Original Article

Comparison of Antioxidant Status between Pilots and Non-flight Staff of the Army Force: Pilots May Need More Vitamin C

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Abstract

Objective To compare the blood antioxidant levels and dietary antioxidant intakes between pilots and non-flight staff of the Army Force in The Islamic Republic of Iran.

Methods Thirty-seven helicopter pilots and 40 non-flight staff were included in this study. Their general characteristics were recorded and their weight, height, and waist circumference were measured. Their daily intake of energy and nutrients including antioxidants was assessed using a semi-quantitative food frequency questionnaire. Serum levels of total antioxidant capacity (TAC), malondialdehyde (MDA), and glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in red blood cells were also measured.

Results The median erythrocytes SOD, serum MDA level and the mean serum level of TAC and erythrocytes GPx were significantly higher in pilots than in non-flight staff. The median vitamin C intake was significantly lower in pilots than in non-flight staff. The serum MDA levels were similar in non-flight staff and pilots when their vitamin C intake was ≤168 mg and significantly lower in non-flight staff than in pilots when their vitamin C intake was >168 mg.

Conclusion The serum MDA level is lower in non-flight staff than in pilots when their vitamin C intake level is high, indicating that pilots need more vitamin C than non-flight staff.

Key words: Antioxidant status; Pilots; Army force

Biomed Environ Sci, 2014; 27(5): 371-377 doi: 10.3967/bes2014.062 ISSN: 0895-3988

www.besjournal.com (full text) CN: 11-2816/Q

INTRODUCTION

Pilots are exposed to 8 types of common stress, including fatigue, vibration, noises, barometric pressure, partial pressure of decreased oxygen, thermal changes, decreased humidity, and accelerative and gravity forces. In addition to these physical stresses and polluting factors, pilots are also faced with mental stress, occasional accidents, and even death[1]. Exposure to different kinds of stress contributes to the production of reactive oxygen species. Moreover, biological or mental stresses can significantly increase the oxidative stress markers, thus

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Received: March 16, 2013; Accepted: July 18, 2013

enhancing the activity of antioxidant defense system[2]. Despite multiple antioxidant systems, excessive generation of reactive oxygen and nitrogen species can inhibit the functions of immune system, thus leading to oxidative stress[3]. Damage caused by free radicals results in cell oxidation and inflammation which is known as an effective causal factor for some diseases like cancer, Alzheimer's disease, arthritis, heart disease, stroke, diabetes, multiple sclerosis, Parkinson's disease, and aging[4]. The effect of antioxidants on different disorders and diseases caused by oxidative stress has been well-documented, and antioxidant capacity determination is now considered as a tool for medical diagnosis and treatment of diseases[5-6]. It has been shown that the serum total antioxidant capacity is relatively stronger in pilots than in non-flight staff[7-8]. It was also reported that the serum level of total antioxidant capacity is significantly higher in pilots than in normal subjects[9]. The increased serum total antioxidant capacity in pilots is often accompanied by the increased level of lipid peroxides and chromosome damage[7]. Although pilots are widely exposed to different stressors, few studies are available on the assessment of the status of their antioxidants in Iran although a few studies have reported the level of blood antioxidants in pilots of other countries[7-9]. However, those studies did not consider the amount of dietary antioxidant intake. Hence, the blood antioxidant status and dietary antioxidant intake in pilots of the Army Force in Islamic Republic of Iran were determined and the difference between pilots and non-flight staff in the Army Force and between their blood antioxidant status and dietary antioxidant intake was compared in this study.

MATERIAL AND METHODS

Subjects

This descriptive-analytic cross-sectional study was performed among 37 male helicopter pilots and 40 non-flight staff of the Army Force in Islamic Republic of Iran between February and March 2011. Their body mass index (BMI) was 18.5-29.9 kg/m^2 and none of them had a history of inflammatory and specific diseases, such as diabetes, heart disease, multiple sclerosis, arthritis, cancer, respiratory and digestive inflammatory diseases, liver or kidney diseases. Those who smoked or consumed mineral and/or vitamin supplements were not included in this study. This study was carried out according to the principles of the Declaration of Helsinki and approved by The Ethics Committee of Tehran University of Medical Sciences (TUMS). The subjects were informed of the objective and methods, anticipated benefits and potential risks of the study, sources of funding and institutional affiliations of the researchers. Each subject signed an informed consent form prior to participation in the study.

General data of the subjects were collected through interviews and questionnaires. Their educational level was also considered and measured as the years of schooling.

Physical Activity Measurement Techniques

This study adopted the classified physical activity questionnaire according to metabolic equivalent (MET), which includes 9 activity levels from sleep/rest (MET=0.9) to high-intensity physical activities (MET>6). The validity of this questionnaire was justified using the daily physical activity record questionnaire and the CSA accelerometer (model 7164 ambulatory monitor)[10]. In Iran, the reliability and validity of this questionnaire has been verified by Kelishadi et al.[11]. The hours spent on each physical activity were multiplied by their MET quantities and the numbers obtained were summed together to calculate the MET.H/day value.

Dietary Analysis

The daily intake of energy, nutrients and servings of fruits and vegetables of the subjects was determined in the month immediately before the study using a semi-quantitative food frequency questionnaire. The reliability and validity of the food frequency questionnaire containg 168 items used in this study was designed and validated as previously described[12]. Since the only available Iranian food composition table includes limited varieties of raw foods[13], the USDA food composition table was employed. The Iranian food composition table was used as an alternative for Iranian foods like Kashk (whey) that is not included in the USDA food composition table.

Anthropometric Measurement Techniques

The weight of the subjects wearing light clothes but no shoes was measured and recorded as 100 gr (Tefal scale, France). Their height was measured without shoes using the Seca height gauge and recorded as 0.5 cm. Their waist circumference between the lower rib and the anterior superior iliac spine was measured at a standing position with a normal breath with a tape. Their BMI was then calculated according to the formula: weight (kg)/height^2 (m).

Laboratory Measurement Techniques

A 10 mL venous blood sample was taken from each subject after 12 h fasting. The TAC was assessed using the modified -di-3-ethyl-benzthiazoline sulphonate, Azino 2' 2 ABTS cation radical decolorization method. Their MDA was measured by spectrophotometry (SATOH 1978)[14]. The GR, GPx, and SOD enzymes of red blood cells were assessed by spectrophotometry with the Randox company kit (Crumlin, UK). The activity of CAT enzyme in red cells was measured by manual assay and spectrophotometry, respectively.

Statistical Analysis

Data were analyzed using the SPSS software version 11.5. The normal distribution of variables was checked by Kolmogorov-Smirnov test. The normal and abnormal distributions of variables were compared by independent *t* and Mann-Whitney tests, respectively. Data were expressed as mean±SD. $P<0.05$ was considered statistically significant.

RESULTS

The median education level of the pilots was higher (P=0.001) than that of the non-flight staff (Table 1).

Table 1. General Characteristics and Anthropometric Measurements of Pilots and Non-flight Staff

Variables	Pilots	Non-flight Staff
Age (year)	42±5*	40±10.75*
Literacy (year)	16±2$^{*,\#}$	16$^{*,\#}$
Years of service (year)	24±6.5*	20.5±11.25*
Flight hour (hour)	1000±830*	-
Physical activity (METs. Time/day)	37.27±5.68	39.9±6.97
Weight (kg)	80.94±9.13	79.97±8.58
Height (cm)	174.5±6.2	173.19±5.85
BMI (Kg/m^2)	26.54±2.27	26.61±1.95
Waist circumference (cm)	92.22±6.52	91.45±5.97

Note. Data expressed as means±SEM or median±inter-quartile range, *median±inter-quartile range, $^\#P<0.001$.

There was not much difference between the two groups in the mean intake of energy and most nutrients, but the pilots intake of Vitamin C and Vitamin B6 was significantly lower (P=0.01 and P=0.03, respectively) than that of the non-flight staff (Table 2).

The median erythrocytes SOD and serum MDA and the mean serum TAC and erythrocytes GPx of the pilots were significantly higher than the non-flight staff (all $P<0.001$) (Table 3). Comparison of the biochemical parameters between the two groups based on their educational level showed that the subjects with a higher level of education had a significantly higher mean red blood cell GR level than those with a lower educational level (Table 4).

The median and inter-quartile ranges of biochemical parameters of the pilots and the non-flight staff based on their Vitamin C intake are shown in Table 5. Based on the median dietary Vitamin C intake of the pilots, namely about 168 mg per day, all of the subjects were divided into two groups (≤168 mg or >168 mg). In the group with the daily Vitamin C intake >168 mg, the levels of TAC, SOD, MDA, and GPx of the pilots were significantly higher than those of the non-flight staff (all $P<0.008$). However, no difference was found in the serum MDA level between the pilots and the non-flight staff in the group with the daily Vitamin C intake ≤168 mg (Table 5).

DISCUSSION

This has been the first study to determine the antioxidant status of pilots in relation to their dietary intake, as previous studies on pilots have not assessed the dietary intake of antioxidants[7-9,15-18]. Generally, food consumed affects the oxidative stress status[8], for the level of dietary intake of antioxidant micronutrients directly influences the circulating level of these nutrients and antioxidant metalloenzyms. Therefore, reduction in intake of the antioxidant micronutrients weakens the immune system against free radical damages[19]. No significant difference was found between the two groups in the intake of energy, most nutrients and servings of fruits and vegetables. However, statistically significant differences were found in the median daily intake of Vitamin C and Vitamin B6 between the two groups. The pilots had a lower Vitamin C intake, which may be related to the amount of their fruit consumption. The daily fruit intake of the pilots was lower than that of the non-flight staff.

Table 2. Dietary Intake of Pilots and Non-flight Staff

Variables	Pilots		Non-flight Staff		*P* Value
	Mean	SD	Mean	SD	
Energy (kcal)	3106.60	865.4	3320.8	1031.10	0.28
Protein (g)	117.15	37.02	126.53	40.06	0.25
Fat (g)	93.2	34.8	103.6	41.5	0.20
Carbohydrate (g)	606.6	1000.3	494.4	139.7	0.45
Vitamin C (mg)	168.01	127.81	237.58	120.02	0.01
Vitamin E (mg)	11.57^{+}	4.03^{*}	12.12^{+}	6.58^{*}	0.32
Vitamin A (μg)	607.3	300.93	579.09	540.56	0.67
Vitamin D (μg)	2.1	1.3	2.06	1.1	0.86
Vitamin K (μg)	378	167.56	1352.4	5699.6	0.25
Vitamin B1 (mg)	2.8	0.8	3	0.9	0.80
Vitamin B2 (mg)	2.5	0.7	2.8	0.9	0.13
Vitamin B3 (mg)	33.28	9.27	35.22	10.37	0.35
Vitamin B6 (mg)	2.46	0.78	2.84	0.87	0.03
Vitamin B12 (μg)	5.2	4.05	5.4	2.66	0.73
Selenium (μg)	165.75	57.91	169.27	50.77	0.76
Calcium (mg)	1603.6	456.74	1824.8	694.12	0.07
Zinc (mg)	20	10.07	20.53	9.85	0.80
Magnesium (mg)	660.8	283.58	701.77	288.76	0.49
Fruit intake (serving/day)	3.32	1.78	3.81	1.49	0.10
Vegetable intake (serving/day)	2.5	1.39	2.75	1.28	0.40

Note. Data showing means±SEM or median±inter-quartile range, $^{+}$median, * interquartile range.

Table 3. Biochemical Parameters of Pilots and Non-flight Staff

Variables	Pilots	Non-flight Staff
TAC (g/Dl Alb)	$4.54\pm0.37^{\#\#\#}$	$3.4\pm0.62^{\#\#\#}$
SOD (U/g Hb)	$1381\pm276^{*,\#\#\#}$	$1082\pm296.25^{*,\#\#\#}$
MDA (nmol/mL)	$3.8\pm0.85^{*,\#\#}$	$3\pm1.2^{*,\#\#}$
GPx (U/g Hb)	$46.6\pm7.86^{\#\#\#}$	$28.39\pm9.1^{\#\#\#}$
GR (U/g Hb)	$4.2\pm1.7^{*}$	$4.4\pm3.05^{*}$
CAT (K/g Hb)	$121\pm37^{*}$	$125.5\pm39.75^{*}$

Note. Data expressed as means±SEM or median±inter-quartile range, *median±inter-quartile range, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$.

However, this difference was not statistically significant. The lower Vitamin C intake of the pilots may be due to their busy work schedule compared to the non-flight staff. This issue needs further investigation. The lower Vitamin C intake exacerbated the oxidative stress of the pilots, which will be discussed later.

Findings of the present study revealed that the median erythrocytes SOD and serum MDA and the mean serum TAC and erythrocytes GPx of the pilots were significantly higher than those of the non-flight staff. The same results were obtained when the biochemical parameters of the pilots and the non-flight staff were compared based on the educational level. In addition, comparison of the same parameters between the two groups based on the amount of Vitamin C intake showed that in the group with a lower Vitamin C intake, the MDA level of the pilots was similar to that of the non-flight staff. However, in the group with a higher Vitamin C intake, the MDA level of the non-flight staff was significantly lower when compared to the pilots. Thus, the serum MDA level decreased as the Vitamin C intake of the non-flight staff increased, while a similar decrease was not observed among the pilots. The mean Vitamin C intake was high for both the pilots and the non-flight staff. The data was collected in winter featuring high consumption of citrus and kiwi fruits. However, the result of the study shows that pilots may need even more Vitamin C. Further studies could be illuminating if they explor the effect of interventions such as increasing of dietary antioxidant intake or provision of supplements on changes of the oxidative stress pattern in pilots, like

Table 4. Biochemical Parameters of Pilots and Non-flight Staff with Different Educational Levels

Education Level	Pilots	Non-flight Staff
≤16 Years	*n*=21	*n*=34
TAC (g/dL Alb)	4.71±0.62*,###	3.52±0.57*,###
SOD (U/g Hb)	1413±313.5*,###	1092±329*,###
MDA (nmol/mL)	3.91±1.07##	3.27±0.82##
GPx (U/g Hb)	46.68±8.63###	27.9±8.91###
GR (U/g Hb)	4.4±2.4*	4.1±2.52*
CAT (K/g Hb)	126±42*	125.5±39.75*
>16 Years	*n*=16	*n*=6
TAC (g/dL Alb)	4.5±0.61*,###	3.56±1.12*,###
SOD (U/g Hb)	1386.25±205.23###	980.33±177.42###
MDA (nmol/mL)	3.76±0.74#	2.85±1.12#
GPx (U/g Hb)	45.5±7.25*,###	29±21.75*,###
GR (U/g Hb)	4.11±1.11###	6.91±2.05###
CAT (K/g Hb)	114±40*	124.5±86.5*

Note. Data expressed as means±SEM or Median±inter-quartile Range, *median±inter-quartile range, #P<0.05, ##P<0.01, ###P<0.001.

Table 5. Biochemical Parameters in Different Levels of Daily Vitamin C Intake in Pilots and Non-flight Staff

Vitamin C ntake	Pilots	Non-flight staff
≤168 mg	*n*=18	*n*=5
TAC (g/dL Alb)	4.5±0.43###	3.31±0.72###
SOD (U/g Hb)	1408±267.2*,#	1098±551*,#
MDA (nmol/mL)	3.65±0.82*	3.3±1.3*
GPx (U/g Hb)	47±7.2*,###	25±9.7*,###
GR (U/g Hb)	4.76±2.52	4.64±1.72
CAT (K/g Hb)	122.5±24.75*	101±23*
>168 mg	*n*=19	*n*=35
TAC (g/dl Alb)	4.57±0.3###	3.42±0.61###
SOD (U/g Hb)	1408.68±220.71###	1019±229.05###
MDA (nmol/mL)	3.99±1.13##	3.2±0.9##
GPx (U/g Hb)	45.68±7.71###	28.52±9.54###
GR (U/g Hb)	4.35±1. 53	5.01±2.03
CAT (K/g Hb)	116±54*	128±56*

Note. Data expressed as means±SEM or median±inter-quartile range, *median±inter-quartile range, #P<0.05, ##P<0.01, ###P<0.001.

decrease in serum MDA. In a study by Ghosh, the oxidative damage as a result of the shortage of ascorbic acid increased lipid peroxidation and MDA concentration in African swine tissue[20]. Also, in a study by Olayaki, Vitamin C intake reduced MDA production in pregnant women[21]. Vitamin C and glutathione play a role in activating the inactive form of Vitamin E, which as an antioxidant, affects the lipid peroxidation system. In this way, Vitamin C influences generation of lipid peroxidation products like MDA[22].

In a study by Liu and on pilots, civilian flight staff and non-flight residents, the mean serum SOD, MDA and TAC significantly differed among the three groups. In this study, the mean serum levels of SOD, MDA and TAC of the pilots and the flight staff were higher than those the non-flight residents[8], showing similar results to the present study.

In a study by Hao, the pilots had a higher mean serum MDA and a lower mean blood GPx level than the control group. The lower GPx level might be due to the time of the examination that was performed immediately after flight, heat and noises in the flight environment might have caused an increase in lipid peroxidation reaction and a decrease in the antioxidant capability[17].

It has also been shown in studies on animals that exposure to acute noise could cause a significant increase in SOD, GPx, and CAT[23].

In Chen's a study similar to ours, a statistically significant difference was reported in mean SOD, MDA, and TAC levels and between the flight and non-flight staff. The mean SOD, MDA, and TAC levels of the flight staff were higher when compared with the non-flight staff. The increase in the levels of these parameters in the flight group indicates that the level of lipid peroxides, as a chromosomal damage factor, increased during the flight, which agrees with the compensatory increase in the antioxidant capacity. In other words, the flight staff were under oxidative stress, resulting in an increase in reactive oxygen species[7].

In a survey conducted by Luca, a significant increase in erythrocytes SOD was found in the pilots as compared to the control group, and such increase was detected in the absence of adjustment of the pilots' CAT and GPx. Changes in circadian rhythms, psycho-behavioral and physical stress led to an increase in stress indicators among these pilots[15].

Therefore, the results of the present study as well as the findings of many previous studies suggest that pilots and flight staff have a higher antioxidant serum level than non-flight staff. The reason is that pilots are exposed to different stressors that result in a compensatory increase in antioxidant enzymes. Oxidative stress occurs when internal resources (including mitochondrial aerobic metabolism and destruction of dopamine by monoamine oxidase) and external sources (such as exposure to ionizing radiation) for producing reactive oxygen species exceed the capacity of the antioxidant defense system. To decrease the destructive effects of oxidative stress, some of the internal antioxidant defense systems (including SOD and GPx) and the external antioxidant resources (including vitamins and antioxidant flavonoids) will function in the body. When the reactive oxygen species produced exceeds the antioxidant capacity of these systems, it will lead to the oxidative stress with symptoms such as aging, neurological disorders, and carcinogenesis increase[24]. No study has considered the antioxidant intake of the pilots as an confounder factor[7-9,15-18]; however, it is of great importance to realize that changes of the antioxidant status are only a result of the oxidative stress or antioxidant intake[24].

Although the pilots in our study had an antioxidant intake level higher than the RDA values, they had higher levels of serum MDA. Consequently, it is probable that they need more antioxidants, such as Vitamin C. Intake of more antioxidant vitamins could be helpful for the pilots by decreasing the oxidative damage to them[3]. More studies are needed to clarify advantages and limitations of antioxidant interventions in pilots.

One of the limitations of the present study was that we used serum MDA assay to assess the oxidative stress. Despite the fact that this assay is common, existence of other aldehydes can simply affect the results. Therefore, using other assays such as isoprostanes could represent the oxidative stress status more precisely. Nevertheless, this study provided valuable information on the dietary antioxidant intake of the pilots as compared to the non-flight staff, and it is one of the few studies to assess the effect of physical activities on pilots' antioxidant status.

Overall, pilots had higher levels of erythrocytes antioxidant enzymes, serum total antioxidant capacity and MDA as compared to the non-flight staff and the intake of dietary Vitamin C was significantly lower in the pilots. The non-flight staff with a higher level of Vitamin C intake had a lower serum MDA level, whereas the same relationship was not found in the pilots. This probably shows that pilots need more Vitamin C intake. Therefore, it is recommended to evaluate the effects of increase in intake of dietary antioxidant and supplements and also fruits and vegetables as well on oxidative stress biomarkers in pilots.

ACKNOWLEDGEMENTS

The authors would like to thank the pilots and the non-flight staff of the Islamic Republic of Iran Army Aviation for their participation in this study. This study was funded by the Medical University of Armed Forces.

The protocol of this study was approved by the Ethics Committee of the Tehran University of Medical Sciences.

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