.	2.08	1.10	1.04	0.82	1.02	0.19
t-value				1.80	1.83	1.54
p-value				< 0,05		

Table 4.

Total serum urate in healthy volunteers over a 4 week period of treatment with singlet oxygen activated air. All values are given in mmol/l .

Individual no	Sampling			Difference		
	Initial I	2 week II	4 week III	II - I	III - I	III - II
1	342	293	314	-49	-28	21
2	314	314	275	0	-39	-39
3	297	311	232	14	-65	-79
4	479	438	420	-41	-59	18
5	367	410	353	43	-14	-57
6	505	342	406	-163	-99	64
7	336	339	312	3	-24	-27
8	363	339	329	-24	-34	-10
9	375	344	341	-31	-34	3
10	360	392	307	32	-53	-85
Mean	374	352	329	-21.6	-44.9	-19.1
S.D.	67.1	46.5	56.0	58.3	24.8	47.5
t-value				1.17	5.73	1.27
p-value				< 0,001		

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2	5.6	6.5	6.8		0.9	1.2	0.3
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4	8.3	7.1	6.1		-0.8	-1.2	-1.0
5	8.7	7.1	7.2		-1.6	-1.5	0.1
6	6.5	4.8	6.2		-1.7	-0.3	1.4
7	7.4	7.7	7.3		0.3	-0.1	-0.4
8	5.2	5.1	4.9		-0.1	-0.3	-0.2
9	6.8	6.2	6.0		-0.6	-0.8	-0.2
10	7.4	6.9	6.7		-0.5	-0.7	-0.2
Mean	6.79	6.28	6.12		-0.47	-0.57	-0.16
S.D.	1.31	1.04	1.04		0.81	0.77	0.68
t-value					0.81	2.35	0.75
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2	1.3	1.2	1.1	-0.1	-0.2	-0.1
3	0.9	0.7	0.7	-0.2	-0.2	0.0
4	7.7	4.3	4.1	-2.6	-3.3	-0.2
5	2.5	2.3	2.4	-0.2	-0.1	0.1
6	0.7	1.0	0.7	0.3	0.0	-0.3
7	1.5	1.5	1.3	0.0	-0.2	-0.2
8	0.8	0.8	0.9	0.0	0.1	-0.1
9	3.1	2.2	1.9	-0.9	-1.2	-0.3
10	2.0	1.7	1.6	-0.3	-0.4	-0.1
Mean	2.19	1.64	1.57	-0.47	-0.59	-0.09
S.D.	2.08	1.10	1.04	0.82	1.02	0.19
t-value				1.80	1.83	1.54
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