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To,

Andang Miatmoko, Ph.D.
The Scientific Committee of The 2nd Surabaya Int. Symp.
Stem Cell Research and Development Centre\
Institute of Tropical Diseases, IInd Flor,
Campus C Universitas Airlangga,
Mulyorejo-Surabaya, 60115
Indonesia

Dated : 25 October 2019

Dear Sir,

I am pleased to inform you that your following 37 papers have been accepted for publication in ***Biochemical and Cellular Archives***, Vol. 19, Supplement 2, December 2019.

1. Natriumdiclofena (ND) degrades fibrosis on human lens epithelial cell (HLEC) in congenital cataract eye to prevent posterior capsule opacification by **Ryski Meirina, Diany Yogiantoro, Ratna Doemilah, Deya Karsari**.
2. The Effects of Lime (*Citrus aurantifolia* christm. swingle) peel Extract on Fibroblast Proliferation and Angiogenesis in Rat's Tooth Extraction Sockets by **I Dewa Gde Agung Nanda Krismaya, Ramadhan Pramudya, Puthi Ylvi Intan Sati and David Buntoro Kamadjaja.**
3. Metformin Exerts Antifibrosis Effect on Human Lens Epithelial Cells through Transforming Growth Factor (TGF) β Inhibition by **Dyah Purwita Trianggadewi, Nurwasis, Deya Karsari, Indri Wahyuni, Maftuchah Rochmanti.**
4. Effect of Alteplase in Preventing Posterior Capsule Opacity by Plasminogen Activator Inhibitor-1 and Type I Collagen Expression Inhibition in Posterior Capsule Opacity Model with Fibrin Reaction *In Vitro* (Experimental Laboratory study) by **Rina Wulandari, Indri Wahyuni, Arifa Mustika, Windhu Purnomo, Nurwasis.**
5. INTRACAMERAL INJECTION OF LIMBAL MESENCHYMAL STEM CELLS CONDITIONED MEDIA (LMSCs-CM) IMPROVE CLINICAL OUTCOME WITH DELAYED ON Na-K ATPase CORNEAL ENDOTHELIAL PUMP RECOVERY IN PHACOEMULSIFIED RABBIT EYES by **Eko Widayanto, Nurwasis, Dicky Hermawan, Arief Fidianto, Paramita Putri, Yuyun Rindiastuti, Igo Syaiful H., Willy Sandhika, Evelyn Komaratih, Hendrian D. Soebagjo.**
6. INTRACAMERAL INJECTION OF LIMBAL MESENCHYMAL STEM CELLS SECRETOME ALLEVIATE INFLAMMATION WITH DELAYED STRUCTURAL RECOVERY ON CORNEAL ENDOTHELIAL CELLS IN PHACOEMULSIFIED RABBIT EYES by **Arief Fidianto, Evelyn Komaratih, Wimbo Sasono, Willy Sandhika and Hari Basuki Notobroto.**
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10. BOVINE BONE XENOGRAFT SCAFFOLD SEEDED WITH HUMAN UMBILICAL CORD MESENCHYMAL STEM CELL TO RECONSTRUCT SEGMENTAL DEFECT IN A DOG'S MANDIBLE (A preliminary study) by **David Buntoro Kamadjaja, Nusdianto Triakoso, Purwati**
11. EFFECTS OF LEMONGRASS LEAF ON MACROPHAGES AFTER TOOTH EXTRACTION OF WISTAR RAT by **T. Devianna, A. Purnama, Y. Purnomo and A. Rizqiawan.**
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P. R. Yadav
Chief Editor

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DOI : 10.35124/bca.2019.19.S2.4703

Biochem. Cell. Arch. Vol. 19, Supplement 2, pp. 4703-4711, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

ERYTHROCYTE-SUPEROXIDE DISMUTASE (SOD1) AMONG ELITE COMBAT SPORT ATHLETES RUNNING AN INTENSIVE TRAINING PROGRAM AND THE ASSOCIATION WITH MICRONUTRIENT INTAKE

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(Received 22 August 2019, Revised 22 October 2019, Accepted 25 October 2019)

ABSTRACT : The improved consumption of high intensity antioxidants and long-term exercise has shown consistent results. Also, there is a possibility that combat sport impacts on the everyday nutritional practices of athletes, including dietary antioxidant.

The objective of this study wasto investigate the correlation between micronutrient intake and erythrocyte SOD among elite combat sport athletes running intensive sport training program. This was a cross sectional study, where 49 professional combat sport athletes (karate, pencaksilat, judo, and wrestling) participated. Furthermore, food record was obtained 3x24 hours, while micronutrient intake and erythrocyte SOD level assessment required measurement by spectrophotometry. The age of subjects were 23.08±4.32 years, encompassing elite athletes performing a routine sport training 20-26 h/w for one years. In addition, everyone demonstrated a high erythrocyte SOD levels, with a mean of 2280.69 ± 285.65 U/g Hb. Meanwhile, most subjects exhibited micronutrient intakes that were lower than the dietary recommendation; 97.5%, 85%, 27.5%, 77%, 47.5%, of vitamin E, vitamin C, vitamin A, Zn, and Cu, respectively, and no significant correlation was reported against erythrocyte SOD levels, except for the females, where a significant positive correlation (r = 0.538, p = 0.04) was established against vitamin C intake. It was established that elite combat sport athletes training intensively at the sport program demonstrated high erythrocyte SOD levels. Also, the low nutrient intake recorded requires the invitation of dietetics professionals as sport nutrition consultant. In addition, it is strongly supported that athletes ingest food rich in antioxidants, especially vitamin C for females, in order to maintain high antioxidant capacity.

Key words : Combat sports, athletes, erythrocyte SOD levels, antioxidant, micronutrient intake, vitamin C, intensive training.

INTRODUCTION

High intensity exercise is a potential source for producing reactive oxygen species (ROS) as metabolism fuel required in muscle activity. Therefore, not following up with an increment in antioxidant capacity tends to generate oxidative stress, which has the propensity to impact on health conditions, subsequently leading to a decline in the athletes' performance (Deaton and Marlin, 2004). Furthermore, most ROS are generated during intensive trainings in the form of radical superoxide (O2), which requires the superoxide dismutase (SOD) to neutralize the O2-in cells as one of the antioxidants often associated with exercise and sport (Metin *et al*, 2003). In addition, a wide distribution in this class, which comprises 90% of the total SOD, includes Cu, Zn superoxide dismutase (SOD1) (Noor *et al*, 2002).

During the exercise process, it is possible to induce oxidative stress through excess oxygen uptake. This makes erythrocyte vulnerable, due to the continuous exposure to oxygen, high concentrations of polyunsaturated fatty acids and also haem iron. Furthermore, the damage ensued has been known to impair erythrocyte deformability, which is responsible to hypoxia in the working muscle, although an increase in its turnover tends to facilitate better efficiency in oxygen transport, followed with the possibility of depleting antioxidants (Smith, 1995). Combat sports are in the category of polycyclic, which involves all the body limbs, encompassing a lot of repetitive movements (short sequences), including; attacking and defending, interrupted by a recovery period. Furthermore, they also involve aerobic and anaerobic metabolism, and the intermittent exercise of high intensity further enhances the potential to increases ROS within the body (Burke and Cox, 2009; Pesic *et al*, 2012).

In response to conditions of strenuous exercise, there is a tendency of temporary decrease in the body's antioxidant capacity during and immediately after training, which increases subsequently, through the recovery period (Fisher and Bloomer, 2009). Meanwhile, studies on antioxidants among athletes have previously been conducted, but the results obtained tend to be inconsistent. Reports have shown a higher level of erythrocyte SOD levels in athletes than in people living a sedentary lifestyle, pursuing regular activities (Metin *et al*, 2003). Jemili *et al* (2017) explained the tendency for intense specific training program to improve prooxidant-antioxidant balance, and also increase superoxide dismutase activity after 3-month in elite karate athletes. However, the conflicting findings were reported by Jurgenson *et al* (2019), which showed a significant decline in antioxidant capacity after 12-week of supervised strength training in competitive powerlifting athletes, while Ho *et al* (2007) revealed a lower erythrocyte SOD during heavy training, in contrast with sedentary individuals. Conversely, Bundo and Anthony (2016) reported the absence of a significant change in SOD activities after 3 months of regulated exercise program on healthy volunteers.

Antioxidant capacity (SOD1) is often influenced by numerous factors, encompassing age, type of sport, modes and intensity of training, experience, as well as the interaction with other antioxidants in the body, including vitamin C, E, and A, and also micro minerals, while zinc (Zn) and copper (Cu) are required by the SOD enzyme (Ho *et al*, 2007; Koury *et al*, 2004; Braakhuis *et al*, 2013). In addition, athletes require exogenous antioxidants from food intake, in order to increase its inherent capacity in their bodies and balance the elevation observed in ROS, resulting from the high intensity of exercise performed (Braakhuis *et al*, 2013; Power and Jackson, 2008; Rosseau *et al*, 2004). Therefore, most participating in combat sports tends to attain a specific weight target during the qualification for an event, which requires the adoption of extreme nutritional practices, geared towards reducing body weight (Burke and Cox, 2009). Furthermore a study by Franchini *et al* (2019) reported a high prevalence of weight loss in combat sports athletes (90% in judo, 70.8% in karate, taekwondo 63.3%, wrestling 89%), estimated to be about 60-90%, which possibly impacts on everyday nutritional practices, including dietary antioxidant.

Despite the conflicting findings on athlete antioxidant capacity, erythrocyte SOD in particular has not been widely studied and the nutritional problem remains a concern. Hence, it is interesting to conduct further studies on nutritional intake in combat sport athletes and also investigate its correlation with erythrocyte SOD among the elite category, running intensive sport training program.

MATERIALS AND METHODS

Participants and study design

This was an observational study with cross sectional methodology design. In addition, data collection was conducted from August to December 2014 at the sports training center of East Java, Indonesia, where athletes of martial arts registered with the athletes groups of Indonesian national sports committee were recruited. Furthermore, the specific combat sports involved in this study include pencaksilat (Indonesian martial arts), Judo (modern martial art), karate and wrestling and only 40 out of a total of 49 professional athletes met the research criteria and were willing to participate in the study. This was ascertained by the signing of an informed consent form, which was arranged according to the ethical standards laid down in the Declaration of Helsinki and the Ethical Committee of Faculty of Public Health, Universitas Airlangga and the protocol of study was approved with ethical number 480-KEPK.

The inclusion criteria were combat sports athletes on an intensive sport training program (termed puslatda) that are physically fit with no health problems, based on medical examination and also not preparing for competition. In addition, it was not possible to restrict all supplements because all athletes were provided a specific type from the Indonesian national sports committee, encompassing B-complex vitamin" 1000mg (vitamin B1 100mg, B6 200mg, B12 200mcg) and glucosamine sulfate 1500 mg, which ought to be ingested once daily. Furthermore, the exclusion criteria include active smokers, consumption of antioxidant supplements within the last 2 weeks, suffering from any inflammatory diseases, e.g., asthma, chronic diarrhea, prolonged cough or allergies. However, four athletes were identified to have taken antioxidant supplements and were consequently excluded.

Data collection and characteristic data of subjects

Data collection was conducted through (1) structured interview, in order to obtain the characteristics data, including age, gender, sport experience and years of training. (2) Food record method of 3 × 24 hours, in an attempt to assess macronutrient and micronutrient intake. (3) Anthropometry measurement for body composition evaluation (4) laboratory tests, including erythrocyte SOD, hemoglobin and malondialdehyde (MDA) plasma.

The basic characteristic data reported in relation with the subjects' activities include (1) sport experience, which is defined by the length of time subjects actively train in the specific sport, right from the first incidence. In addition, there is also a probability that they have been involved in childhood. (2) Years of training (total training), which refers to the number of years of participation in this exercise as a competitive combater.

Intensive Training Program

All subjects were professional and elite athletes, ready to partake in the sport competition, both at the national and international level. This also involved those preparing for the 2016 National Sports Week (Indonesian: *Pekan Olahraga Nasional*, *PON*), which is a multi-sport event held every four years in Indonesia. In addition, the national sports committee at East Java obligated them to follow up intensive sport training program, hence, all subjects participated right from a year ago. Therefore, further information based on duration, frequency and intensity were obtained through direct interviews with athletes, and also from secondary data sources, *e.g.*, the training schedule of each sport. Moreover, the coach of an individual game arranged a draft of one-week and similarities were identified in the exercise trend amongst all as they contained physical and specific exercises, encompassing combat techniques and coordination, balance, flexibility exercises and muscular power. Therefore training commenced every day, except for Sunday, with two sessions per day, encompassing physical activities in the morning for 2h and specific drills in the afternoon for 2-3h, with medium to high intensity. These were all initiated with a warm up, and terminated with cooling down, and the draft example for Judo sport is reported in Table 1.

Anthropometry measurements

Body composition was measured using bioelectric impedance analysis (BIA), with seca brand 515/514 type of stainless steel electrodes, then anthropometry was calculated in the morning after a 8h-overnight fasting, prior to before blood sampling evaluation. Furthermore, weight, height, body mass index (BMI), fat free mass, and fat mass were recorded from all subjects and analyzed.

Blood sampling Measurements

Blood sampling was conducted at 8 a.mafter an 8h-overnight fasting into "BD VacutainerTM" test-tubes and placed in a 4°C compartment at all time. Therefore, plasma was obtained from the heparinized-treated samples within 30 min by centrifugation (15 min, 1000×g, 4°C), thus separating the erythrocytes, which were then washed three times with 0.9% NaCl solution, and hemolyzed with four volumes of cold distilled water. These were further maintained or stored at –20°C, prior to subsequent analyses.

SOD activity was evaluated in the erythrocyte samples (cell lysates), using the commercially available RANSOD Kit (Randox Laboratories) and the levels recorded were expressed in unit per gram of hemoglobin (U/g Hb), utilizing the spectrophotometric method with a multiple wavelength spectrophotometer tool (Kakkar *et al*, 1984). Furthermore, its concentration was determined using the cyanmethemoglobin method, where the color of cyanmethemoglobin was read in a photoelectric colorimeter against a standard solution at 540 nm (Kikukawa and Kobayashi, 2002).

Dietary intake

Macronutrient and micronutrient intake assessment was conducted by food record at 3×24 hours, as all subjects were provided with a sheet for this purpose a week prior to the commencement of sampling. Therefore, the nutritionist passed the information on how to correctly input data, including details on portion and size consumed, by demonstrating a food model and the Indonesian food book. In addition, the subjects were asked to record all cuisine and drinks ingested three days of a week (not necessarily consecutive), which was clarified at the day data collection by the nutritionist through direct questioning. In addition to mean and standard deviation, nutrient based on adequate intake in comparison with the Indonesian recommended dietary allowance (RDA) were adopted in micronutrient intake analysis.

Statistical analyses

SPSS 21 was employed in statistical analysis during this study, with the consideration of a significance limit less than 5%. Therefore, the determination of whether the data distribution is normal or abnormal required the use of Shapiro-wilk test. Meanwhile, categorically scaled data were presented as number and percentages, while continuous variables were reported as mean and standard deviation. Furthermore, independent samples t-test and Mann–Whitney were adopted in the analysis of sex differences for anthropometry and also data obtained from laboratory measurements. Subsequently, one-way ANOVA and post hoc tests were performed to identify the type sport difference for the variables evaluated, while the correlation between SOD levels with micronutrient intake was analyzed using the conducted Pearson test or Spearman test.

RESULTS

A total of 40 athletes out of 49 from four different sports, registered as members of training center of East Java Indonesia met the study criteria and were willing to participate in the research by signing informed consent. In addition, out of the nine that did not meet the

Table 1 : A schedule of an intense training program by judo athletes.

Days in one weeks	Time (duration)	Training Type	Physical training	Specific exercise /techniques
Monday	Morning (a.m) 05.30-07.30 (2h) Afternoon (p.m) 16.00-19.00 (3h)	Physical training Specific training	Running 8x400m Speed reaction training Core balance power Hand core endurance Leg training Splits step jump Leg jump squat Push up Squat trash	Uchikomi slam road Randori Randorinewaza Pull technique
Tuesday	Morning 05.30-07.30 (2h) Afternoon 16.00-19.00 (3h)	Physical training Specific training		
Wednesday	Morning 05.30-07.30 (2h) Afternoon 16.00-18.00 (2h)	Physical training Specific training		
Thursday	Morning 05.30-07.30 (2h) Afternoon 16.00-18.00 (2h)	Physical training Specific training		
Friday	Morning 05.30-07.30 (2h) Afternoon 16.00-18.00 (2h)	Physical training Specific training		
Saturday	Morning 05.30-07.30 (2h) Afternoon 16.00-18.00 (2h)	Physical training Specific training		
Sunday	Free	Active recovery		

Table 2 : Subject characteristics and body composition stratified by gender and type of sports.

Variables	Total (n=40)	Male (n=26)	Female (n=14)	Pencaksilat (n=14)	Wrestling (n=11)	Judo (n=8)	Karate (n=7)	P[1]
Gender								
Male (n=26)				8	10	2	6	
Female (n=14)				6	1	6	1	
Avarage training (h/w)				22-24	20-22	24-26	20-22	
Age (y)	23.08±4.32	22.96±3.98	23.29±5.03	25.21±4.09[1]	21.55±2.34	24.75±5.52	19.29±2.14	0.000[2]
Total training (y)	4.05±2.69	3.54±2.58	5±2.72	4.21±2.61	3.36±2.8	6.25±2.2[#]	2.29±1.6	0.02
Sport experience (y)	10.62±2.9	10.18±2.29	11.43±3.76	10±2.32	10.09±2.21	12.25±4.56	10.83±2.37	0.32
Body Weight (kg)	65.61±24.29	71.36±18.09[*]	54.93±5.39	60.91±19.07	72.05±18.13	61.2±14.38	69.9±9.11	0.3
Body Height (m)	1.63±0.98	1.67±0.93[*]	1.56±0.06	1.62±0.11	1.65±0.88	1.69±0.24	1.69±0.25	0.133
BMI (kg/m^2)	24.29±3.72	25.21±4.0[*]	22.59±2.4	22.66±3.74	26.15±4.14	25.1±4.02	24.5±2.86	0.138
Body fat (%)	19.37±8.47	16.66±8.85[*]	24.4±4.75	18.06±8.26	17.52±11.11	24.2±4.79	19.39±6.67	0.335
Fat Free Mass (kg)	52.98±12.39	58.41±10.55[*]	43.67±9.63	49.37±13.4	58.94±10.35	50.34±14.78	55.02±6.88	0.264

specification, four were currently on antioxidant supplements, while others had travelled overseas to participate in competitions during the time of data collection. Furthermore, all subjects were identified as professional athletes routinely performing sport training for 4.05±2.69 years and had a mean sport experience of 10.62±2.9 years. In addition, 26 participants (65%) were male, mostly from the wrestling sport, although they were dominant in most, except Judo and the mean age of subjects was 23.08 ± 4.32 years, with the majority (70%) being between 20 and 29. Also, the mean BMI was recorded as 24.29±3.72 kg/m^2 and most subjects (62.5%) exhibited normal levels, while the value obtained for body fat on all subjects were 19.37±8.47%, with the females having a relatively higher value, especially those in the game of Judoka. Furthermore, the characteristics data and body composition are reported in Table 2.

The dietary method indicates the macronutrient and micronutrient consumed by subjects, where the mean energy intake was 2408.04±801.96 mg/d, which was higher in male than female, and similar results were demonstrated with carbohydrate, fat and protein. In addition, the micronutrient assessed included antioxidants (vitamins A, E, C), Cu and Zn, which are known components of enzymatic erythrocyte (SOD) and no significant different in consumption was identified in accordance with gender, although wrestlers tend to portray superiority in contrast with others. Furthermore, most participants also had vitamin E, and C, as well as zinc intakes that were less than the RDA specification, while the value obtained for vitamin A and copper indicated that 72.5% and 52.5% of subjects respectively ingested sufficient amounts (Table 3).

The mean erythrocyte SOD for all participants was 2280.69 ± 285.65 U/g Hb, which did not differ significantly ($p > 0.05$) between males and females, as well as amongst sports type. In addition, the mean Hb was 15.53±1.37, and all subjects were assessed as non-anemic, based on

Table 3 : Intake of macronutrients and micronutrients based on the food record 3 x 24 hours.

Variable	Total (40)	Male (26)	Female (14)	Wrestling (11)	Karate (7)	Judo (8)	PencakSilat (14)
Macronutrients							
Energy (kcal /d)	2408.04±801.96	2523.76±819.41	2193.12±749.45	3080.09±962.01	2016.73±348.37	2160.03±836.48	2217.37±493.07
Carbohydrate (g/d)	230.63±116.95	237.62±115.12	217.64±123.58	323.71±137.51	165.76±37.42	232.29±132.32	188.98±70.35
Fat (g/d)	130.46±41.27	133.65±43.85	124.56±36.8	159.94±54.64	110.61±18.95	120.23±33.13	123.08±31.4
Protein (g/d)	89.71±33.98	94.08±37.08	81.58±26.69	112.33±42.9	77.59±19.41	74.64±19.27	86.6±31.84
Micronutrients							
Vitamin A (mcg/d)	873.63±398.03	926.82±426.66)	774.84±330.21	1207.08±401.37	725.36±422.26	657.49±251.41	809.28±309.67
Less	11 (27.5%)	6 (23.1%)	5 (35.7%)	11 (100%)	4(57.1%)	3 (37.5%)	4 (28.6%)
Sufficient	29 (72.5%)	20 (76.9%)	9 (64.3%)	0	3 (42.9%)	5 (62.5%)	10 (71.4%)
Vitamin E (mg/d)	8.46±2.54	8.58±2.4	8.21±2.84	10.28±3.28	7.23±1.34	7.66±1.65	8.09±2.14
Less	39 (97.5%)	26(100%)	13 (92.9%)	10 (90.9%)	7 (100%)	8 (100%)	14 (100%)
Sufficient	1 (2.5%)	0	1(7.1%)	1 (9.1%)	0	0	0
Vitamin C (mg/d)	48.85±39.36	50.68±37.82	45.45±43.33	57.89±44.17	37.11±30.13	35.98±24.09	54.98±46.14
Less	34 (85%)	22 (84.6%)	12 (85.7%)	9 (81.8%)	6 (85.7%)	8 (100%)	11 (78.6%)
Sufficient	6 (15%)	4 (15.4%)	2 (14.3%)	2 (18.2%)	1(14.3%)	0	3 (21.4%)
Zinc (mg/d)	10.36±3.98	11.09±4.46	8.98±2.51	13.05±4.64	9.57±3.48	8.73±3.85	9.56±2.95
Less	31(77.5%)	18 (69.2%)	13 (92.9%)	5 (45.5%)	6 (85.7%)	7 (87.5%)	13 (92.9%)
Sufficient	9 (22.5%)	8 (30.8%)	1(7.1%)	6 (54.5%)	1(14.3%)	1(12.5%)	1 (7.1%)
Cuprum (mg/d)	1.18±0.52	1.23±0.55	1.08±0.47	1.39±0.47	0.84±0.27	1.14±0.57	1.21±0.58
Less	19 (47.5%)	12 (46.2%)	7 (50%)	3 (27.3%)	5 (71.4%)	4 (50%)	7 (50%)
Sufficient	21 (52.5%)	14 (53.8%)	7 (50%)	8 (72.7%)	2 (28.6%)	4 (50%)	7 (50%)

the report of blood sampling shown in Table 4.

Ranking using the Spearman correlation test, showed no significant association between the micronutrient intake and erythrocyte SOD levels, based on both gender and sports type, However, there was a substantial correlation between vitamin C and SOD levels (r=0.538, p=0.047), especially with the females.

DISCUSSION

This study investigated some types of combat sports (karate, pencaksilat, judo and wrestling), in an attempt to provide a general description on nutrient intake among these athletes, based on the prevalence of body weight and nutritional problems. Furthermore, erythrocyte SOD (SOD1) was specifically measured as a parameter for antioxidant capacity, and subsequently associated with micronutrient intake, as a result of its wide distribution, with 90% Cu, Zn superoxide dismutase (SOD1) content (Noor *et al*, 2002).

This research involved the recruitment of combat sport athletes (karate, pencaksilat, judo and wrestling) that were registered as members of the Indonesian national sports committee, East Java, Indonesia. Furthermore, all subjects were evaluated to have been participating in an intensive exercise program at the sport training center, during the period of data collection. Prior to entering a sport-training center, a series of medical and laboratory examinations were conducted by professional doctor, in order to prove physical and mental health. In addition, most participants (92.5%) were less than 30 years of age, with a mean age of 23.08 ± 4.32 years, representing the young and healthy elite athletes.

This findings are similar to most studies on combat sports, which involves young participants, as observed by Radovanovic *et al* (2012) on judo athletes aged 20±1.3 years, Pesic *et al* (2012) on karate sportspersons aged 16 to 30, Rynkiewicz *et al* (2010) on sumo wrestlers with an average age of 23±6.6 years. In addition, the mean age 20.8±1.1 years of weight lifters was studied by Ho *et al* (2007) and Rousseau *et al* (2004) on the competitive athletes age 26.8±6.8 of those that

Table 4 : The erythrocyte SOD level by gender and type of sports.

Variable	Total (40)	Male (26)	Female (14)	Wrestling (11)	Karate (7)	Judo (8)	Silat (14)
Hb	15.53±1.37	16.32±0.99	14.08±0.55	16.14±1.17	16.37±1.25	14.58±1.17	15.17±1.31
SOD	352.75±41.4	366.46±39.77	327.29±32.01	366.73±45.65	363.43±11.03	342.25±41.08	342.48±46.52
SOD1 (U/g Hb)	2280.69±285.65	2255.8±311.14	2326.91±234.36	2276.03±296.60	2232.11±201.18	2357.8±318.53	2264.58±313.72

Table 5 : The correlation between micronutrients intake and erytrocyte SOD level.

Variables	Total (40)	Male (26)	Female (14)	Wrestling (11)	Karate (7)	Judo(8)	PencakSilat (14)
Vitamin A	0.697	0.424	0.383	0.096	0.535	0.736	0.493
Vitamin E	0.944	0.7	0.774	0.184	0.645	0.417	0.233
Vitamin C	0.462	0.805	r=0.538, p=0.04*	0.915	0.819	0.086	0.946
Zinc	0.934	0.710	0.306	0.081	0.819	0.867	0.115
Cuprum	0.166	0.171	0.644	0.438	0.877	0.713	0.185

follow routine training. Moreover, youth is a golden period for athletes, where the age range about 20 years has been established to be the most productive for being the best and obtain the highest achievements.

This was confirmed by the report of Indonesian national sports, which stated that a bulk of the numerous gold trophies was attained by young athletes (Record, 2014).

This study involved 40 athletes, where 26 (65%) were male and 14 (35%) were females, which is reinforced by the data obtained from the Indonesian national sports, showing the dominance of males. Meanwhile, there are actually no restrictions or gender specificity in combat sports, although this gender discrepancy was observed in all four types studied, except Judo, which had more female participants. Furthermore, the measurement of body composition demonstrated 24.29±3.72 kg/m² as the mean body mass index (BMI) and an average body fat of about 19.37±8.47% was recorded in both male and female participants, although only 30% were observed to be within the normal limits, while others surpassed. This category of "high fat mass" was probably due to the high intake of fat, which contributed to about 35.95 ± 8.18% of the total energy (data was shown), therefore, concerns about obtaining ideal values and the consequent lean body mass ought to be the attention for health and the best performance (Burke and Cox, 2009).

Each sport had a training program with active exercise schedule conducted 5-6 days per week for an average period of 4-5 hours daily, hence, the total duration was about 20-26 hours per week. These data based on duration, frequency and intensity demonstrates the characteristics of the subject as professional athletes. Furthermore, questions were asked about sport experience, centered on the length of time from when the athletes first participated in the specific sport, and an average value of 10.62±2.9 years was obtained, depicting their active participation as children. In addition, combat sport is considered as one of the highly preferred in Indonesia, often followed by children, and it also confers numerous health advantages (Burke and Cox, 2009; Record, 2014). Meanwhile, previous study represented it as an effective method for enhancing muscular power and flexibility in young athletes aged between 8 and 12 years (Padulo *et al*, 2014) and Ju *et al* (2018) reported the propensity to facilitated the onset of an earlier secondary saccade in children aged 9-12 years.

Based on the data obtained from food records of 3×24h, it was established that dietary carbohydrate, fat, and protein were 230.63±116.95 g, 130.46±41.27 g and 89.71±33.98 g, respectively. These were generally lower than the records obtained from previous studies, including Braakhuis *et al* (2013) on professional rower athletes with carbohydrate, protein and fat intake of 510±190 g, 170±70 g and 110±45 g, respectively. Also, a study conducted by Pettersson (2013) on combat sport athletes exhibited a total 5.5±3.5 g/kgBW, 1.4±0.8 g/kgBW and 1.1±0.8 g/KgBW, for carbohydrates, protein and fat, respectively. According to the sports nutrition guidelines, these findings indicate that fat consumption is relatively high, while that for carbohydrate is slightly lower, and it has been established that combat athletes often adopted restrictions in diet in an attempt to lose weight, by limiting carbohydrate (Petterson *et al*, 2013). Data obtained from the interview showed the possibility to conclude tha almost all participants applied the weight loss program only on the day before the competition. Furthermore, recall clearly showed the incorrect application of nutrition by combat sport athletes, although the guideline explained the importance in goals achievement. This specifically requires fuel obtained from energy and macronutrient consumption, which is derived from a variety of food in everyday diet. In addition, days where high intensity training is undertaken demands the usefulness of fuel supplies, in order to support performance (Burke and Cox, 2009).

The analysis of antioxidant intake, based on the recommended dietary allowance (RDA) demonstrated 85% for vitamin C and 77.5% for zinc and all subjects were classified in the "less" category and a 97.5% for vitamin E was also less than the requirement. These findings are similar to Rousseau *et al* (2004), showing vitamin C intake to be only 40% of RDA, while vitamin E was 81% for athletes, which is under 2/3 of the recommendation, as well as beta carotene at 43%. Meanwhile, 60% of athletes tend to not reach the specification for vitamin C, and E. However, the finding of this study is lower than the value recorded in the investigation conducted on karate athletes, which showed vitamin E and C at 22.4±9.8 mg/day and 215±79mg/day, respectively and also beta carotene at 4.5±3.2 mg/day (Pesic *et al*, 2012). In addition, a study on rowers demonstrated a vitamin C consumption rate of 210±249 mg/day, vitamin E at 14±8 mg/day and beta carotene of 4.9±2.5 mg/day (Braakhuis *et al*, 2013) and it has been established that the restrictions in diet for participants of combat sports result in the reduction of energy an macronutrient intake, subsequently leading to a decline in the antioxidant present (Petterson *et al*, 2013; Carlshon *et al*, 2010).

Erythrocyte SOD levels evaluated in this study was in the form of SOD isoenzymes located in the cytosol, including CuZn-SOD (SOD1), thus, based on its composition of Cu and Zn, these trace mineral therefore play an important role in its activities. Furthermore, only 52.5% of copper and 22.5% of zinc intake were recorded, and subsequently classified in the "sufficient" category, although the value is lower than the record of Koury *et al* (2004), which showed 27% and 2% of athletes had a lesser zinc and copper intake, in contrast with recommended values. Also, low SOD activities in long distance runners were evaluated to be probably caused by the deficiency of these elements, and the loss was estimated to have ensued through sweat and urine excretion (Ho *et al*, 2007). In addition, another report also showed a significant increase of their average levels in urine, measured after exercise (Kikukawa *et al*, 2002), while Resina *et al* (1990) testified that male runners had lower serum levels than the control group/ non-athletes.

Erythrocyte SOD levels recorded were a representation for athletes running an intensive training program, of which all participants demonstrated a high value (> 1601 U/g Hb), with mean of 2280.69±285.65 U/g Hb. These results obtained suggest the adequate endogen antioxidant defence response towards the strenuous exercise, and this elevation in capacity was also revealed by previous studies, both in athletes and non-athletes. Carlshon *et al* (2010) showed the propensity for regular exercise to increase blood levels in younger athletes, while Jemili *et al* (2017) reported the capacity for intense specific training programs to improved prooxidant-antioxidant balance, subsequently increasing the activity of superoxide dismutase after 3-month in elite karate athletes. However, the intensification of antioxidant capacity in non-athletes was proven in a study by Berzosa *et al* (2011), which showed the enhanced probability for acute exercise (cycloergometric tests) to augment the antioxidant enzyme activities of untrained men. Also, the elevation of SOD activity after a single bout exercise in healthy women was also proven in an investigation conducted by Yimcharoen *et al* (2019).

The increase in antioxidant capacity related exercises have been observed to show inconsistent results, as Jurgenson *et al* (2019) revealed a significant decline in the antioxidant volume recorded after 12-week of supervised strength training in competitive powerlifting athletes. Also, Bundo and Anthony (2016) reported the absence of a significant change in SOD activity after 3 months of a supervised exercise program in healthy volunteers, while Pesic *et al* (2012) established that there was no significant change in oxidative stress and SOD activity during a training process, both in state of rest and after loading and also that the elevations observed were not as a result of long-time intensive exercise, but high physical loading.

The analysis of SOD activity based on gender and sport types showed no statistically significant difference, which differs from the report by Dopsaj *et al* (2013) where a variation in values was observed between the karate professionals and wrestlers (73 ± 37 vs. 103 ± 30, $p < 0.05$). Therefore, the high activity in wrestlers is possibly associated with the long-term impact of the sport, being a type of strenuous exercise. Moreover, other reasons for the high values obtained in this current study are probably due to the discrepancy in the production of ROS formed, the variation in modes and intensity of training performed, as well as the interaction between SOD and other antioxidants within the body, encompassing vitamin C, E and A. This was also due to its collaboration with micro minerals, including zinc (Zn) and copper (Cu) required by the enzyme, as well as the elevation in the potential loss of minerals through sweat and urine (Metin *et al*, 2003; Ho *et al*, 2007; Bundo and Anthony, 2016).

The use of Spearman rank correlation test showed no significant correlation between the consumption of micronutrients and erythrocyte SOD levels, although a substantial positive relationship ($r=0.538$, $p=0.047$) was identified with vitamin C in female participants. In addition, numerous athletes, were considered to be at a greater risk of iron depletion, with a possibility of leading into deficiency (with or without anemia), although the mean Hb of 15.53±1.37 recorded from all subjects were assessed as non-anemic, and the females had a significantly lower value in contrast with male (Table 5). Hence, the results obtained are possibly explained by the capability for vitamin C to enhance iron absorption, and the closely related mechanism of hemoglobin and red blood cell (Alaunyte *et al*, 2015). Furthermore, another reason is centered of the fact that vitamin C is stored in the adrenal gland and is subsequently released during stressful periods, in an attempt to confer protection against oxidative stress. This assumption is backed up by a previous investigation, which showed its correlation with total antioxidant capacity amongst competitive rowers (Ho *et al*, 2007). Yimcharoen *et al* (2019) reported the probability of improved antioxidant capacity in healthy women that perform moderate intensity cycling, using supplementations containing ascorbic acid.

An important aspect of this study is the established fact that all participants consumed vitamin B supplements (B1, B6 and B12) daily during the sport programming. Furthermore, these have also been identified as responsible for the change in SOD activity, which was categorized as high, although the intake of micronutrient was low. Ford *et al* (2018) provided evidence for the efficacy of high-dose B-group supplementation in reducing oxidative stress and subsequently increasing the affiliated metabolism. Therefore, the ease of tolerating antioxidant-rich foods and its impact on performance makes it a preferred choice over supplements (Koivisto *et al*, 2018).

The fact that the study participants represent the young and healthy elite athletes determines the erythrocyte quality, as its deformability is highly influenced by age. Also, the endurance rate in sport tends to suggest the capability of the erythrocyte system to adapt to changing conditions, including adolescence, with the onset of sex hormones or physical exercise effects (Tomschi *et al*, 2018).

Strength

This is the first study providing data on SOD1 among elite athletes running an intensive training program for a long time (one year). In addition, variations were identified in the characteristics of each combat sport, especially in terms of specific exercises, but the duration and frequency of training were similar, indicating the control of these variables. Moreover, most studies tend to only focus on athletes involved in one type of combat sport, but this current investigation entailed recruiting a variety, including karate, pencaksilat, judo and wrestling, in an order to provide a general description. Finally, erythrocyte SOD (SOD1) was specifically measured as an indicator of antioxidant capacity, which was further analyzed in association with the intake of micronutrient, including vitamin C, vitamin E, vitamin A and micro minerals (zinc (Zn) and copper (Cu)) required by the SOD enzyme, which collectively serve as sources of antioxidants

Limitations

Erythrocyte SOD levels recorded was measured only once, exactly one year after the inception of the intensive training program, and no prior data or information was made available. Therefore, it is not possible to draw conclusions on its increase or decline. Moreover, the study only adopted SOD in the evaluation of antioxidant capacity in response to high intensity and the longtime training program, where it is plausible to measure other markers, encompassing glutathione peroxidase (GPx) and catalase (CAT) based on the records from previous studies which reported a marked increase in the their individual concentrations (Jemili *et al*, 2017; Braakhuis *et al*, 2013).

CONCLUSION

This study involved young elite combat sport athletes participating in an intensive sport training program, and it was established that all subjects had high erythrocyte SOD levels. In addition, most participants were observed to have incorrectly applied the stipulated sport nutrition, encompassing the intake of macronutrient and micronutrients, which were lower than the recommendation. This shortfall ought to attract the sport committee attention, therefore requiring the invitation of dietetics professionals as nutrition consultant, in an attempt to solve the problem with diet. Furthermore, there is a possibility for the total low intake to cause depletion in vitamin/mineral status, especially with vitamin C in female athletes, which is why the intake of food rich in antioxidants is highly recommended to maintain high activity.

ACKNOWLEDGEMENTS

The authors are grateful to all participants in this study, including the athletes, coaches and the sport committee, as well as the research assistants that collected and analyzed the relevant data. This study was funded by Universitas Airlangga, through the Faculty Prime Research Grant in 2014.

Conflict of interest

The authors have no conflicts of interest to report.

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