Judul Artikel: Erythrocyte-superoxide dismutase (SOD1) among elite combat sport athletes running intensive training program and the association with micronutrient intake

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

ABSTRACT

Background: The improved consumption of high intensity antioxidants ~~of high intensity~~ and long-term exercise ~~seems still~~ has~~ve~~ shown ~~any~~ consistent results. Also, there is a possibility that ~~C~~combat sport ~~athletes may have an~~ impacts on the~~ir~~ everyday nutritional practices of athletes, including dietary antioxidant.

Objective: to investigate the correlation between micronutrient intake and erythrocyte SOD among elite combat sport athletes running intensive sport training program

Methods: ~~In~~This was a cross sectional study, where 49 professional combat sport athletes (karate, pencak silat, judo, and wrestling) participated. Furthermore, ~~F~~food record ~~method~~was obtained 3x24 hours, ~~to~~while~~ assess~~ micronutrient intake and erythrocyte SOD level assessment required~~were~~ measurement~~d~~ by spectrophotometry.

Results: The ~~subjects~~ age of subjects were 23.08±4.32 years, ~~were~~encompassing elite athletes performing a routine sport training ~~routinely~~ 20-26 h/w for one years. In addition, ~~All subjects~~everyone demonstrated a high erythrocyte SOD levels, with a mean of 2280.69 ± 285.65 U/g Hb. Meanwhile, ~~M~~most subjects ~~showed~~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 ~~N~~no significant correlation was reported ~~between micronutrients intake~~ against~~nd~~ erythrocyte SOD levels~~ was reported~~, except~~specially~~ for the females~~ subjects,~~ ~~showed~~where a significant positive correlation (r = 0.538, p = 0.04) was established ~~between~~ against vitamin C intake~~ and erythrocyte SOD levels~~.

Conclusions: It was established that ~~E~~elite combat sport athletes ~~who~~ training intensively at the sport ~~training~~ program demonstrated high erythrocyte SOD levels.~~T~~ Also, the low nutrient intake ~~should be needed~~recorded requires the ~~to~~ invitation of~~e~~ dietetics professionals as sport nutrition consultant. In addition, it is strongly supported ~~for~~that athletes ~~to intake~~ingest food rich in antioxidants, especially vitamin C for females~~ athletes~~, in order to maintain high antioxidant capacity.

Keywords: combat sports; athletes; erythrocyte SOD levels; antioxidant; micronutrient intake; Vitamin C; intensive training

INTRODUCTION

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

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During the exercise process, it is possible to induce oxidative stress can be induced throughby excessover oxygen uptake. This makes Eerythrocyte is vulnerable, to oxidative stress sincedue to the it continuouses exposure to oxygen, and high concentrations of polyunsaturated fatty acids, and also haem iron. Furthermore, oxidativethe damage ensued has been known tocan impair erythrocyte deformability, that which is responsible tocause hypoxia in the working muscle during exercise. , Alalthough an increase in erythrocyteits turnover increase tends to to facilitate better efficiency in oxygen transport oxygen more efficient during exercise, followed with the possibility of depleting antioxidants in erythrocyte can be depleted during that condition (Smith 1995). CCombat sports are in the category of polycyclic sports, which involvesing all the body limbs, encompassingwith a lot of repetitive movements (short sequences), including; attacking and defending, movements interrupted by a recovery period. Furthermore,Combat sportsthey also involve aerobic and anaerobic metabolism, and nd the the high intensity of intermittent exercise of high intensity further enhances thein these sports potential toly increases ROS within the body (Burke & Cox 2009; Pesic *et.* *al* 2012).

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In response to conditions of strenuous exercise, there is a tendency of temporary decrease in the body's antioxidant capacity may be temporarily decreased during and immediately post exerciseafter training, whichand increases againsubsequently, during through the recovery period (Fisher&Bloomer 2009). HoweverMeanwhile, studies abouton antioxidants among athletes have previously been conducted, but the results obtained tend to bey still showed inconsistent results. Metin *et al.*, study showedReports have shown a higher level of erythrocyte SOD levels inof athletes were higher than those of in people living a sedentary people lifestyle, pursuing regular activities (Metin *et.al* 2003). Jemili *et al.*, (2017) studyexplained the tendency for found intense specific training program to improved the prooxidant-antioxidant balance, and also increase in superoxide dismutase activity after 3-

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month specific training in elite karate athletes (Jemili et.al 2017) . However, the conflicting findings were reported by Jurgenson, *et al*., (2019), thatwhich showed a significant decline in antioxidant capacity decreased significantly after 12-week of supervised strength training in competitive powerlifting athletes (Jurgensen et.al 2019)., while Ho *et al*., (2007)study also revealed a lower erythrocyte SOD during heavy training had lower erythrocyte SOD, in contrast withan sedentary peopleindividuals (Ho *et.al* 2007). Conversely, Bundo and Anthony, study reported there was noabsence of a significant change in SOD activities after 3 months of supervised regulated exercise program ion healthy volunteers (Bundo &Anthony T 2016).

Antioxidant capacity (SOD1) is often influenced by manynumerous factors, such asencompassing age, type of sport, the modes and intensity of training performed, sport experience, as well as the interaction with other antioxidants in the body, such asincluding vitamin C, vitamin E, vitaminand A, and also micro minerals, while zinc (Zn) and copper (Cu) are required by the SOD enzyme , namely zinc (Zn) and copper (Cu) (Ho *et.al* 2007, Koury *et. al* 2004, Braakhuis *et. al* 2013). In addition, A athletes require exogenous antioxidants from their food intake, in order to increase antioxidantits inherent capacity in their bodies, in order andto balance the increaseelevation observed inof ROS, resulting fromdue to the high intensity of exercise performed (Braakhuis *et. al* 2013, Power & Jackson 2008, Rosseau *et. al* 2004). Therefore, Mmost athletes who compete inparticipating in combat sports sports will tends to meetattain a specific weight target during theto qualificationy for theiran –event, andwhich requires the adoption of extreme nutritional practices, geared towards may be undertaken to reducinge body weight (Burke & Cox 2009). Furthermore a Sstudy 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%). The high

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~~prevalence of rapid weight loss~~, estimated to be ~~(~~about 60-90%,~~)~~ ~~among combat sport athletes~~ which possibly~~may have an~~ impacts on ~~their~~ everyday nutritional practices, including dietary antioxidant ~~(Franchini et.al 2019)~~.

~~Since~~Despite the ~~antioxidant capacity in athletes still show~~ conflicting findings on athlete antioxidant capacity,~~and~~ ~~particularly~~ erythrocyte SOD in particular ~~i~~has not been~~yet~~ widely studied, and the nutritional problem ~~among combat sport athletes is still~~remains a concern~~.~~. Hence, ~~I~~it is interesting to conduct further studies~~y~~ ~~a~~on~~bout~~ nutritional intake in combat sport athletes, and also investigate its~~he~~ correlation ~~between micronutrient intake~~ with~~and~~ erythrocyte SOD among the elite ~~combat sport~~category~~ athletes~~, running intensive sport training program

MATERIALS AND METHODS

Participants and Study Design

This ~~i~~was an observational study with ~~methodology design is~~ cross sectional methodology design. In addition, ~~D~~data collection was conducted from August to December 2014 at the sports training center ~~in~~of East Java, Indonesia, where~~. We recruited~~ athletes of martial arts ~~that~~ registered ~~as~~with the athletes groups of Indonesian national sports committee were recruited ~~at East Java, Indonesia~~. Furthermore, ~~T~~the specific combat sports involved in this study ~~were~~include pencak silat (Indonesian martial arts), Judo (modern martial art), karate, and wrestling, and~~.~~ ~~From~~only 40 out of a total of 49 professional ~~combat sport~~ athletes~~, 40~~ met the research criteria and were willing to participate in the ~~research~~study~~,~~. ~~by signing informed consent form~~. Thise~~ Informed~~ was ascertained by the signing of an informed consent form, which~~consent~~ 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 approved the protocol of study was approved with ethical number is 480-KEPK and signature of informed consent was obtained from all subjects.

The inclusion criteria includedwere combat sports athletes followingon an intensive sport training program (name istermed puslatda), havethat are physically fit andwith no health problems, bybased on medicaldoctor examination, and also not preparing for competition. In this studyaddition, we cannotit was not possible to restrict all supplements because during an intense training program, all athletes gotwere provided thea specific supplementstype from the Indonesian national sports committee. The supplements , wereencompassing B-complex vitamin" 1000mg (vitamin B1 100mg, B6 200mg, B12 200mcg) and glucosamine sulfate 1500 mg, thatwhich mustought to be takeningested once daily for every day. SoFurthermore, the exclusion criteria wasinclude active smokers, consumption ofing antioxidant supplements within the last 2 weeks, suffering from any inflammatory diseases, such ase.g., asthma, chronic diarrhea, asthma, chronic prolonged cough or allergies. However, we found four athletes were identified to have taken antioxidant supplements, and were consequently have excluded these participants.

Data collection and characteristic data of subjects

Data collection was conducted through (1) structured interview, in order to getobtain the characteristics data, including age, gender, sport experience, and years of training. (2) Food record method of 3x24 hours, in an attempt to assess macronutrient and micronutrient intake. (3) Anthropometry measurement tfor assess body composition evaluation (4) laboratory tests, including erythrocyte SOD, hemoglobin, and malondialdehyde (MDA) plasma.

The Bbasic characteristic data were reported in this studyin relationed witho the subjects' activities areinclude (1) sport experience and total training. The , which is definedition of sport experience iby thes how length ofong time is subjectss in the first time actively training in the specific sport, right from the first incidence. In addition, there is also a probability that t may be they have been involved in the sport since childhoodren. (2) Years of training (total training), which refers to the number of years the athlete had ofbeen training participation in this exercise as a competitive combater

Intensive Training Program

All subjects in this study awere professional and elite athletes, that ready to partake part in the sport competition, both at the national and international level. This also involved those Ppreparing for the 2016 The National Sports Week (Indonesian: *Pekan Olahraga Nasional*, *PON*), which is a multi-sport event held every four years in Indonesia.; In addition, theIndonesian national sports committee at East Java, obligated elite athletesthem to follow up intensive sport training program., Hhence, all subjects participated in this program sinceright from onea year ago. Therefore, further Iinformation aboutbased on duration, frequency, and intensity of intensive training were obtained through direct interviews with athletes, and also from secondary data sources, ofe.g., the training schedule of each sport. Moreover, Tthe coach of eaan ch individualtype sports game arranged a draft of one-week intensive training program., Howeverand, there is the similarities were identified in the trainingexercise trend amongst allat each type's sports as they; it contained physical exercise and specific exercises, encompassing—each combat sport techniquess as well ands coordination, balance, and flexibility exercises, and muscular power. ThereforeAthletes training commenceded every day, except for Sunday, with two sessions per day;, encompassing physical trainingactivities atin the morning for 2h and specific training drills

~~at~~in the afternoon for 2-3h, with medium to~~until~~ high intensity. These were ~~A~~all ~~trainings~~ ~~started~~initiated with a warm up, and terminated~~end final~~ with cooling down~~.~~, and ~~For~~the draft example ~~o~~for Judo sport~~, the draft of intense training program~~ is reported in table 1

Table 1

Anthropometry~~i~~ Measurements

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

Blood sampling Measurements

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

~~The~~ SOD activity was evaluated in the erythrocyte samples (cell lysates),~~ by~~ using the commercially available RANSOD Kit (Randox Laboratories), and ~~SOD~~ the levels recorded were expressed ~~as~~in unit per gram of hemoglobin (U/g Hb)~~.The examination of Erythrocyte SOD level is conducted by~~, utilizing the spectrophotometric method ~~by using~~with a multiple wavelength spectrophotometer tool [18]. Furthermore, its ~~C~~concentration ~~of hemoglobin~~ was determined ~~by~~using the cyanmethemoglobin method, where ~~and~~ the color of cyanmethemoglobin ~~i~~was read in a photoelectric colorimeter ~~at 540 nm~~ against a standard solution, at 540 nm [19].

Dietary intake

Macronutrient and micronutrient intake assessment was ~~carried~~conducted by food record at 3x24 hours~~. A week before blood sampling began;~~ , as all subjects were ~~given~~provided with a ~~food record~~ sheet for this purpose a week prior to the commencement of sampling. Therefore, the nutritionist ~~gave~~passed the information on how to ~~record foods~~ correctly input data, including details on portion and size ~~of the foods~~ consumed, by demonstrating a food model and the Indonesian food book. In addition, ~~T~~the subjects were asked to record all ~~food~~cuisine and drinks ~~consumed~~ ingested~~in~~ three days of~~in~~ ~~one~~a week (not necessarily consecutive) ~~on the food record sheet.~~ , which was clarified ~~A~~at the day data collection ~~of food record,~~ by the nutritionist ~~clarified the data by asking~~through direct questioning~~ly to subjects~~. In addition ~~For micronutrient intake analysis, beside~~ to mean and standard deviation, ~~we also reported those~~ nutrient based on adequate intake in comparison with~~ed to~~ the Indonesian recommended dietary allowance (RDA) were adopted in micronutrient intake analysis.

Statistical analyses

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

RESULTS

From A total of 4049 athletes out of 49 ofrom four different sports, registered as members of sports training center inof East Java Indonesia, 40 met the study criteria and were willing to participate in the research by signing informed consent. In addition, outA total of the nine athletes did that did not meet study criteriathe specification,: four subjects werereas currently taking on antioxidant supplements, and while others had gonetravelled overseas to run participate in competitions whenduring the time of data collection was implemented. Furthermore, Aall subjects awere identified as professional athletes routinely performing sport training routinely for 4.05±2.69 years, and had a mean of sport experience of 10.62±2.9 years. In addition, A total of 26 athletesparticipants (65%) were male, and mostly came from the wrestling athletessport. Male is , although they were dominant in almost of eachmost, sports except Judo., and Tthe mean age of subjects was 23.08 ± 4.32 years, with the majority of subjects (70%) ibeings in the age group ofbetween 20 and -29 years. Also, Tthe mean of BMI iwas recorded as 24.29±3.72 kg/m^2, and most subjects (62.5%) hadexhibited normal BMIlevels., while Tthe mean ofvalue obtained for body fat on all subjects were 19.37 ± 8.47%, with higher fat mass found inthe females having a relatively higher valuethan male athletes. Since fat mass is more prevalent in female athletes, it is not somewhat surprising that , especially those in the game of Judoka was dominant in women had the highest averages of fat mass. Furthermore, Tthe characteristics data and body composition weare reported atin Table 2

Table 2

From The dietary method, it can beindicates the seen macronutrient and micronutrient consumedintake byby subjects., where Tthe mean energy intake was 2408.04±801.96 mg/d;, which those intakeswas were higher in male than female., and– Ssimilar results were showndemonstrated by with carbohydrate, fat and protein that higher significantly in male athletes. In addition, the Mmicronutrient intake assessed in this study included antioxidants (vitamins A, E, C), as well as the Cu and Zn, intake which are known components of enzymatic erythrocyte (SOD)., and There is no significant different in consumption wasof micronutrient intake identified in accordanceing withe gender, although and wrestlers tend to portrayhad superiority intake of both macronutrient and micronutrient in contrast withthan others. Furthermore, Mmost of participants subjectsalso had vitamin E, vitamin and C, as well asnd zinc intakes that were less than the RDA specification., while the value obtained Ffor vitamin A and copper, indicated that 72.5% and 52.5% of subjects respectively wereingested sufficient classified amountsas sufficient intake (Table 3)

Table 3

The mean erythrocyte SOD of for all subjectsparticipants wasere 2280.69 ± 285.65 U/g Hb, and thesewhich values did not differ significantly (p> 0.05) between males and females, subjects as well as betweenamongst type sports type. In addition, Tthe mean Hb was 15.53±1.37, and all subjects were assessed as notn--anemicca, based on. Tthe reported of blood sampling can be showneen in Table 4

Table 4

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

Table 5

DISCUSSION

~~In t~~This study~~, we~~ investigated~~volved~~ some types of combat sports (karate, pencak silat, judo, and wrestling), in ~~order~~an attempt to ~~give~~provide a general description~~ about combat sp~~ on nutrient intake among these athletes,~~ort generally. Since~~ based on the prevalence of body weight and nutritional problems~~ is prevalent in almost type of combat sport, we tried to describe nutrient intake among these athletes~~. Furthermore, ~~we specifically measured~~ erythrocyte SOD (SOD1) was specifically measured as a parameter for antioxidant capacity, and ~~and~~subsequently associated with micronutrient intake, ~~because~~as a result of its wide~~ly~~ distribution,~~ed and comprises~~ with 90% ~~of total SOD is~~ Cu, Zn superoxide dismutase (SOD1) content (Noor *et.al* 2002).

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~~In this study, we~~This research involved the recruitment of~~ed~~ combat sport athletes (karate, pencak silat, judo, and wrestling) that were registered as members of the Indonesian national sports committee,~~ at~~ East Java, Indonesia~~.~~. Furthermore, ~~A~~all subjects were evaluated to have been ~~running~~participating in an intensive exercise program at the sport training center, ~~when~~during the period of data collection~~ conducted~~. ~~Before~~Prior to entering a sport-training center, ~~all athletes must follow~~ a series of medical and laboratory examinations were conducted by professional doctor, in order to prove ~~that they are~~ physical~~ly~~ and mental~~ly~~

healthyy. FurthermoreIn addition, almost of subjectsparticipants (92.5%) awere less than 30 years of aged, with a mean age of 23.08 ± 4.32 years., It means the study participants representinged the young and healthy elite athletes.

This findings are similar to most studies on combat sports, which involves young participants, such as studiesas observed by Radovanovic *et al.*, (2012) on judo athletes aged 20±1.3 years (Radovanovic et.al 2012), Pesic *et al.*, (2012) on karate athletes sportspersons aged 16 to 30 years (Pesic et.al 2012), Rynkiewicz *et al*., (2010) on sumo wrestlers with an average age of 23±6.6 years (Rynkiewicz et.al 2010). In addition, Tthe mean age 20.8±1.1 years ofn 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 thatwho follow routine training similar to those in study ((Ho et.al 2007, Rosseau et.al 2004). Moreover, Yyouth is a golden period for an athletes, where the age range ofabout 20 years has been established to beis the most productive age for athletes tfor being the best and getobtain their highest achievements. This was confirmed by Tthe reported of Indonesian national sports, which confirmedstated that thata mostbulk of the numerous gold trophies wasere achieved attained by young athletes (Record M 2014).

This study involved 40 athletes;, where 26 athletes (65%) awere male, while and 14 athletes (35%) awere females., which is reinforced by the data obtained from the Indonesian national sports data, showinged theat more sports are dominancent followed byof males athletes than female ones. MeanwhileIn combat sports, there are actually no restrictions orto agender specificity gender for participain combat sportsnts., although this gender discrepancy was observed in From the all four combat sports types studied in this research, male athletes were more dominant;, except Judo,only in one combat sport athletes, which werehad more female athletes participantsted, namely, judo. Furthermore, Tthe measurement of body composition showeddemonstrated 24.29±3.72 kg/m² as the mean body mass index (BMI), was 24.29±3.72 kg/m² andand an average body fat of all athletes wabouts 19.37 ± 8:47% was recorded in, wiboth higher fat mass categories found both in male and female athletesparticipants, although. Oonly 30% revealed fat masswere observed to be within the normal limits, while others surpassed. Thise category of "high fat mass" in this study might bewas probably due to the high fat intake of fat, whichsince it contributed to about 35.95 ± 8.18% of the total energy (data was shown)., therefore, concernsing about obtaining ideal levelsvalues of body fat and the consequent lean body mass shouldought to be the attention for for healthy and the best performance (Burke & Cox 2009).

Each sport in this study hads each a training program with active exercise schedule were activeconducted 5-6 days per week for an average of training period ofwas 4-5 hours per daily., Hhence, the total duration of training was about 20-26 hours per week. These Ddata based onabout duration, frequency and training intensity in this study demonstratesd that the characteristics of the subjects asre professional athletes. Furthermore, wequestions were asked about sport experience, that centered on themeans lengthongtime of time sincefrom when the athletes first participated in the specificir sports. The mean of sport experience was, and an average value of 10.62±2.9 years was obtained. It supposed that they, depicting their actively in the sport participation asince children. In Indonesiaaddition, combat sport is considered as one of the highly most favoritespreferred sport in Indonesia, often followed by children, and it also and confershave many numerous health advantages for health (Burke & Cox 2009, Record M 2014). Meanwhile, Pprevious study reported combat sport 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)., Furthermoreand Ju *et al.*, reported combat

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trainingthe propensity to facilitated the onset of an earlier secondary saccade onset in children aged 9-12 years [24].

Based on the data obtainedF from food records of 3x24h, it can bewas seenestablished that dietary of carbohydrate, fat, and protein were 230.63±116.95 g, 130.46±41.27 g, and 89.71±33.98 g, respectively.. TheseThese findings awere generally lower than the records obtained from previous studies., including Braakhuis *et al*, (2013) on professional rower athletes with carbohydrate intake of 510±190 g, 170±70 g, protein, and 110±45 g fat intake of 510±190 g, 170±70 g and 110±45 g, respectively(Braakhuis et.al 2013). Also, a study conducted by Pettersson, (2013) study aton combat sport athletes withexhibited a total intake of carbohydrates, protein and fat are 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 (Petterson et.al 2013). According to the sports nutrition guidelines, theseis findings showedindicate that fat intakeconsumption is relatively high, while that fore carbohydrate intake is slightly lower., and it has been established that Ccombat sport athletes often adoptedpplied the restrictions ofin diet food intake in an attempt ftor loseing weight, by limiting their carbohydrate intake as low as possible (Petterson *et.al* 2013). Data obtained Ffrom the interview, showed it can the possibility beto concluded tha almost all subjectsparticipants applied the weight loss program only on the day before the competition. Furthermore, from recall it clearly showed the incorrect application ofat nutrition by combat sport athletes have not applied yet sport nutrition correctly. , although Tthe guideline of nutrition in combat sport explained theat it is importancet forin goals achievementing goals. This specifically requires for fuel obtained from energy and macronutrient intakeconsumption, which is derived from a variety of food in the everyday diet. In addition,On days wheren high intensity training is undertaken, demands fuel

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requirements concerning macronutrient intake is very the usefulness of fuel supplies, in order to support training performance (Burke & Cox 2009).

The Aanalysis of antioxidant intake, based on the recommendedation dietary allowance (RDA) demonstrated 85% for vitamin C intake and 77.5% for zinc, and allintake of the subjects awere classified in the "less" category, indeed all subjectsand a (97.5%) for showed vitamin E was intakealso less than the recommended requirementamount. Theseis findings are similar to Rousseau *et al.*, (2004), which foundshowing that vitamin C intake to beis only 40% of RDA, while vitamin E intake inwas 81% ofor athletes, which is under 2/3 of theRDA recommendation, as well asnd beta carotene intake at 43% under 2/3 RDA. Meanwhile,, and 60% of athletes tend todo not reach theRDA specification for vitamin C, and 81% for vitamin E (Rosseau et.al 2004). However, ourthe finding of this study is lower than Pesic *et al.*, the value recorded in the investigation conductedstudy on karate athletes, which showeds vitamin E intake at 22.4±9.8 mg/day, andvitamin 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). Braakhuis *et al.*, 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 Rrestrictions ofin food intakediet for participants of combat sports athletes will result in lowthe reduction of energy oanr macronutrient intake, alsosubsequently affectleading to a decline in thes low antioxidant intakepresent (Petterson *et. al* 2013;, . Carlshon *et. al* 2010)

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Erythrocyte SOD levels studiedevaluated in this researchstudy arewas in thea form of SOD isoenzymes located in the cytosol, namelyincluding, CuZn-SOD (SOD1)., Since CuZn thus, based on its compositionSOD consists of Cu and Zn, theseis trace mineral therefore plays an important role in erythrocyte SOD its activities. Our Furthermore, findings showed only 52.5% of copper and 22.5% of -zinc intake were recorded, and subsequently classified in the “sufficient” category. This finding, although the value is lower than the record of Koury *et al.*, (2004), which study showed 27% and 2% of athletes had a lesser zinc and copper intake, in contrast lower than with recommended valuesation, respectively (Koury et.al 2004). Ho *et al.*, study demonstrated Also, low SOD activities in long distance runners athletes were evaluated to be probably caused by the copper and zinc deficiency of these elements;, and those minerals were estimated thetobe loss was estimated to have ensuedt through sweat and urine excretion (Ho *et.al* 2007). Kikukawa and Kobayashi,In addition, another report study also showed that a significant increase of their average levels of Zn and Cu in urine, increased significantly measured after exercise (kikukawa *et.al* 2002), whileand Resina *et al.*, (1990) reported testified that male runners had lower serum levels of serum than the control group/ non-athletes (Resina et.al 1990).

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Erythrocyte SOD levels in this study recorded were a representations SOD levels ofor athletes runninghave an anrun intensive training program. In this study, , of which all subjects participants representeddemonstrated a high erythrocyte SOD levelsvalue (> 1601 U/g Hb), with mean of 2280.69 ± 285.65 U/g Hb. These results obtained suggest that the adequate endogen antioxidant defence responseded adequately to intensive training program towards the strenuous exercise., The increasedand this elevation in antioxidant capacity relating towas also sports activities is also revealed by previous studies, both in athletes and non--athletes. Carlshon *et al.*, (2010) studyshowed the propensity forat regular exercise to increases blood levelsantioxidant capacity in younger athletes (Carlshon et.al 2010), while. Jemili *et al.*, study(2017) foundreported the capacity for intense specific training programs to improved the prooxidant-antioxidant balance, subsequently and increasinge theiin activity of superoxide dismutase activity after 3-month specific training in elite karate athletes (Jemili et.al 2017). However, theThe increasingintensification of antioxidant capascity in non--athletes wasere proven in a study by Berzosa *et al.*, (2011)study that, which showed the enhanced probability for acute exercise (cycloergometric tests) led to an augmentation ofthe antioxidant enzyme activities iofn untrained men (Berzosa et.al 2011). Also, Tthe elevationed of SOD activity after a single bout exercise in healthy women iwas also proven byin an investigation conducted by Yimcharoen *et al.*, (Yimcharoen et.al 2019).

The increase ofin antioxidant capacity related exercises seems still have been observed to shown inconsistent results., as Jurgenson *et al.*, (2019) revealed a significant decline in the antioxidant capacity volume recorded decreased significantly after 12-week of supervised strength training in competitive powerlifting athletes (Jurgenson et.al 2019). Also, Bundo and Anthony, (2016)study reported there was noabsence of a significant change in SOD activityies after 3 months of a supervised exercise program in healthy volunteers (Bundo & Anthony T 2016)., while Pesic *et al.*, (2012)study revealed established thatduring training process both in the state of rest and after the loading, there was no significantly changed toin oxidative stress and SOD activity during a training process, both in state of rest and after loading(Pesic et.al 2012), and also that. Tthe increasingelevations of SOD levelsobserved were not causedas a result byof long-time intensive trainingexercise, but caused by high physical loading.

The Aanalysis of SOD activity based on gender and sport types in this study showed no statistically significant differenceece. Our findings, which differs from the report by Dopsaj *et al.*, stwhereudy showed significanta variation in values difference of SOD activity was observed between the karate professionals and wrestlers (73 ± 37 vs. 103 ± 30, $p < 0.05$);.

Therefore, the high ~~SOD~~ activity in wrestlers ~~could~~is possibly ~~be~~ associated with the long-term impact of ~~wrestling~~the sport, ~~as~~being a type of strenuous exercise (Dopsaj *et.al* 2013). Moreover, ~~O~~other reasons ~~o~~for the high ~~SOD levels~~values obtained in this current study ~~differs from previous studies~~ are ~~likely~~probably due to the ~~differences~~discrepancy in the production of ROS formed, the ~~different~~variation in modes and intensity of training performed, as well as the interaction between~~of~~ SOD ~~with~~and other antioxidants within the body, ~~such as~~encompassing vitamin C, ~~vitamin~~ E, and~~vitamin~~ A. This ~~and~~was also due to its collaboration with micro minerals, including zinc (Zn) and copper (Cu) required by the ~~SOD~~ enzyme, ~~namely zinc (Zn) and copper (Cu),~~ as well as~~nd also~~ the ~~increasing~~elevation in the potential loss of minerals through~~in~~ sweat and urine (Metin *et. al* 2003, Ho *et. al* 2007, Bundo & Anthony T 2016).

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The~~By~~ useing of ~~the~~ Spearman rank correlation test~~, there is~~ showed no significant correlation between the consumption of micronutrients ~~intake~~ and erythrocyte SOD levels~~. However~~, although~~the significant result seems on female subjects,~~ a ~~where there is a significant~~ substantial positive ~~correlation~~relationship (r=0.538, p=0.047) was identified ~~between~~with vitamin C ~~with erythrocyte SOD Level~~in female participants. In addition, ~~Many~~numerous athletes, ~~particularly female, a~~were considered to be at~~a~~ a greater risk of iron depletion,~~ed~~ ~~which may~~with a possibility of leading into ~~iron~~ deficiency (with or without anemia),~~.~~ ~~A~~although the mean Hb ~~were~~of 15.53±1.37 recorded from~~and~~ all subjects ~~were~~ were assessed as non-~~t-~~anemic~~a~~, ~~but mean Hb at~~and the females~~ subjects~~ had a significantly lower value ~~than~~ in contrast with~~ose~~ male (Table 5). Hence, the results obtained are possibly explained by the capability for~~Since~~ vitamin C to enhance~~s~~ iron absorption, and the closely related mechanism ~~between~~of hemoglobin and red blood cell~~, it might explain the result~~ (Alaunyte *et.al* 2015). Furthermore,~~A~~ another reason is centered of the fact that vitamin C is

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stored in the adrenal gland, and is subsequently released during periods of stressful periods, in an attempt to confer protection against oxidative stress.. This assumption is backed up by a Ppreviousevious studyinvestigation, which showeded vitamin C intake its correlatione witho total antioxidant capacity amongst competitive rowers [14]. Furthermore, Yimcharoen *et al.*, (2019)study reported the probability of improved antioxidant capacity inat healthy women that performed moderate intensity cycling, reportedusing supplementations withcontaining ascorbic acid improve antioxidant capacity (Yimcharoen et.al 2019).

AnOne of the important things inaspect of this study is the established fact that all subjectsparticipants consumed multivitamin B supplements (Vitamin B1, B6, and B12) every daily duringas the sport programming. FurthermoreIn this finding, these have also been identified as responsible for the change in SOD activity, which was is categorized as high, although the intake of micronutrient intake iwas low, migh be caused the effect of this supplement. Ford *et al.*, provided evidence for the efficacy of high-dose B-group supplementation in reducing oxidative stress, and subsequentlythough increasing the affiliated oxidative metabolism. SinceTherefore, the ease of tolerating antioxidant-rich foods is well tolerated and haveits impact on performance .., it is supposed to ingested makes it aantioxidant-rich foods preferred choice ratherover than supplements (Koivisto *et. al* 2018)

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The fact that the study participants represent theing young and healthy elite athletes might determines the erythrocyte quality., as its Erythrocyte deformability is highly influenced by age. Also, andthe endurance rate of thein sport thattends to suggests the capability of the erythrocyte system mayto adapt to changing conditions, such asincluding adolescence, with the onset effects of sex hormones or physical exercise effects (Tomschi *et.al* 2018).

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Strength

To the best of our knowledge, tThis is the first study providingsupplying data on SOD1 among elite athletes running an intensive training program for a long time (one year). AlthoughIn addition, variations were identifiedthe in the characteristics of each combat sport are different, especially in terms ofon specific exercises, but the duration and frequency of training iswere similar each other, it indicatingmeans wthe controlled of these variables. Moreover,For most studies, it is tend to only focus and involved on athletes involved infrom one type of combat sport, in this study, but this current investigation entailedwe recruitinged somea typesvariety, including of combat sports (karate, pencak silat, judo, and wrestling), in orderan order to provide a general give description about combat sport generally. Finally, we specifically measured erythrocyte SOD (SOD1) was specifically measured as an indicator of antioxidant capacity at combat sport athletes, which was and further analyzed thein association with the intake of micronutrient intake as antioxidants, including vitamin C, vitamin E, vitamin A and micro minerals (zinc (Zn) and copper (Cu)) required by the SOD enzyme, namely zinc (Zn) and copper (which collectively Cu)serve as sources of antioxidants

Limitations.

Erythrocyte SOD levels recordedin this study wasere measured only once, exactly one year after athletes havethe- inception of thetrained intensive training program, and. Nno prior data or information about was made availablethese values before training. IndeedTherefore, weit is can nnot possible to draw conclusionsded whetheron SOD1 levels its increase or declinerease. Moreover, the study only adopted SOD in the evaluation of antioxidant capacity in response to high intensity and the longtime training program is not only SOD, where it is plausible to measure other antioxidant markers, such asencompassing glutathione peroxidase (GPx), and catalase (CAT), based on, sincethe records from previous studies whichy reported a marked~~the~~ increase~~ing~~ in the their individual concentrations~~of SOD, CAT, GPx concentration in response to regular high-intensity and or prolong duration exercise~~ (Jemili *et.al* 2017, Braakhuis *et.al* 2013).

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Conclusions

This study involved young elite combat sport athletes ~~that run~~participating in an intensive sport training program~~.~~, and it was established that ~~A~~all subjects had~~d~~ high erythrocyte SOD levels.~~ A high intensity and long-term exercise training program might be associated with high erythrocyte SOD levels in combat sports athletes.~~ In addition, ~~M~~most ~~subjects~~participants were observed to have ~~not~~incorrectly applied ~~yet~~the ~~sport~~ stipulated sport nutrition~~ correctly;~~, encompassing the intake of macronutrient and micronutrients, ~~intake is still~~ which were lower than the recommendation. ~~The~~This ~~low~~ shortfall~~nutrient intake both macronutrient and micronutrient intake~~ ~~should~~ought to ~~be~~attract the ~~attention by~~ sport committee attention, therefore requiring the~~to~~ invitation of~~e~~ dietetics professionals as ~~sport~~ nutrition consultant, in an attempt to solve the ~~nutrition~~ problem with diet. Furthermore, ~~T~~there is a possibility for the total ~~of~~ low intake to ~~could~~ cause depletion ~~of~~in vitamin/mineral status, especially with vitamin C ~~for~~in female athletes~~.~~, which is why ~~It is recommended for athletes~~ the~~to~~ intake of food rich in antioxidants is highly recommended to maintain high ~~antioxidant capacity~~activity.

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Conflict of interest

The au