〈Original Article〉

Serum SH group-mediated elimination of oxidative stress and exercise stress caused by various concentrations of oxygen and swimming load in mice

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Summary Excess intake of high concentrations of oxygen and extreme exercise loads induce oxidative and exercise stress, which detrimentally affect the body. Antioxidants resolve these effects, and the serum SH group is one such antioxidant in the body. The serum SH group can be classified into free-SH (F-SH) with a strong reduction action and bound-SH (B-SH), to which various substances are bound. The current study used mice to investigate the action of serum SH on the elimination of oxidative stress under various oxygen concentrations and relaxation conditions, and on the elimination of exercise and oxidative stress caused by swimming exercises.

The results indicated that F-SH increased and B-SH decreased in an environment of high-concentration oxygen in the control group. High oxygen concentrations split the S-S bonds in B-SH, thereby increasing F-SH. F-SH showed the highest levels of production at 40% oxygen in the swimming group. In a comparison between the control group and swimming group at various oxygen concentrations, F-SH in the swimming group decreased significantly at 40% to 100% oxygen. F-SH eliminated oxidative stress and exercise stress produced by swimming load.

Key words: High-concentration oxygen, Swimming load, Serum SH group, Oxidation stress, Exercise stress

1. Introduction

Aerobic organisms require large volumes of adenosine triphosphate (ATP) to support life, and to facilitate the generation of ATP, humans consume about 500 L of oxygen per day. However, excess intake of oxygen leads to elevated production of harmful reactive oxygen species and free radicals. Therefore, the living body has antioxidant defenses against reactive oxygen species and free radicals. The

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Received for Publication September 16, 2011
Accepted for Publication September 21, 2011

substances that demonstrate antioxidant action include antioxidant enzymes, such as SOD and catalase, and antioxidant compounds, such as ascorbic acid, uric acid, alpha-lipoic acid and ceruloplasmin[1].

Recently, in addition to the above antioxidants, albumin has been studied[2, 3]. Albumin has a highly reactive SH group (cysteine residue; Cys-34) at the 34th position counting from the N terminus of the amino acid sequence. Albumin[4] in the free state, in which in this SH group is not bound to any substance, has an oxidoreductive function that provides and accepts H^+. Sogami et al.[5] separated and measured reduced albumin (human mercaptalbumin; HMA) and oxidized albumin (human non-mercaptalbumin; HNA) by high-performance liquid chromatography (HPLC). Subsequently, Imai et al.[6] reported that, when using this method, HMA decreased and as a result, the SH group of HMA acted to eliminate exercise and oxidative stress.

We developed an improved[7] colorimetric method[8-10] to measure the SH group using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and using this method, classified the serum SH group into: free-SH (F-SH) to which no other substance is bound; bound-SH (B-SH) to which cysteine, glutathione or other substances are bound; and total-SH (T-SH), which is the sum of these[11].

Furthermore, we reported that F-SH eliminates exercise and oxidative stress and is converted to B-SH, based on the following findings: long-distance running increases F-SH in humans[12] and aerobic exercise increases F-SH[12], whereas anaerobic exercise decreases F-SH and increases B-SH in mice[13]. Measurement of changes in serum SH groups enables the measurement of degree of relaxation and elimination of exercise and oxidative stress.

In recent years, aggressive and careless inhalation of high concentrations of oxygen has been seen during and after exercise in order to assist in recovery from fatigue. However, detailed investigations have not been conducted on the elimination of related exercise and oxidative stress. This study, using the serum SH measurement method, evaluated the exercise and oxidative stress produced in a living body, as well as the elimination of their effects, which may occur as a biological reaction, by breeding mice in a high oxygen environment with further loading from swimming exercise.

2. Materials and methods

1) Supply of high-concentration oxygen

Oxygen at concentrations of 20% to 100% flowed into the breeding box. Gas was allowed to flow in at 5 L/min for the initial 5 minutes, and the inflow was then continued at 1 L/min. We used atmospheric air for 20% oxygen, a pure oxygen cylinder for 100% oxygen, and prepared mixed gas cylinders containing oxygen and nitrogen for 40%, 60% and 80% oxygen (Eba Medical Gas Industry, Nagoya, Japan) (Table 1).

2) Mice and exercise load

Male ddY mice aged 7 weeks (Japan SLC Inc., Hamamatsu, Japan) were exposed to an exercise load of swimming, whereas the control group was simply bred without any load in the same environment as

Table 1 Composition of gas cylinder at various concentrations of oxygen

		Oxygen 20%	Oxygen 40%	Oxygen 60%	Oxygen 80%	Oxygen 100%
Composition of gas cylinder	Oxygen	atmosphere	40%	60%	80%	100%
	Nitrogen		60%	40%	20%	—

The atmosphere used was a 20% concentration of oxygen. Gas cylinders were prepared at ratios ranging from 40% to 80% concentrations of oxygen with nitrogen gas mixed with oxygen gas at each proportion. A pure oxygen gas cylinder was used for the 100% concentration of oxygen.

the swimming group. The swimming group completed 6 sets of swimming for 10 minutes and 5 minutes of rest in from 10:00 a.m. and 6 identical sets from 2:30 p.m. with 3 hours rest in between, for a total of 2 hours swimming per day. We prepared a water tank that was sufficiently deep to submerge the mice if they did not continue to swim, and the water temperature was maintained at 30℃. This swimming load was imposed for 3 days. We washed the mice with neutral detergent in order to prevent floating on the surface of the water due to air trapped in the hair.

The control group mice were divided into 20% oxygen group (n=36), 40% oxygen group (n=13), 60% oxygen group (n=13), 80% oxygen group (n=10), and 100% oxygen group (n= 14). Also, the swimming group mice were divided into 20% oxygen group (n=7), 40% oxygen group (n=13), 60% oxygen group (n=7), 80% oxygen group (n=14), and 100% oxygen group (n=8).

3) Breeding environment and closed breeding box

Mice were bred in the following environment: under a normal atmosphere until swimming load began; and after swimming load began, mice were kept under a normal atmosphere during swimming load, and under various oxygen concentrations during other resting times until blood was collected. Closed breeding boxes were used by modifying acrylic desiccators (Sanplatec #0097; Sanplatec, Osaka, Japan). That is, after drilling a hole through both side panels, a gas flowmeter (Sanplatec #5076; Sanplatec, Osaka, Japan) was attached to one side, allowing an inflow of oxygen, and an outlet was prepared on the other side. During breeding, the whole mouse breeding cage was placed in the breeding box. Room temperature was maintained at 25℃, and water and food were provided ad libitum.

4) Collection of blood and lactic acid measurement

Blood was collected via the tail vein on the morning after the last swimming load in the swimming group (day 4 of the experiment), and the level of lactic acid was measured using Lactate Pro (Arkray, Kyoto, Japan). Subsequently, heart blood was collected under etherization, and serum SH group concentrations were measured from the obtained serum. Similarly for the control group, after breeding for 3 days at each oxygen concentration, the lactic acid value was measured on the morning of day 4, heart blood was collected under etherization, and serum SH group concentration was measured.

Animal experiments were conducted in accordance with the Guidelines for the Management of Laboratory Animals at Fujita Health University

5) Serum SH group concentration measurement

We used a modified DTNB method[11] developed for serum SH group concentration measurement. We

Table 2 Result of F-SH, B-SH, T-SH and lactic acid concentration at each concentration of oxygen

		Oxygen 20%	Oxygen 40%	Oxygen 60%	Oxygen 80%	Oxygen 100%
F-SH (mg/dl)	control group	2.13 ± 0.20	4.45 ± 0.26	4.28 ± 0.20	4.18 ± 0.35	3.26 ± 0.28
	swimming group	2.03 ± 0.53	2.87 ± 0.23	1.83 ± 0.14	2.11 ± 0.12	1.95 ± 0.21
B-SH (mg/dl)	control group	5.74 ± 0.18	3.40 ± 0.21	4.25 ± 0.18	4.12 ± 0.17	4.25 ± 0.14
	swimming group	5.89 ± 0.53	6.14 ± 0.16	6.24 ± 0.36	5.20 ± 0.40	6.13 ± 0.56
T-SH (mg/dl)	control group	7.87 ± 0.14	8.72 ± 0.15	8.53 ± 0.24	8.30 ± 0.27	7.51 ± 0.21
	swimming group	7.92 ± 0.32	9.01 ± 0.23	8.11 ± 0.25	7.31 ± 0.44	8.08 ± 0.43
Lactic Acid (mmol)	control group	2.05 ± 0.10	1.94 ± 0.19	2.12 ± 0.10	2.59 ± 0.21	3.46 ± 0.45
	swimming group	1.74 ± 0.13	2.35 ± 0.15	2.21 ± 0.14	1.81 ± 0.09	2.58 ± 0.19

Values are means ± S.E.

simultaneously measured a cysteine solution at a known concentration and converted this data into SH group concentrations. The F-SH concentration was calculated by colorimetry at 420 nm via direct reaction between the serum and DTNB reagent followed by 10-min incubation at 37℃.

With regard to B-SH concentration, serum SH concentration was calculated using colorimetry measurement via oxalic acid treatment of serum followed by 5-min centrifugation at 5,600 × g for the reaction between the supernatant and DTNB reagent. Furthermore, the final B-SH concentration was calculated by dividing the above calculated results by the mixing ratio (0.9) between serum and oxalic acid. The T-SH concentration was calculated by adding the F-SH and B-SH concentrations.

6) Statistical methods

We assessed the results obtained for the control group and the swimming group using multiple comparison testing. The results were deemed significant when the risk rate was less than 5%.

3. Results

1) Changes in F-SH levels

In the control group, F-SH increased significantly at 40%, 60%, 80% and 100% oxygen, as compared with 20% oxygen. There were no significant differences between 40%, 60% and 80% oxygen, but F-SH at 100% oxygen concentration showed significantly lower values than at 40%, 60% and 80% oxygen. In the swimming group, there was a significant increase at 40% oxygen. However, there were no significant differences at 20%, 60%, 80% and 100% oxygen (Table 2, Fig. 1). As compared to the control group, F-SH was significantly lower in the swimming group at 40%, 60%, 80% and 100% oxygen.

2) Changes in B-SH levels

In the control group, B-SH was significantly lower at 40%, 60%, 80% and 100% oxygen, as compared to 20% oxygen, and at 40% oxygen, B-SH was significantly lower than at 60%, 80% and 100% oxygen.

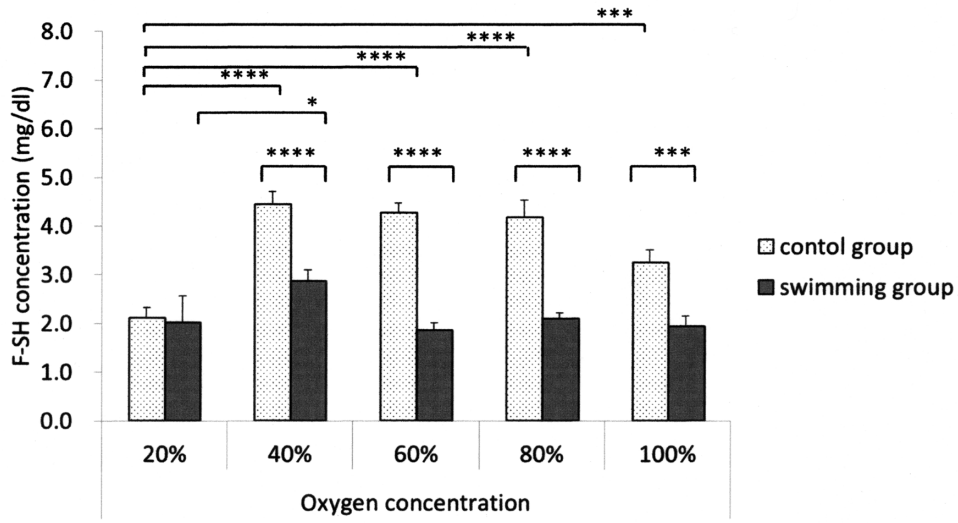

Fig. 1 Changes in F-SH levels

The control group showed significant increases at each concentration of oxygen against a 20% concentration of oxygen. There was a significant increase only at 40% against other concentrations of oxygen in the swimming group. In comparisons between the control group and the swimming group, a significant difference was not recognized only at a 20% concentration of oxygen, but the swimming group showed significant decreases at all other concentrations of oxygen.

Values are means + S.E. *: $p<0.05$, ***: $p<0.005$, ****: $p<0.001$

There were no significant differences between 60%, 80% and 100% oxygen. In the swimming group, there were no significant differences between the various oxygen concentrations (Table 2, Fig. 2). As compared to the control group, there were significant increases in B-SH at 40%, 60%, 80% and 100% oxygen in the swimming group.

3) Changes in T-SH levels

In the control group, T-SH was significantly higher at 40% and 60% oxygen, as compared with 20% oxygen, and significantly higher values were seen at 40%, 60% and 80%, as compared with 100% oxygen. There were no significant differences between 40%, 60% and 80% oxygen. In the swimming group, there were significant differences between 40% and 80% oxygen (Table 2, Fig. 3). There were no significant differences between the control group and the swimming group at any oxygen concentration.

4) Changes in lactic acid levels

In the control group, there were no significant differences at 20%, 40%, 60% and 80% oxygen, but lactic acid at 100% oxygen was significantly higher. In the swimming groups, there were significant increases in lactic acid at 40% and 100% oxygen, as compared to 20% and 80% oxygen (Table 2, Fig. 4). As compared to the control group, the swimming group had significantly lower levels of lactic acid at 80% oxygen.

4. Discussion

In the control group, when the concentration of oxygen in the breeding environment was increased from 20% to between 40% and 100%, F-SH increased significantly, whereas B-SH decreased significantly. This suggests that high concentrations of oxygen

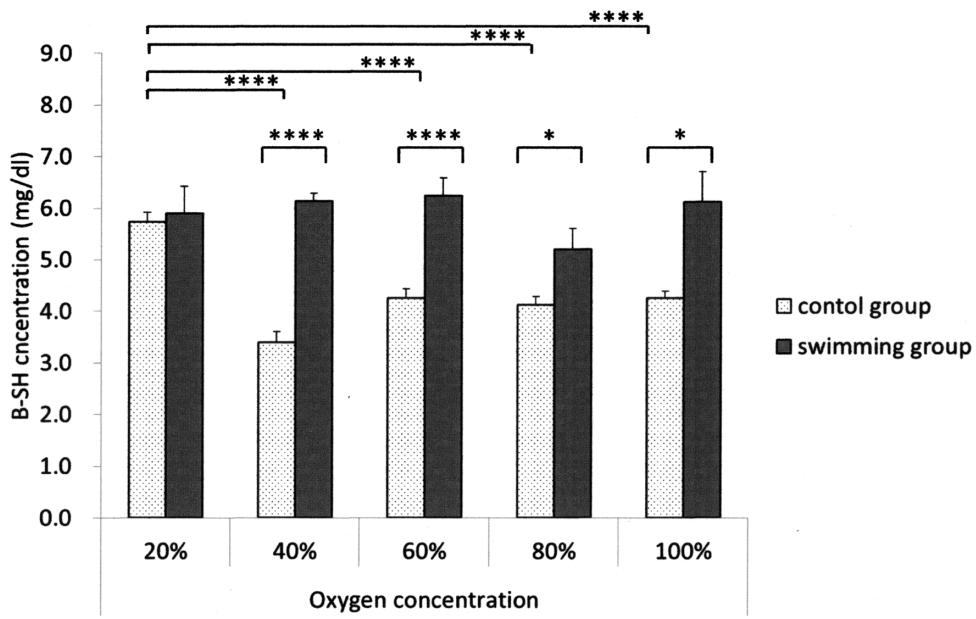

Fig. 2 Changes in B-SH levels

The control group showed significant decreases at each concentration of oxygen against a 20% concentration of oxygen. There were no significant differences between each concentration of oxygen in the swimming group. In comparisons between the control group and the swimming group, a significant difference was not recognized only at a 20% concentration of oxygen, but the swimming group showed significant increases at all other concentrations of oxygen.

Values are means + S.E. *: $p<0.05$, ****: $p<0.001$

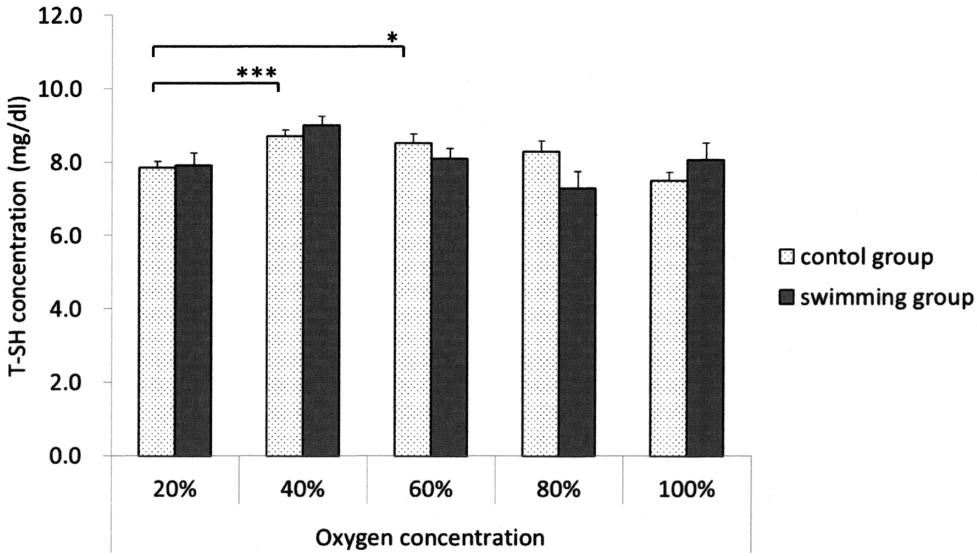

Fig. 3 Changes in T-SH levels

The control group showed significant increases at 40% and 60% against a 20% concentration of oxygen. There was a significant decrease at 100% against 40%, 60%, and 80% concentrations of oxygen. There was a significant difference between 40% and 80% in the swimming group. In comparisons between the control group and the swimming group, there were no significant differences at each concentration of oxygen

Values are means + S.E. *: $p<0.05$, ***: $p<0.005$

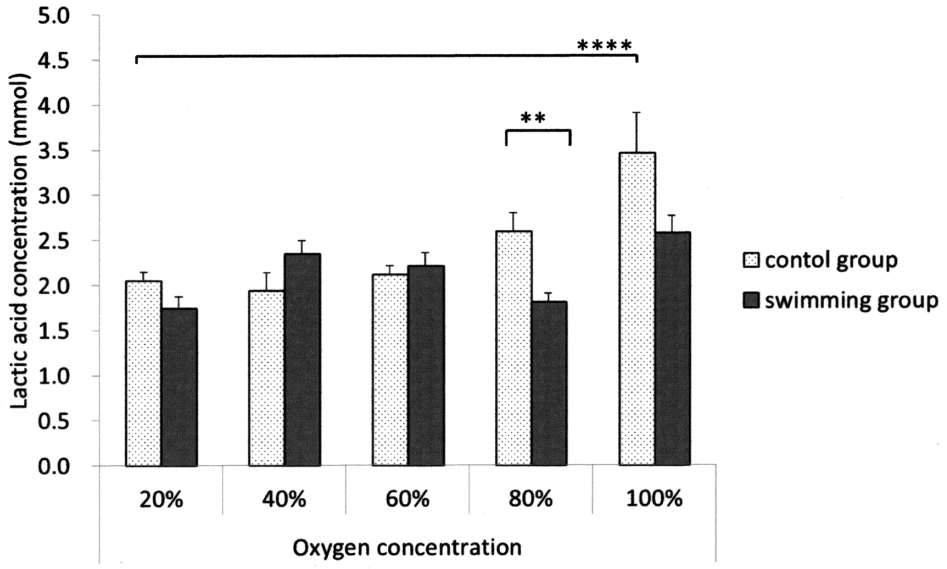

Fig. 4 Changes in lactic acid levels

The control group showed a significant increase only at 100% against each concentration of oxygen. There were significant increases in the swimming group at 40% and 100% against a 20% concentration of oxygen. There were significant differences between 40% and 80% concentrations of oxygen and between 80% and 100%. In comparisons between the control group and the swimming group, there was a significant difference only at an 80% concentration of oxygen.

Values are means + S.E. **: $p<0.01$, ****: $p<0.001$

splits the S-S bond of B-SH for conversion into F-SH[12]. In the swimming group, a significant increase in F-SH was only seen when levels peaked at 40% oxygen. As compared to the control group, significant decreases in F-SH were seen in the swimming group at 40% to 100% oxygen, which suggests that F-SH acted to eliminate exercise and oxidative stress produced by the swimming load.

We believe that elevated beta-oxidation levels resulted in high values of lactic acid under 80% and 100% oxygen. The presence of excess oxygen promoted fatty acid degradation and produced acetyl-CoA, which was supplied to the TCA cycle. The TCA cycle operated at the maximum rate to process excess acetyl-CoA. On the other hand, excess pyruvic acid was produced by the Embden-Meyerhof pathway and failed to proceed to the TCA cycle, thus producing lactic acid[14), 15)].

The observation that lactic acid decreased significantly at 80% oxygen in the swimming group, and the declining trend observed at 100% oxygen, suggest that F-SH is produced at high levels under high-concentration oxygen, and this acted to eliminate lactic acid.

In addition to albumin, cysteine and glutathione play a role as blood substances that possess the SH group. However, the serum concentrations are 3.8-5.3 g/dl for albumin vs. 45-160 μ g/dl for cysteine and 15-300 μ g/dl for glutathione; thus, the total levels of cysteine and glutathione are less than 3% that of albumin. This suggests that most of the serum SH groups originate from the SH groups in albumin. Imai et al.[6)] reported that an intensive exercise load decreased HMA and increased HNA, which is similar to our observation that swimming load decreased F-SH and increased B-SH, suggesting that HMA to HNA conversion is similar the F-SH to B-SH conversion[11)].

5. Conclusion

Breeding under 100% oxygen results in elevated lactic acid values. This is passive oxidative stress. If anaerobic exercise load is added to this, production of lactic acid (marker of oxidative stress) increase, causing further elevated lactic acid values. However, the swimming group under 100% oxygen also showed decreased lactic acid values. This suggests that increased F-SH due to high-concentration oxygen eliminates lactic acid. Therefore, the intake of high-concentration oxygen for many hours is a double-edged sword involving the production of damaging reactive oxygen species and elevated production of protective F-SH.

Reference

1) Sies H: Oxidative stress: oxidants and antioxidants. Exp Physiol, 82: 291-295, 1997.
2) Bourdon E, Loreau N and Blache D: Glucose and free radicals impair the antioxidant properties of serum albumin. FASEB J, 13: 233-244, 1999.
3) Halliwell B and Gutteridge JM: Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys, 246: 501-514, 1986.
4) Carter DC and Ho JX: Structure of serum albumin. Adv Protein Chem, 45: 153-203, 1994.
5) Sogami M, Nagoka S, Era S, Honda M and Noguchi K: Resolution of human mercapt- and nonmercaptalbumin by high-performance liquid chromatography. Int J Peptide Protein Res, 24: 96-103, 1984.
6) Imai H, Hayashi T, Negawa T, Nakamura K, Tomida M, Koda K, Tajima T, Koda Y, Suda K and Era S: Strenuous exercise-induced change in redox state of human serum albumin during intensive kendo training. Jpn J Physiol, 52: 135-140, 2002.
7) Ishiguro Y, Nakagami Y, Maruta K, Ito Y, Ohashi A, Nagamura Y, Kato M and Sugiyama S: Changes of serum total-SH in hemodialysis patients. J Anal Bio-Sci, 27: 385-390, 2004.
8) Ellman GL: A colorimetric method for determining low concentrations of mercaptans. Arch Biochem Biophys, 74: 443-450, 1958.
9) Ellman GL: Tissue sulfhydryl groups. Arch Biochem Biophys, 82: 70-77, 1959.
10) MacQueen J, Plaut D, Borges J and Anido G: Manual colorinetric method for pseudocholinesterase and real cell(true) cholinesterase. Clin Chem, 7: 481-485, 1971.
11) Nakagami Y, Maruta K, Ito Y, Uchida Y, Kato M and Sugiyama S: Modification and application of thiocholine method for serum SH group measurement. J Anal Bio-Sci, 30: 352-356, 2007.
12) Nakagmi Y, Ito Y and Maruta K: Effects of aerobic exercise and high oxygen concentration on serum sulfhydryl-group levels. Medicine and Biology, in print,

2011.

13) Nakagami Y, Maruta K, Uchida Y, Ito Y and Nagamura Y: Change of serum SH under various conditions. Jpn J Physiol, 55 (Suppl), 2005.

14) Knnedy EP: Metabolism of lipides. Annu Rev Biochem, 26: 119-48, 1957.

15) Edited by JW. Salih: JW. Salih: Fatty acid metabolism. Lipid metabolism, 1-48, Academic Press, New York and London (1970)