The Effects of Red Guava Juice Consumption (*Psidium Guajava L.* Cultivar Red) on Erythrocyte Fragility during Aerobic Exercise for The Beginner

Sugiarto[1]*, Achmad Djunaidi[2] and Zaenal Muttaqien Sofro[2]

[1] *Department of Sport Science, Faculty of Sport Science, Semarang State University-Indonesia*
[2] *Department of Physiology, Faculty of Medicine, Gadjah Mada University-Indonesia*

**Corresponding author, email: sugiartocahcilik@staff.unnes.ac.id*

Abstract

Aerobic exercise for the beginner is potentially causing increase production of free radical compound. Free radicals damage erythrocyte membranes, decreasing the number of erythrocytes. The exogenous antioxidant supplement is necessary, one of which is vitamin C from the guava. The research was designed as experimental-nonrandomized pre-post test control group. Sixteen students were divided into 2 groups red guava juice and mineral water treated groups. Both groups were asked to perform aerobic exercise of 30-minute, every 2 days for 27 days. Blood samples were taken 4 times. T-test and Repeated Anova test were used for data analysis. There is not significant difference on the level of erythrocyte fragility in guava juice and mineral water treated groups (repetead anova p>0,05). Consumption of red guava juice of aerobic exercise does not significantly inhibit erythrocyte fragility.

Keywords: *aerobic exercise, red guava juice, erythrocyte fragility.*

INTRODUCTION

Physical exercise is a form of physical activity which increase physical fitness (Giam and Teh, 1993; Kartawa, 1997). Physical exercise is advantageous, but also risky (Rost, 1993). Tissue damage may occur because of either maximum or submaximum physical exercise (Gervino and Douglas, 1993). Light until moderate intensity of aerobic exercise will boost physical health and fitness. Moderate intensity of physical exercise generates cardiovascular capacity and minimizes injury. On the other hand, hard aerobic exercise can cause injury and harm body immunity. During maximum physical exercise, body's oxygen consump-tion increases 20 times and muscle's oxygen consumption increases 100 times. In-creasing oxygen consumption leads to a release of free radicals which can damage cells. In order to prevent cell damage, antioxidant is needed (Hajar, 2004).

Physical exercise which emphasizes on increasing physical performance can trigger body homeostasis disruption, as well as tissue and cell membrane damage which is caused by malondi-aldehyde (MDA) compound (Balakrishnan and Anuradha, 1998; Ji, 1999; Halliwell and Gutteridge, 1999). Tissues damage is caused by oxygen free radicals gained from physical exercise (Sjodin *et al*., 1990). Free radical is also one of the factors which damages erythrocyte. In the period of physical exercise, mechanical trauma which is caused by muscle contraction happens to erythrocyte. In the period of physical exercise, body temperature increases, body hydrational fluids decrease, hemoconcentration and oxidation stress happen and cause erythrocyte hemolysis during aerobic exercise or cooling down (Senturk *et al*., 2001). Aerobic exercise for the beginner can cause excessive muscle contraction, so that additional motor unit recruitment is needed. Musculosceletal contraction is an electric, chemical, and mechanical process which consists of six steps called cross bridge cycle. For an untrained person, power stroke, sliding filament, and disconnecting on cross bridge cycle are mechanical traumas which can trigger muscle injury (Len *et al*., 2002).

The increase of free radicals and tissues damage in physical exercise are caused by increasing energy consumption during the exercise. Where as energy supply in intracellular is limited, energy production process happens continuously through the process of oxidation, Krebs cycle, and electron transportation. The process of energy production needs oxygen, so the need of oxygen drastically increases (Sjodin *et al*., 1990). The increase of energy need during aerobic exercise often multiplies the oxygen need. This oxygen need approximately rises by 10-15 times more than normal condition (Sen, 1995). Physical exercise is related to oxygen consumption especially in active tissues, and oxygen increase is related to the increase of free radicals production (Brites *et al*., 1999).

Hydrogen peroxide free radicals compound can damage erythrocyte membrane, so that the age of erythrocyte becomes shorter. If the production of hydrogen peroxide continues, it will cause anemia due to erythrocyte lysis. Anemic condition causes oxygen transportation to cells to decrease, so that it affects cells metabolism which makes cells difficult to regenerate (Guyton and Hall, 2008).

Body has protection systems which are endogenous antioxidant components in the form of enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase which can remove free radicals enzymatically to fight free radicals. Body also has exogenous antioxidants which amount depends on diet consumption. Although body can naturally overcome the increase of free radicals, but in a hard condition such as maximum physical exercise, endogenous antioxidants are not sufficient, so that body needs exogenous antioxidants. Inspite of it, some research shows that moderate and regular physical exercise can improve endogenous antioxidants defense system and protect the body from free radicals which are produced by the body during its physical exercise (Harjanto, 2004).

The increase of erythrocyte fragility is also possible to happen because of disturbance in fulfilling energy needed for maintaining erythrocyte's structure and function integrity, so that cells are unable to prevent oxidative damage. Now a days, people tend to use natural substances to boost physical stamina in doing physical exercise. People especially like fruit as natural supplement. Red guava is widely consumed, easily got, and well known by the people of Indonesia. Red guava contains vitamin C, kalium, β-carotene, vitamin E, Fe, Se, Cu, zinc, lycopene, lutein, xanthine, cryptoxanthin, zeaxanthin, ellagic acid, anthocyanidin, quercetin, and lignin (Astawan, 2008). The objective of this research is to assess the ability of guava to lower the levels of plasma MDA, increase plasma vitamin C and inhibit the increase of erythrocyte fragility during aerobic exercise for the beginner.

Aerobic exercise is physical exercise which is done with oxygen, it is the ability of the body to use sufficient oxygen to fulfill the body's need in doing physical exercise. Excessive physical exercise can damage erythrocyte cell membrane. The evidence of the damage because of oxidation during physical exercise depends on parts of body trained and levels of practice. Hard physical exercise for untrained person enables the damage due to oxidative stress. Hard physical exercise produces extra free radicals, so that the body needs antioxidants (Cooper, 2001).

A 30-60 minute physical exercise is more effective compared to a 10-15 minute physical exercise. The suggested duration of minimal physical exercise is 20 minutes, and it will be more effective if it is done for 30-60 minutes. It is suggested to do physical exercise from 3 to 5 times a week. The duration of the exercise program can be for 3-4 weeks. However, most of researchers suggest that physical exercise program is done for 5-10 weeks (Murray and Udermann, 2003).

Erythrocyte has simpler structure compared to other human cells. Erythrocyte is a membrane that covers hemoglobin solution and does not have cell organs such as mitochondria, lysosomes, or golgi apparatus (Temiz *et al*., 2000). The normal number of erythrocytes of a man is 4,6-6,2 million/µL. The age of a normal erythrocyte is 120 days, so less than 1% of erythrocyte population is replaced every day. Erythrocyte cell membrane has double layers which consist of less than 50% lipid and 50% protein. Erythrocyte's internal composition is protected by a mechanism which needs energy (Guyton and Hall, 2008). In an aerobic exercise, erythrocyte may experience oxidative stress due to the increase of MDA (Temiz *et al*., 2000) level and erythrolysis (Harjanto, 2004; Duthie *et al*., 1990). The recovery from erythrocyte oxidative stress due to physical exercise may need long time. Erythrocyte is still vulnerable to oxidative stress up to 5 days after physical exercise (Duthie *et al*., 1990).

Body has antioxidant defense mechanism to prevent oxidative stress. Antioxidants which belong to enzymatic antioxidants are SOD, glutathione peroxidase, and catalase, while the non-enzymatic ones are vitamin E (α-tocopherol), vitamin C (ascorbic), β-carotene, glutathione, flavonoids, uric acid, and ceruloplasmin. Aerobic exercise can cause oxidative stress on many kinds of antioxidants like reduced Glutathione (GSH), catalase, Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and vitamin C. This can lower antioxidants activity and its recovery may take varied length of time. Plasma vitamin C increases during physical exercise, but it can decrease for 48 hours after the exercise (Clarkson and Thompson, 2000).

Vitamin C decomposes superoxide and hydrogen peroxide well. Human cannot self-synthesize vitamin C, so human needs sufficient supply of it. Vitamin C is soluble in water. Vitamin C is easily damaged by heat, light, oxidation, and alkaline condition (Murray and Udermann, 2003).Vitamin C can decompose oxidants including H_2O_2, O_2, OH^-, and ROO^- (Nielsen *et al*., 1997).

Orally consumed, vitamin C with low dose is well absorbed, but its absorption decreases as the dose increases. Its bioavailability after oral consumption is 87% for 30 mgs, 80% for 100 mgs, 72% for 200 mgs, and 63% for 500 mgs. If the dosage is 1250 mgs, less than 50% of it will be absorbed and most of the absorbed dosage will be discarded through urine. Vitamin C does not bind protein and is filtered and reabsorbed by kidney in healthy condition, but it disappears in hemodialysized patient. Vitamin C can be seen in urine when the dosage is more than 100 mgs/day. Decreasing bioavailability and excretion through kidney preserve the level of plasma vitamin C to be less than 100 µmol/L, even in oral consumption of 1000 mgs (Padayatty and Levine, 2001).

Taking 60 mgs of vitamin C every day will preserve 1,5 mg supply in the body. Bigger supply can be gained by taking more doses of vitamin C, but excessive dosage of vitamin C will be excreted through urine. In this condition the concentration of plasma vitamin C is 1,5 mgs/dL. When its consumption is insufficient or somebody suffers from thrush, excretion of vitamin C through urine decreases up to the undetected level (WHO, 2004).

Fresh red guava contains high nutrition and complete composition. Red guava contains quite large amount of vitamin C. The amount of vitamin C in red guava is twice bigger than in lemon which is only 49 mgs per 100 grams and vitamin C in red guava is a very effective antioxidant (Parimin, 2006).

MATERIAL AND METHOD

This design research is experimental, nonrandomized pre-post test control group. The subjects of the research are 2 groups of 8 people who are healthy volunteers followings are the inclusion and the exclusion criteria. The research material is red guava (*P. Guajava L.* Cultivar Red) which is almost ripe with yellowish peel. The subjects are given red guava juice made from 800 grams of red guava and 1200 mLs of water (trade mark: Aqua), so that every subject consumes 240 mLs red guava juice.

The determination of erythrocytes fragility is with Osmotic Fragility Test method (Kraus *et al.*,1997). Equipment used is reaction tube which size is 1 x 12 cm, measuring pipette of 5 mL, U-1800 Spectrofotometer Hitachi, micropipet, beaker, parafin, 10 mL syringe volume. Reagent used is buffer saline emulsion which concentrations are 0,10%, 0,20%, 0,30%, 0,35%, 0,40%, 0,45%, 0,50%, 0,55%, 0,60%, 0,65%, 0,75% and 0,90%, aquades, tissue and blood sample which edta coagulant has been given. The procedure is preparing 13 reaction tubes which size is 1 x 12 cm, 50 µL blood sample is put into each tube, putting 5 mL buffer saline mixture into reaction tube 1 up to 12 which concentrations are as follow; reaction tube 1 (0,90%), reaction tube 2 (0,75%), reaction tube 3 (0,65%), reaction tube 4 (0,60%), reaction tube 5 (0,55%), reaction tube 6 (0,50%), reaction tube 7 (0,45%), reaction tube 8 (0,40%), reaction tube 9 (0,35%), reaction tube 10 (0,30%), reaction tube 11 (0,20%), reaction tube 12 (0,10%), reaction tube 13 (5 mL aquades), reaction tube is covered with parafin, then sample and mixture of buffer saline are inverted, incubation in a room temperature for 30 minutes, sentrifuse for 5 minutes which speed is 1200 g, lysis red blood cell fraction for each concentration of buffer saline mixture is measured at spectrofotometer which wave length is 540 nm.

Sample absorbance data is processed to get lysis percentage from each red blood cell fraction. Lysis percentage pattern is (absorbance sample reduced by minimum absorbance) divided by (maximum absorbance reduced by minimum absorbance) multiplied with 100%. Lysis starting mixture is signed with the beginning of the appearance of red color (<50% lysis) and lysis is completed if the color of the mixture is red (>50%). The percentage of completed lysis is seen at buffer saline mixture concentration of 0,40% which is on the 8^{th} tube.

The result of the research is analyzed by using SPSS program for Windows. Erythrocyte fragility of the two groups are analyzed by using t-test (p<0,05). The disparity in measurement of each group is analyzed by using Repeated Anova test.

RESULTS AND DISCUSSION

Average value, the erythrocyte fragility of red guava group and mineral water group decreases from the condition before the first aerobic exercise to the condition after the 7^{th} and 14^{th} aerobic exercises. The results of t-test shows that there are differences in erythrocyte fragility between two groups (p>0,05) and Repeated Anova test analysis show that there are disparities between two groups (p>0,05).

Aerobic exercises during the research are done in surroundings temperature of 26°C-27°C. After the aerobic exercise, the subjects from red guava juice lose their weight by 0,35% and the subjects from mineral water group lose their weight by 0,33%. The body temperature of both groups after the exercise increases by 0,2°C and 0,3°C. The increase of temperature and perspiration as the main mechanism of body regulation is caused by the increase of body metabolism when doing aerobic exercise (Grucza *et al.*, 1987).

Table 1. Analysis results of erythrocyte fragility (%)

Result	Group		p value*	p value**	
	G-1 (n=8)	G-2 (n=8)		G-1 (n=8)	G-2 (n=8)
Before the1st AE	81,89±10,56	81,97±16,42	0,991	0,800	0,897
After the 1st AE	83,85±10,17	83,23±3,08	0,873		
After the 7th AE	79,89±13,57	85,44±14,08	0,435		
After the 14th AE	80,48±8,81	85,39±7,29	0,245		

** t-test; ** Repeated Anova Test; AE=Aerobic Exercises*

Water and electrolyte discharged along with the sweat is hypotonic relative to the body hydrational fluids and causing the increase of extracellular hydrational fluids osmotic pressure. It makes intracellular hydrate moves to extracellular and then, it causes dehydration in all body compartments (Casaburi, 1992; Wilmore and Costill, 2004; Williams, 2004).

The imbalance between free radicals and antioxidant defense in the body starts oxidative stress. Low level of antioxidants can increase the oxidative cellular damage, so that it increases lipid peroxidation in the form of malondialdehyde. Free radicals damage cells by attacking and destroying PUFA chain, the main component of either cellular or subcellular membrane phosphor lipid.

The increase of oxygen consumption which is caused by aerobic exercise, 2-5% of the oxygen is transported by hemoglobin and processed in mitochondria is predicted to be changed into peroxide compound (Halliwell and Gutteridge, 1999). In this research, the MDA levels increase before the subjects are given vitamin C which is gained from red guava juice. It is estimated that chain process of free radicals formation which is from polyunsaturated fatty acid undergoes degradation. This process starts from free radicals which take hydrogen from PUFA to form lipid radical L^- (initiation phase). Lipid radicals (L^-) which are released to membranes cause free radicals chain process which is with the existence of oxygen (O^2) produces abundant peroxyl radicals (LO_2^-) (propagation phase). If there is vitamin E, LO_2^- is transfomed into LOOH and EOOL which are less reactive, and then, peroxidation reaction stops.

The increase of vitamin C significantly correlates with cortisol increase. The researches assume that ascorbic acid correlates with discharge of cortisol and adrenal gland (Clarkson and Thompson, 2000). Ascorbic acid increases 5 minutes after half-marathon run and it is back to normal after 24 hours. Some studies say that 5 minutes after physical exercise will decrease 6% concentration (Siswanto *et al*., 2001).

The higher MDA level after the first aerobic exercise causes cell membrane unprotected from peroxidation by free radicals, so that it brings about erythrocyte fragility. But after the consumption of red guava juice for 2 weeks, fragility increase can be prevented, so that the fragility decreases although it is not statistically significant.

Erythrocyte fragility of red guava juice group is lower than the one of mineral water group because vitamin C and vitamin E in red guava protect them from the process of polyunsaturated fatty acid peroxidation. If the vitamin C and E are not sufficient to fight free radicals, cell membranes become vulnerable to oxidative damage. Membranes become fragile and stiff, and erythrocyte will be fragile on its way through narrow vessels.

Additional vitamin C as antioxidants is needed to inhibit and prevent oxidative stress which leads to cells damage (Cooper, 2001). Vitamin C plays a role in membrane stability, so that it can prevent erythrocyte membrane damage and keep the erythrocyte fragility low. In aerobic exercise for the beginner, erythrocyte may under go oxidative stress as the result of MDA levels increase and erythrolysis. Erythrocyte oxidative recovery due to physical exercise takes 5 days (Harjanto, 2004).

Influencial factors of erythrocyte membrane fragility are nutrient status, environment temperature and genes. Nutrient status will affect the composition of erythrocyte membrane structure and as a result, it affects the type and the nature of protein of the person. In this research, the number of erythrocytes and the level of hemoglobin do not change significantly.

CONCLUSION

From this research, it is concluded that consumption of red guava juice for the beginner of aerobic exercise does not significantly inhibit erythrocyte fragility.

ACKNOWLEDGMENT

The writer thanks all people who have helped the writer do this research- Achmad Djunaidi, dr., S.U., AIFM., Zaenal Muttaqien Sofro, dr., M.D., Sport&Circ. Med., AIFM., Denny Agustiningsih, dr., M.Kes., AIFM. as an advisor, Prof. Dr. Endriatmo Soetarto, M.A. as the head of STPN Yogyakarta and H.M. Soewarno M. Serad as the Public Affairs Director of PT. Djarum. The writer also thanks Ms. Budi Lestari, Ms. Ida Dorojatun, Ms. Yuan Leni, Mr. Dwi Nuh Riyadi, and Mr. Yuswibowo as a laboratory assistant of biochemistry laboratory, Faculty of Medicine, Gadjah Mada University.

REFERENCES:

Astawan, M., 2008, *Sari Kesehatan Keluarga: Sehat dengan Buah*, PT. Dian Rakyat, Jakarta.

Balakrishnan, S.D., Anuradha, C.V., 1998, Exercise Depletion of Antioxidants and Antioxidant Manipulation, *Cell. Biochem. Funct.,* 16(*4*):269-275.

Brites, F.D., Avelson, P.A., Christiansen, M.G., Nicol, F.M., Basilico, J.M., Wikinski, R.W., and Liesuy, S,F., 1999, Soccer Player under Regular Training Show Oxidative Stress but an Improved Plasma Antioxidant Status, *Clin. Sci.,* 96(*4*):381-385.

Casaburi, R., 1992, *Principles of Exercise Training*, American College of Chest Physician.

Clarkson, P.M. and Thompson, H.S., 2000, Antioxidant: What Role do they Play in Physical Activity and Health, *Am. J. Clin. Nutr.,* 72(*2*):637s-646s.

Cooper, K.H., 2001, *Sehat tanpa Obat: 4 Langkah Revolusi Antioksidan,* translate from Textbook of Antioxidant Revolution, Kaifa, Bandung.

Duthie, G.G., Robertson, J.D., Maughan, R.J., and Morrice, 1990, Blood Antioxidant Status and Erythrocyte Lipid Peroxidation following Distance Running, *Arch. Biochem. Biophys.,* 282(*1*):78-83.

Duthie, G.G., Robertson, J.D., Maughan, R.J., and Morrice, 1990, Blood Antioxidant Status and Erythrocyte Lipid Peroxidation following Distance Running, *Arch. Biochem. Biophys*., 282(*1*):78-83.

Gervino, E.V. and Douglas, P.S., 1993, The Benefits and Risk of Endurance Exercise, *Int. J. Sport. Cardiol.,* (*2*):73-78.

Giam, C.K. and Teh, K.C., 1993, *Ilmu Kedokteran Olahraga,* Binarupa Aksara, Jakarta.

Grucza, R., Szezypaczewska, M. and Kozlowski, S., 1987, Thermoregulation in Hyperhydrated Men during Physical Exercise, *Eur. J. Appl. Physiol*., 56(*5*):603-607.

Guyton, A.C. and Hall, J.E., 2008, *Buku Ajar Fisiologi Kedokteran (Textbook of Medical Physiology),* 11th ed., Alih Bahasa: Irawati, dkk., EGC, Jakarta.

Hajar, S., 2004, *Kadar MDA Plasma dan Hemoglobin Darah setelah Pemberian Vitamin C Pada Manula di Daerah Dataran Tinggi dan Dataran Rendah* [thesis], Program Pascasarjana Universitas Gadjah Mada, Yogyakarta.

Halliwell, B. and Gutteridge, J.M.C., 1999, *Free Radical in Biology and Medicine*, 3rd Edition, Oxford University Press Inc., New York.

Harjanto, 2004, Recovery From Oxidative Stress in Physical Exercise, *JKY,* 12(*3*): 81-87.

Hubner, W.E., Panczenko, K.B., Lerezak, K., and Po'Snik, J., 1994, Effects of Graded Treadmill Exercises on the Activity of Blood Antioxidant Enzymes, Lipid Peroxides and non Enzymatic Antioxidant in long Distance Skiers, *Biol. Sport*., 11(*4*):217-226.

Ji, L.L., 1999, Antioxidants and Oxidative Stress in Exercise, *Society for Experimental Biology and Medicine*, (*222*): 283-292.

Kanter, M.M., Lesmes, G.R., Kaminsky, L.A., La Ham-Saeger, J. and Nequin, N.D., 1988, Serum Creatinin Kinase and Lactate Dehydrogenase Changes following an Eighty Kilometer Race, *Eur. J. Appl. Physiol*., 57(*1*):60-63.

Kanter, M.M., Nolte, L.A. and Holloszy, J.O., 1993, Effect of Antioxidant Vitamin Mixture on Lipid Peroxidation at rest and post Exercises, *J. Appl. Physiol*., 74(*2*): 965-969.

Kartawa, H., 1997, Pengaruh Program Senam Kesegaran Jasmani terhadap Tingkat Kesegaran Jasmani Usia Pertumbuhan Cepat dan Usia Dewasa, *M. Med. Indones.,* 32(*4*);191-196.

Kraus, A., Roth, H.P. and Kirchgessner, M., 1997, Supplementation with Vitamin C, Vitamin E or β-Carotene Influences Osmotic Fragility and Oxidative Damage of Erythrocytes Zinc Deficient Rats, *J. Nutr*., 127:1290-1296.

Len, J., Davies, C.T., Young, K., 2002, Changes in Indicators of Inflamation after Eccentric Exercise of the Elbow Flexors, *Med. Sci. Sports. Exerc.,* (*25*):236-239.

Maughan, R.J., Donnelly, A.E., Gleeson, M., Whiting, P.H., and Walker, K.A., 1989, Delayed Onset Muscle Damage and Lipid Peroxidation in Man after a Downhill Run, *Muscle Nerve*, 12(*4*):332-336.

Maxwell, S.R.J., Jakeman, P., Thompson, H., 1993, Changes in Plasma Antioxidant Status during Eccentric Exercise and the Effect of Vitamin Supplementation, *Free. Radic. Res. Commun*., 19:191-202.

Murray and Udermann, E.B., 2003, Fluid Replacement: A Historical Prespective and Critical Review, *Int. Sports. J*., 7(2):58-64.

Nielsen, F., Mikkelsen, B.B., Nielsen, J.B., Andersen, H.R. and Grandjean, P., 1997, Plasma Malondialdehyde as Biomarker for Oxidative Stress: Reference Interval and Effects of Life-Style Factors, *Clin. Chem*., 43(*7*):1209-1214.

Padayatty, J. And Levine, M., 2001, New Insight into the Physiology and Pharmacology of Vitamin C, *Can. Med. Assoc. J*., 164(*3*):353-355.

Parimin, S.P., 2006, *Jambu Biji, Budidaya dan Ragam Pemanfaatannya*, Penebar Swadaya, Jakarta.

Rokitzki, L., Logemann, E., Sagredos, A.N., Murphy, M., Wetzel-Roth, W., and Keul, J., 1994, Lipid Peroxidation and Antioxidant Vitamins under Extreme Endurance Stress, *Acta. Physiol. Scand*., 151(*2*):149-158.

Rost, R.E., 1993, Cardiovascular Incidents during Physical Activity, *Int. J. Sport Cardiol.,* (*2*):11-18.

Sahlin, K., Cizinsky, S., Warholm, M., and Hoberg, J., 1992, Repetitive Static Muscle Contractions in Humans: a Trigger of Metabolic and Oxidative Stress, *Eur. J. Appl. Physiol*., 64(*3*):228-236.

Sen, C., 1995, Oxidants and Antioxidants in Exercise, *J. Appl. Physiol*., 79(*3*):675-686.

Senturk, U. K., Gunduz, F., Kuru, O., Aktekin, M. R., Kipmen, D., Yalcin, O., BorKucukatay, M., Yesilkaya, A. and Baskurt, O. K., 2001, Exercise-

Induced Oxidative Stress affects Erythrocytes in Sedentary Rats but not Exercise Trained Rats, *J. Appl. Physiol.,* 91:1999-2004.

Sjodin, B., Hellsten-Westing, Y., Apple, F.S., 1990, Biochemical Mechanism for Oxygen Free Radicals Formation during Exercise, *Sports. Med.,* 10(*4*):236-254.

Sumida, S., Tanaka, T., Kitao, H., and Nakadomo, F., 1989, Exercise-Induced Lipid Peroxidation and Leakage of Enzyme before and after Vitamin E Supplementation, *Int. J. Biochem*., 21(*8*):835-838.

Tiezt, N.W., Bhagavan, N.V., Conn, R.B., Kachmar, J.F., Pruden, E.L. and Whitley, R.J., 1986, *Texbook of Clinical Chemistry*, W.B. Saunders Company, Philadelphia.

WHO, 2004, *Vitamin and Mineral Requirements in Human Nutrition*, 2th Ed., Thailand, Bangkok.

Williams, M.H., 2005, *Nutrition for Health, Fitness & Sport,* 7th Ed., MacGraw-Hill, New York.

Wilmore, J.H. and Costill, D.L., 2004, *Physiology of Sport and Exercise*, 3th Ed., Human Kinetics Publisher, United States.

Winarsi, H., 2007, *Antioksidan Alami dan Radikal Bebas*, Kanisius, Yogyakarta.