Original Articles

Juntendo Medical Journal
2011. 57 (6) , 624~629

Dietary Factors Alter the Oxygen Affinity of Hemoglobin

HIROSHI SAKURAMOTO*1)2)

JIN UCHIMARU*3)4)

HISASHI NAITO*4)

TOSHIKI WAGA*5)

SATOSHI SUNAYAMA*6)

TAKAHICO SHIMIZU*1)7)

HISASHI KUROSAWA*2)

TAKUJI SHIRASAWA*1)8)

Objective : In a previous report, we suggested that the oxygen affinity of hemoglobin (Hb) regulates the level of erythropoiesis, tissue O₂ consumption, physical activity, and behavior in mice. Here, we explore the possibility that dietary factors alter the oxygen affinity of Hb.

Material and Methods : A blood sample was collected from a healthy volunteer. Then, the hemolysate was mixed with various plant extracts, and P₅₀ was measured with the HEMOX-ANALYZER *in vitro*. *Allium sativum* extract was also administered orally to healthy volunteers, and the P₅₀ and blood concentrations of 2,3-diphosphoglycerate (2,3-DPG) were measured.

Results : Based on screening test, various plant extracts produced changes in P₅₀ *in vitro*. We selected a candidate extract, *Allium sativum*, and it increased P₅₀ significantly *in vitro*. Furthermore, P₅₀ was significantly increased 2 hours after the single administration of *Allium sativum* extract *in vivo*. P₅₀ was increased significantly at 2 and 3 weeks after the start of the once-daily administration of *Allium sativum* extract, while the blood concentrations of 2,3-DPG were decreased *in vivo*.

Conclusions : The oxygen affinity of Hb is altered by dietary factors including *Allium sativum*.

Key words : hemoglobin oxygen affinity, dietary factor, plant extract, *Allium sativum*, 2,3-DPG

Introduction

Hemoglobin (Hb) is a tetrameric protein composed of α - and β -globin subunits that transports oxygen from the lungs to peripheral tissues. The oxygen dissociation curve (ODC) represents the association and dissociation of Hb with and from oxygen, and the oxygen affinity of Hb can be expressed by the half saturation pressure of Hb (P₅₀).

When the oxygen affinity of Hb is decreased, the ODC shifts rightward and P₅₀ increases. A decrease in the oxygen affinity of Hb enhances O₂ release in the peripheral tissues but in some conditions it reduces O₂ binding with Hb in the alveoli of the lung.

We previously reported that the oxygen affinity of Hb regulates the level of erythropoiesis, tissue O₂ consumption, physical activity, and behavior in mice¹⁾. Furthermore, previous reports showed that

* 1) *Molecular Gerontology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*

* 2) *Department of Orthopedics, Juntendo University Faculty of Medicine, Tokyo, Japan*

* 3) *Department of Medicine and Science in Sports Exercise, Tohoku University School of Medicine, Miyagi, Japan*

* 4) *Department of Exercise Physiology, School of Health and Sports Science, Juntendo University, Chiba, Japan*

* 5) *Development Laboratory for New Business, Asahi Breweries, Ltd., Ibaraki, Japan*

* 6) *Department of Cardiology, Juntendo University Faculty of Medicine, Tokyo, Japan*

* 7) *Applied Biological Chemistry, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo, Japan*

* 8) *Department of Aging Control Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan*

[Received July 30, 2010] [Accepted May 2, 2011]

the P_{50} changes altered cardiac index^{2) 3)}. Thus, we suggest that the oxygen affinity of Hb plays an important role in reducing cardiac workload and improving physical activity in peripheral tissues. Previous reports have also shown that P_{50} is altered *in vivo* by temperature, pH, PCO_2 , 2, 3-diphosphoglycerate (2, 3-DPG), sex, age, critical diseases, structural changes in Hb, living altitude, and synthetic allosteric modifiers such as [2-(4-[(3, 5-dimethylanilino) carbonyl] methylphenoxy)-2-methyl propionic acid] (RSR13)^{4) ~12)}.

Previous reports have shown that bezafibrate, diphosphonate with fructose, propranolol, and RSR13 modulate the oxygen affinity of Hb^{7) 13) ~15)}. However, there have been no reports about whether dietary factors or supplements decrease the oxygen affinity of Hb.

In the present study, we investigated whether dietary factors extracted from plants can modulate P_{50} and revealed that *Allium sativum* decreased the oxygen affinity of Hb *in vitro*. We also demonstrated that *Allium sativum* extract decreased the oxygen affinity of Hb and the blood concentration of 2, 3-DPG *in vivo*.

Materials and Methods

The screening of various plant extracts *in vitro*. Blood samples were collected from a healthy volunteer into heparin-treated tubes. Erythrocytes were collected by centrifugation of 2 ml of whole blood, washed in 0.9% NaCl, and lysed in 4 ml distilled water. The hemolysate was then collected by centrifugation at 15,000 rpm for 30 min. Then, 40 μ l of hemolysate were mixed with 5 ml of buffer [50 mM HEPES and 10 mM NaCl buffer (pH 7.4)] and 10 μ l of 10% dimethylpolysiloxane antifoaming agent. Various plants were extracted using hot water and dried. The plant extracts were dissolved in HEPES buffer and added to the hemolysate solutions at a final concentration of 1 mg/ml. P_{50} was measured with the HEMOX-ANALYZER (TCS, Medical Products Division, Southampton, PA) with or without the plant extracts, as described previously⁹⁾.

Administration of plant extracts *in vivo*. Six healthy volunteers were fasted for 12 h, and their blood was collected. Two hours after the oral administration of 1,000 mg *Allium sativum*, samples

of their whole blood were collected, and P_{50} was measured with a HEMOX-ANALYZER. The blood concentrations of 2, 3-DPG were measured after the deproteinization of the whole blood samples with a UV kit and expressed in μ mol/ml of blood (Sigma, St. Louis, MO, USA). Next, eight healthy volunteers took 1,000 mg *Allium sativum* every day for 3 weeks, and their whole blood was collected and their P_{50} and blood concentrations of 2, 3-DPG were measured every week.

Role of the funding source. The sponsor of the study played no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit it for publication.

Statistical analysis. All data are expressed as the mean $\pm$ SD. The statistical significance of differences was assessed by the unpaired *t* test.

Ethics. The ethics committees of Tokyo Metropolitan Institute of Gerontology and Juntendo University approved this study. Informed consent was obtained from all subjects.

Results

Effects of various plant extracts on the oxygen affinity of Hb *in vitro*. Synthetic allosteric modifiers of Hb, such as bezafibrate, diphosphonate with fructose, propranolol, and RSR13, modulate the oxygen affinity of Hb^{7) 13) 14)}. However, no dietary factors have been reported to alter the oxygen affinity of Hb; therefore, we investigated whether dietary factors affected the oxygen affinity of Hb *in vitro*. We screened 136 extracts from 102 plants *in vitro* to find dietary allosteric modifiers of Hb. Hb was prepared from a healthy volunteer, mixed with each plant extract, and the changes in P_{50} were measured. Interestingly, we found that a variety of plant extracts modified P_{50} *in vitro* (Figure-1A and Table-1). By screening, one hundred and sixteen extracts increased P_{50} , and 20 extracts decreased P_{50} . Among these extracts, *Allium sativum* increased P_{50} most strongly (Figure-1A and Table-1). Furthermore, we confirmed *Allium sativum* increased P_{50} significantly by three times (Figure-1B).

Effects of *Allium sativum* extract on the oxygen

Table-1 The list of plant extracts

No.	PlantExtract	Manufacturer	ΔP_{50} (mmHg)	No.	PlantExtract	Manufacturer	ΔP_{50} (mmHg)
1	<i>Allium sativum</i>	Bizen Chemical Co., Ltd.	22.25	69	<i>Sedum erythrostictum</i>	NIPPON Funmatsu Yakuhin co, ltd	1.22
2	<i>Terminalia catappa</i>	Ryukyu Bio Resource co. ltd	20.03	70	<i>Cynara scolymus</i>	ASK INTERCITY CO., LTD.	1.22
3	<i>Terminalia catappa</i>	Ryukyu Bio Resource co. ltd	19.26	71	<i>Crataegus cuneata</i>	NIPPON Funmatsu Yakuhin co, ltd	1.21
4	<i>Melissa officinalis</i>	ASK INTERCITY CO., LTD.	14.47	72	<i>Solidago gigantea</i>	ASK INTERCITY CO., LTD.	1.19
5	<i>Lagerstroemia speciosa</i> Pars	Use Techno Corporation	13.12	73	<i>Morus bombycis</i> Koidz.	NIPPON Funmatsu Yakuhin co, ltd	1.17
6	<i>Rubus suavissimus</i> S. Lee	NIPPON Funmatsu Yakuhin co, ltd	12.90	74	<i>Corchorus olitorius</i> L.	Matsuura Yakugyou Co. Ltd.	1.14
7	<i>Mallotus japonicus</i>	NIPPON Funmatsu Yakuhin co, ltd	11.83	75	<i>Glycine max</i> (L.) MERR.	SHONAN FLAVORS, INC.	1.13
8	<i>Daucus carota</i> L. var. <i>sativa</i> DC.	Matsuura Yakugyou Co. Ltd.	11.13	76	<i>Cimicifuga racemosa</i>	TOKIWA PHYTOCHEMICAL CO., LTD.	1.10
9	<i>Embelia officinalis</i> Gaertn.	ASK INTERCITY CO., LTD.	9.37	77	<i>Agrostodon blomhoffii</i>	NIPPON Funmatsu Yakuhin co, ltd	1.10
10	<i>Rubus suavissimus</i> S. Lee	Suntory Holdings Limited	9.20	78	<i>Echinacea purpurea</i>	NIPPON Funmatsu Yakuhin co, ltd	1.07
11	<i>Ginseng Radix Rubra</i>	NIPPON Funmatsu Yakuhin co, ltd	8.47	79	<i>Centella asiatica</i>	ASK INTERCITY CO., LTD.	1.03
12	<i>Orthosiphon stamineus</i>	Cognis Japan Ltd.	7.41	80	<i>Urtica dioica</i>	ASK INTERCITY CO., LTD.	0.93
13	<i>Mentha arvensis</i>	Ogawa & Co. Ltd.	7.32	81	<i>Echinacea purpurea</i>	ASK INTERCITY CO., LTD.	0.91
14	<i>Mentha piperita</i>	ASK INTERCITY CO., LTD.	6.99	82	<i>Humulus lupulus</i> L.	ASK INTERCITY CO., LTD.	0.82
15	<i>Psidium guajava</i> L.	NIPPON Funmatsu Yakuhin co, ltd	6.59	83	<i>Vitex agnus castus</i>	NIPPON Funmatsu Yakuhin co, ltd	0.76
16	<i>Alpinia zerumbet</i>	Ryukyu Bio Resource co. ltd	6.27	84	<i>Euphrasia species</i>	NIPPON Funmatsu Yakuhin co, ltd	0.75
17	<i>Perilla frutescens</i>	NIPPON Funmatsu Yakuhin co, ltd	5.98	85	<i>Gynostemma pentaphyllum</i>	NIPPON Funmatsu Yakuhin co, ltd	0.75
18	<i>Eucalyptus globulus</i>	ASK INTERCITY CO., LTD.	5.67	86	<i>Coix lacryma-jobi</i> L.	NIPPON Funmatsu Yakuhin co, ltd	0.75
19	<i>Thymus vulgaris</i>	NIPPON Funmatsu Yakuhin co, ltd	5.56	87	<i>Daucus carota</i> L. var. <i>sativa</i> DC.	NIPPON Funmatsu Yakuhin co, ltd	0.75
20	<i>Rubus suavissimus</i> S. Lee	NIPPON Funmatsu Yakuhin co, ltd	4.87	88	<i>Maticaria chamomile</i>	Kenko Corporation	0.73
21	<i>Apocynum venetum</i> L.	TOKIWA PHYTOCHEMICAL CO., LTD.	4.57	89	<i>Lycium rhombifolium</i>	NIPPON Funmatsu Yakuhin co, ltd	0.66
22	<i>Polymnia sonchifolia</i>	MARUZEN PHARMACEUTICALS CO., LTD.	4.48	90	<i>Zanthoxylum piperitum</i>	NIPPON Funmatsu Yakuhin co, ltd	0.65
23	<i>Psidium guajava</i> L.	NIPPON Funmatsu Yakuhin co, ltd	4.24	91	<i>Plantago ovata</i>	MARUZEN PHARMACEUTICALS CO., LTD.	0.63
24	<i>Cassia obtusifolia</i>	NIPPON Funmatsu Yakuhin co, ltd	4.08	92	<i>Acanthopanax senticosus</i>	NIPPON Funmatsu Yakuhin co, ltd	0.61
25	<i>Scaphium affine</i>	NIPPON Funmatsu Yakuhin co, ltd	4.08	93	<i>Medicago sativa</i>	ASK INTERCITY CO., LTD.	0.60
26	<i>Rosmarinus officinalis</i> L.	ASK INTERCITY CO., LTD.	4.08	94	<i>Ilex paraguariensis</i> A.	ASK INTERCITY CO., LTD.	0.57
27	<i>Psidium guajava</i> L.	Ryukyu Bio Resource co. ltd	3.72	95	<i>Pseudocyonia sinensis</i>	NIPPON Funmatsu Yakuhin co, ltd	0.51
28	<i>Cornus officinalis</i>	NIPPON Funmatsu Yakuhin co, ltd	3.64	96	<i>Lepidium meyenii</i>	TOKIWA PHYTOCHEMICAL CO., LTD.	0.50
29	<i>Phellinus Linteus</i>	NIPPON Funmatsu Yakuhin co, ltd	3.58	97	<i>Valeriana officinalis</i> L.	ASK INTERCITY CO., LTD.	0.42
30	<i>Echinacea purpurea</i>	ASK INTERCITY CO., LTD.	3.56	98	<i>Piper methysticum</i>	ASK INTERCITY CO., LTD.	0.40
31	<i>Psidium guajava</i> L.	NIPPON Funmatsu Yakuhin co, ltd	3.51	99	<i>Aloe arborescens</i> Mill.	MARUZEN PHARMACEUTICALS CO., LTD.	0.36
32	<i>Psidium guajava</i> L.	OS INDUSTRY CO., LTD.	3.45	100	<i>Valeriana officinalis</i>	NIPPON Funmatsu Yakuhin co, ltd	0.35
33	<i>Psidium guajava</i> L.	MARUZEN PHARMACEUTICALS CO., LTD.	3.44	101	<i>Paullinia cupana Kunth</i>	NIPPON Funmatsu Yakuhin co, ltd	0.32
34	<i>Psidium guajava</i> L.	Bizen Chemical Co., Ltd.	3.32	102	<i>Sasa veitchii</i>	Matsuura Yakugyou Co. Ltd.	0.32
35	<i>Uncaria Tomentosa</i>	NIPPON Funmatsu Yakuhin co, ltd	3.3	103	<i>Maticaria chamomile</i>	NIPPON Funmatsu Yakuhin co, ltd	0.30
36	<i>Zingiber officinale</i>	NIPPON Funmatsu Yakuhin co, ltd	3.05	104	<i>Psidium guajava</i> L.	Ryukyu Bio Resource co. ltd	0.27
37	<i>Gardenia jasminoides</i>	NIPPON Funmatsu Yakuhin co, ltd	2.83	105	<i>Citrus unshiu</i>	NIPPON Funmatsu Yakuhin co, ltd	0.25
38	<i>Perilla frutescens</i>	Nippon Shinyaku Co. Ltd.	2.80	106	<i>Passiflora incarnata</i> L.	ASK INTERCITY CO., LTD.	0.23
39	<i>Panax notoginseng</i>	Matsuura Yakugyou Co. Ltd.	2.78	107	<i>Ziziphus zizyphus</i>	NIPPON Funmatsu Yakuhin co, ltd	0.22
40	<i>Glycyrrhiza glabra</i>	Matsuura Yakugyou Co. Ltd.	2.76	108	<i>Sasa veitchii</i>	MARUZEN PHARMACEUTICALS CO., LTD.	0.17
41	<i>Acanthopanax senticosus</i> Harms	Kenko Corporation	2.71	109	<i>Cucurbita</i>	NIPPON Funmatsu Yakuhin co, ltd	0.15
42	<i>Apocynum venetum</i> L.	Matsuura Yakugyou Co. Ltd.	2.68	110	<i>Melilotus officinalis</i>	NIPPON Funmatsu Yakuhin co, ltd	0.12
43	<i>Ficus carica</i>	NIPPON Funmatsu Yakuhin co, ltd	2.54	111	<i>Chelidonium majus</i> var. <i>asiaticum</i>	ASK INTERCITY CO., LTD.	0.10
44	<i>Camellia sinensis</i> (L.) Kuntze	NIPPON Funmatsu Yakuhin co, ltd	2.27	112	<i>Apium graveolens</i>	ASK INTERCITY CO., LTD.	0.08
45	<i>Arctium lappa</i> L.	ASK INTERCITY CO., LTD.	2.26	113	<i>Tilia europaea</i>	ASK INTERCITY CO., LTD.	0.08
46	<i>Salvia officinalis</i>	ASK INTERCITY CO., LTD.	2.25	114	<i>Aesculus hippocastanum</i>	ASK INTERCITY CO., LTD.	0.06
47	<i>Aspalathus linearis</i>	MARUZEN PHARMACEUTICALS CO., LTD.	2.18	115	<i>Urtica dioica</i>	ASK INTERCITY CO., LTD.	0.05
48	<i>Viscum album</i> L.	ASK INTERCITY CO., LTD.	2.14	116	<i>Zingiber officinale</i>	MARUZEN PHARMACEUTICALS CO., LTD.	0.05
49	<i>Equisetum arvense</i>	ASK INTERCITY CO., LTD.	1.97	117	<i>Lepidium meyenii</i>	NIPPON Funmatsu Yakuhin co, ltd	-0.02
50	<i>Acer nikoense</i> Maximowicz	NIPPON Funmatsu Yakuhin co, ltd	1.87	118	<i>Olea europaea</i>	ASK INTERCITY CO., LTD.	-0.14
51	<i>Rubus idaeus</i>	ASK INTERCITY CO., LTD.	1.85	119	<i>Garcinia cambogia</i>	ASK INTERCITY CO., LTD.	-0.17
52	<i>Artemisia princeps</i> Pampan.	MARUZEN PHARMACEUTICALS CO., LTD.	1.81	120	<i>Aloe arborescens</i> Mill.	MARUZEN PHARMACEUTICALS CO., LTD.	-0.18
53	<i>Houttuynia cordata</i>	MARUZEN PHARMACEUTICALS CO., LTD.	1.77	121	<i>Plantago asiatica</i> L.	NIPPON Funmatsu Yakuhin co, ltd	-0.25
54	<i>Cimicifuga racemosa</i>	Kenko Corporation	1.75	122	<i>Plantago lanceolata</i>	ASK INTERCITY CO., LTD.	-0.34
55	<i>Althaea officinalis</i> L.	ASK INTERCITY CO., LTD.	1.73	123	<i>Petroselinum crispum</i>	Matsuura Yakugyou Co. Ltd.	-0.36
56	<i>Diospyros kaki</i>	NIPPON Funmatsu Yakuhin co, ltd	1.69	124	<i>Valeriana officinalis</i>	NIPPON Funmatsu Yakuhin co, ltd	-0.52
57	<i>Cimicifuga racemosa</i>	Matsuura Yakugyou Co. Ltd.	1.66	125	<i>Harpagophytum procumbens</i>	NIPPON Funmatsu Yakuhin co, ltd	-0.54
58	<i>Eucommia ulmoides</i>	NIPPON Funmatsu Yakuhin co, ltd	1.64	126	<i>Perilla frutescens</i>	ORYZA OIL & FAT CHEMICAL CO., LTD.	-0.55
59	<i>Panicum miliaceum</i>	ASK INTERCITY CO., LTD.	1.50	127	<i>Hedera helix</i>	ASK INTERCITY CO., LTD.	-0.56
60	<i>Carthamus tinctorius</i> L.	NIPPON Funmatsu Yakuhin co, ltd	1.48	128	<i>Houttuynia cordata</i>	NIPPON Funmatsu Yakuhin co, ltd	-0.69
61	<i>Tilia europaea</i>	Kenko Corporation	1.36	129	<i>Psidium guajava</i> L.	Bizen Chemical Co., Ltd.	-0.70
62	<i>Avena fatua</i>	ASK INTERCITY CO., LTD.	1.33	130	<i>Daucus carota</i> L. var. <i>sativa</i> DC.	ALPS Pharmaceutical Ind. Co, Ltd	-0.77
63	<i>Humulus lupulus</i> L.	NIPPON Funmatsu Yakuhin co, ltd	1.29	131	<i>Gymnema sylvestre</i>	MARUZEN PHARMACEUTICALS CO., LTD.	-0.79
64	<i>Agaricus blazei</i> Murill	NIPPON Funmatsu Yakuhin co, ltd	1.28	132	<i>Cassia obtusifolia</i>	MARUZEN PHARMACEUTICALS CO., LTD.	-1.15
65	<i>Ginkgo biloba</i>	Matsuura Yakugyou Co. Ltd.	1.28	133	<i>Panax ginseng</i> C.A. Mey.	MARUZEN PHARMACEUTICALS CO., LTD.	-1.36
66	<i>Morus bombycis</i> Koidz.	NIPPON Funmatsu Yakuhin co, ltd	1.27	134	<i>Citrus unshiu</i>	MARUZEN PHARMACEUTICALS CO., LTD.	-1.51
67	<i>Pfafia paniculata</i>	NIPPON Funmatsu Yakuhin co, ltd	1.26	135	<i>Urtica dioica</i>	NIPPON Funmatsu Yakuhin co, ltd	-2.75
68	<i>Cichorium intybus</i>	NIPPON Funmatsu Yakuhin co, ltd	1.23	136	<i>Echeveria glauca</i>	Beijing Kingsun Aima Technology Company Limited	-3.70

affinity of Hb *in vivo*. *Allium sativum* is a harmless spice that is commonly used in meals throughout the world. To verify whether *Allium sativum* affects the oxygen affinity of Hb *in vivo*, we conducted an oral administration test on volunteers. Healthy male volunteers (n=6) took 1,000mg of *Allium sativum* extract, and we measured their P_{50} before and 2 hours after the oral administration of *Allium sativum* extract. P_{50} was significantly higher at 2 hours after the oral administration of *Allium sativum* ex-

tract than before it (Figure-2A) . We next investigated the long-term effects of *Allium sativum* in human subjects. Healthy male volunteers (n=8) took 1,000 mg of *Allium sativum* extracts every day for 3 weeks, and their P_{50} and blood concentrations of 2,3-DPG were measured every week for 4 weeks. Interestingly, P_{50} was increased at 2 and 3 weeks after the start of the oral administration of *Allium sativum* extract and had returned to the baseline level at a week after the cessation of the

Figure-1 A, Effects of various plant extracts on the oxygen affinity of Hb. ΔP_{50} after treatment with 136 plant extracts *in vitro* screening. B, Effects of *Allium sativum* on the oxygen affinity of Hb (n=3). * p<0.05.

administration of *Allium sativum* (Figure-2B upper). Concomitantly, the blood concentrations of 2, 3-DPG were decreased at 3 weeks after the start of *Allium sativum* extract administration and had recovered to the baseline at a week after the cessation of *Allium sativum* extract administration (Figure-2B bottom). These results suggested that *Allium sativum* extract elevated P₅₀ and decreased the blood concentrations of 2, 3-DPG after its oral administration in human subjects.

Discussion

Previous reports have shown that bezafibrate, propranolol, and RSR13 modulate the oxygen affinity of Hb^{7) 13) 14)}. In the present study, we found that some plant extracts altered the oxygen affinity of Hb (Figure-1A and Table-1). Among them, *Allium sativum* most strongly decreased the oxygen affinity of Hb (Figure-1A, B and Table-1). Furthermore, *Allium sativum* elevated P₅₀ and decreased the blood concentration of 2, 3-DPG after its oral administration *in vivo* (Figure-2A). These results suggest that *Allium sativum* extract decreases the oxygen affinity of Hb as an allosteric modifier. Since the oxygen affinity of Hb was altered by many factors *in vivo*^{4)~12)}, we can not rule out the possibility that *Allium sativum* extract modulates metabolic parameters *in vivo*.

The decrease in the oxygen affinity of Hb enhances O₂ release in the peripheral tissues, but in

some conditions it reduces O₂ binding in the alveoli of the lung. Farber *et al.*³⁾ argued that the cardiac index is inversely correlated with P₅₀ during hard work, and thus more O₂ is utilized to produce the less cardiac work under low Hb oxygen affinity conditions in healthy humans. In the present study, *Allium sativum* slightly but significantly decreased the oxygen affinity of Hb and the blood concentrations of 2, 3-DPG (Figure-2B). We suggest that the oxygen affinity of Hb plays an important role in reducing cardiac workload and improving physical activity in peripheral tissues. We also suggest that P₅₀ is optimized *in vivo*, which maximizes the delivery of oxygen from the lungs to the peripheral tissues.

Allium sativum may be beneficial for therapy in patients with impaired cardiac function. Furthermore, the reduction in the oxygen affinity of Hb induced by *Allium sativum* can be applied to improving physical activities of daily living and cellular metabolism.

In conclusion, we have demonstrated that some dietary factors extracted from plants alter the oxygen affinity of Hb and the blood concentrations of 2, 3-DPG. These findings imply that environmental factors such as exercise, dietary factors, and allosteric modifiers are able to enhance physical activity by modulating the oxygen affinity of Hb.

Acknowledgements

We thank Drs. H. Nojiri, T. Baba, H. Kuwahara,

Figure-2 Effects of *Allium sativum* on the oxygen affinity of Hb and the blood concentrations of 2, 3-DPG *in vivo*.

A, P₅₀ at 2 hours after the oral administration of *Allium sativum* extract in the control subjects. B, Long-term changes in P₅₀ (Upper) and the blood concentrations of 2, 3-DPG (Bottom) after the oral administration of *Allium sativum* extract in the control subjects. Values are shown as means $\pm$ SD. (statistical significance indicated by an asterisk.) *p<0.05.

S. Kawakami, Y. Abe, C. Koyama, Y. Kondo, and T. Ikegami for their technical assistance and helpful discussions. We are grateful to W. Zhou for preparing the manuscript.

Grants

This study was supported by a Grant-in-Aid for the Development of Innovative Technologies from the Japan Science and Technology Corporation.

References

- 1) Shirasawa T, Izumizaki M, Suzuki Y, *et al* : Oxygen affinity of hemoglobin regulates O₂ consumption, metabolism, and physical activity. *J Biol Chem*, 2003 ; **278** : 5035~5043.
- 2) Eichelbronner O, Sielenkamper A, D'Almeida M, *et al* : Effects of f(i) on hemodynamic responses and O₂ transport during rsr13-induced reduction in p(50). *Am J Physiol*, 1999 ; **277** : H290~H298.
- 3) Farber MO, Sullivan TY, Fineberg N, *et al* : Effect of decreased O₂ affinity of hemoglobin on work performance during exercise in healthy humans. *J Lab Clin Med*, 1984 ; **104** : 166~175.
- 4) Hsia CC : Respiratory function of hemoglobin. *N Engl J Med*, 1998 ; **338** : 239~247.
- 5) Benesch R, Benesch RE : The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. *Biochem Biophys Res Commun*, 1967 ; **26** : 162~167.
- 6) Humpeler E, Vogel S, Schobersberger W, *et al* : Red cell oxygen transport in man in relation to gender and age. *Mech Ageing Dev*, 1989 ; **47** : 229~239.
- 7) Abraham DJ, Wireko FC, Randad RS, *et al* : Allosteric modifiers of hemoglobin : 2-[4-[(3, 5-disubstituted anilino) carbonyl] methyl] phenoxy]-2-methylpropionic acid derivatives that lower the oxygen affinity of hemoglobin in red cell suspensions, in whole blood, and in vivo in rats. *Biochemistry*, 1992 ; **31** : 9141~9149.
- 8) Veicsteinas A, Samaja M, Gussoni M, *et al* : Blood O₂ affinity and maximal O₂ consumption in elite bicycle racers. *J Appl Physiol*, 1984 ; **57** : 52~58.
- 9) Guarnone R, Centenara E, Barosi G : Performance characteristics of hemox-analyzer for assessment of the hemoglobin dissociation curve. *Haematologica*, 1995 ; **80** : 426~430.
- 10) Schmidt W, Dahnert HW, Correa R, *et al* : Blood gas transport properties in endurance-trained athletes living at different altitudes. *Int J Sports Med*, 1990 ; **11** : 15~21.
- 11) Braumann KM, Boning D, Trost F : Oxygen dissociation curves in trained and untrained subjects. *Eur J Appl Physiol Occup Physiol*, 1979 ; **42** : 51~60.
- 12) Takagi Y : [2, 3-diphosphoglycerate (2, 3-dpg)]. *Nippon Rinsho*, 2004 ; **62** : S820~S822.
- 13) Perutz MF, Poyart C : Bezafibrate lowers oxygen affinity of haemoglobin. *Lancet*, 1983 ; **2** : 881~882.
- 14) Schrumpf JD, Sheps DS, Wolfson S, *et al* : Altered hemoglobin-oxygen affinity with long-term propranolol therapy in patients with coronary artery disease. *Am J Cardiol*, 1977 ; **40** : 76~82.

1) Shirasawa T, Izumizaki M, Suzuki Y, *et al* : Oxygen af-

抄 録

Dietary factors alter the oxygen affinity of hemoglobin

ヘモグロビンの酸素親和性に影響を及ぼす食物由来の因子に関する研究

櫻 本 浩 司
HIROSHI SAKURAMOTO

内 丸 仁
JIN UCHIMARU

内 藤 久 士
HISASHI NAITO

和 賀 俊 明
TOSHIKI WAGA

砂 山 聡
SATOSHI SUNAYAMA

清 水 孝 彦
TAKAHICO SHIMIZU

黒 澤 尚
HISASHI KUROSAWA

白 澤 卓 二
TAKUJI SHIRASAWA

目的：ヘモグロビンの酸素親和性の変化が生体に及ぼす役割は、いまだ議論のあるところである。われわれはマウスにおいてヘモグロビンの酸素親和性が、赤血球生成、末梢組織での酸素消費および身体活動性を制御している可能性を報告した。そこでわれわれはヘモグロビンの酸素親和性を変動させる因子を探るため、環境因子、特に食物由来の因子を探索した。

対象および方法：健康男性ボランティアから血液を採取し、生成したヘモグロビンと様々な服用可能な植物抽出液を混ぜ、 P_{50} 値をHEMOX-ANALYZERで測定した。さらに、健康男性ボランティアが*Allium sativum*抽出物質1,000mgを服用し、その前後での P_{50} 値および2,3-diphosphoglycerate (2,3-DPG)値を測定した。

結果：スクリーニングテストにおいて、多種の植物由来の抽出物質が P_{50} 値を変動させた。この中から*Allium sativum*を候補として選択し、*in vitro*において有意に P_{50} 値を上昇させることを認めた。さらに*in vivo*において*Allium sativum*抽出物質1,000mg服用後2時間で P_{50} 値は有意に上昇した。さらに*Allium sativum*抽出物質1,000mgを長期服用後 P_{50} は上昇し2,3-DPG値は低下し、服用中止後には P_{50} 値は減少し2,3-DPG値は上昇した。

結論：ヘモグロビン酸素親和性は*Allium sativum*抽出物質のような食物因子によって変化していることが示された。この作用はヘモグロビンへの直接的作用の可能性が示唆された。

キーワード：ヘモグロビン酸素親和性、食物因子、植物抽出物質、*Allium sativum*、2,3-DPG