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THE EFFECT OF VITAMIN C SUPPLEMENTATION ON LEUCOCYTE COUNTS AND EXERCISE PERFORMANCE

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ABSTRACT

THE EFFECT OF VITAMIN C SUPPLEMENTATION ON LEUCOCYTE COUNTS AND EXERCISE PERFORMANCE. **M. Özaslan, T. Aytekin, İH. Kiliç, AI. Bozkurt, E. Güldür, B. Cengiz, C. Bağci**. **JEP**online. 2004;7(2):101-105. We studied the effects of vitamin C supplementation on the leucocyte counts and exercise performance of mice. Mice were divided into 4 groups; 1 control and 3 experimental. We supplemented the diet of the mice from each experimental group with different doses of ascorbic acid (4 mg, 8.8 mg, and 13 mg/day; C4, C9, C13, respectively) by intra-peritoneal injection. Physiological serum was given to the control group (CON) via the same procedure. Exercise performance was based on swim time to fatigue. Blood samples were taken and evaluated at day 7, 14, 21, and 28. At the end of day 28, tissue samples were taken from different organs for pathological examination. The lymphocyte percentage was 40.2 ± 6 % for CON on day 28. For all C groups, the range of the lymphocyte percentage was 47.5 %-57.1 % ($p < 0.001$). Swim time was 1.6 ± 0.3 min at day 28 for CON. This value was increased to 4.6- 8.8 min for the C groups ($p < 0.001$). No pathological differences in organs (skeleton muscle, stomach, spleen, kidney, liver, heart, skin, brain) were determined between the control and experimental groups. In conclusion, it was found that vitamin C supplementation increased the lymphocyte level in blood and improved swim time to fatigue.

Key Words: Diet, Lymphocyte, Swimming, Mice

INTRODUCTION

Vitamins are organic micro food materials that are needed for optimal cell, tissue, organ and body functions. For example, vitamins activate some enzymes which have important roles in protein, carbohydrate and fat metabolisms (1,2). An important water-soluble vitamin is vitamin C, which has diverse functions including being an antioxidant, exerting positive effects on lipid and iron metabolism (4), and promoting improved immune function (4,5).

We investigated the influence of different doses of vitamin C supplementation on relative changes in mice leucocyte counts and swim time to fatigue. It was hypothesized that the immune system and exercise performance would be improved by each dose of vitamin C, and that pathological investigation of organ tissue would reveal morphological differences caused by vitamin C supplementation.

METHODS

Forty mice (32 ± 4 g) were assigned to 4 groups of ten, as explained and detailed below. All animals were housed in an environmental controlled room with a 12 hr light and dark cycle. Hydration was provided ad-libitum using normal tap water.

The four groups of mice were fed according to the recipe given below.
Group 1: Control group, standard mice feed, and isotonic water given as 13 mg/day intraperitoneal injection (CON).
Group 2: Standard mice feed, and 4 mg/day vitamin of C by intraperitoneal injection (125 mg/kg) (C4).
Group 3: Standard mice feed, and 8.8 mg/day vitamin of C by intraperitoneal injection (275 mg/kg) (C9).
Group 4: Standard mice feed, and 13 mg/day of vitamin C by intraperitoneal injection (406 mg/kg) (C13).

The standard mice feed was composed of water, crude protein, crude cellulose, crude ash, ash that is insoluble in HCl, NaCl, calcium, phosphorus, sodium, lysine, methionine (Korkutelli Feed and Food Ltd. Co Antalya, Turkey). The vitamin C source was ampules containing 500mg/5mL vitamin C (Redoxon®, Roche Pharmaceuticals).

Blood samples were taken at days 7,14, 21 and 28, and blood smears were achieved and stained with giemsa stain. Lymphocyte, monocyte and neutrophil counts were done using standard manual count and formula methods (6).

For exercise performance measurements, a swimming test was applied to the mice at the end of the 4th week. Mice swam alone in pools with 10 cm length and 25 cm diameter dimensions. Swim time was recorded as the time to the first sign of an inability to maintain the head above water.

For pathological examinations, tissue samples were taken from liver, kidney, spleen, stomach, heart, tail, fury skin, brain, and leg muscles after day 28. The preparations were examined using a Hematoxilen-Eosin stain.

Statistical analyses were performed using SPSS 6.0 for the personal computer. ANOVA tests were used for the comparison of groups according to the parametric conditions. For the comparison of the values of the same groups at different weeks, the Friedman test was used. All data are reported as Mean ± SD.

RESULTS

The results of lymphocyte, monocyte and neutrophl percentages of blood obtained at blood smear from control and experimental groups at the end of days 7, 14, 21 and 28 are given at Table 1. There were no differences in lymphocyte, monocyte and neutrophil percentages across days for each group. However, significant differences were obtained when comparing data between CON and experimental groups. Lymphocyte percentages of each of C4, C9, and C13 increased significantly, whereas neutrophil percentages decreased.

Swim time was significantly increased in all experimental groups, and also increased with increasing dosages of vitamin C (Table 2). Although slight inflammation was detected in tissue samples from the kidney, liver, spleen and heart, similar results were evident for both control and all experimental group. As such, vitamin C supplementation did not cause alterations to tissue morphology.

Table 1.The percent of lymphocyte, neutrophil and monocyte counts of each group.

		CON	*C4*	*C9*	*C13*	*Between group comparisons*
Lymphocyte %						
	7	41.6 ± 5	47.1±7	49.8±6	57.1±5	p=0.000
	14	40.5±4	46.6±6	51.3±7	54.3±5	p=0.001
	21	41.8±3	47.7±6	51.4±5	55.3±3	p=0.000
	28	40.2±6	47.5±5	51.1±4	57.1±4	p=0.000
Monocyte %						
	7	4.2±3	6.4±3	3.8±3	5.8±3	p=0.21
	14	6.4±2	6.4±2	5.1±2	5.4±2	p=0.57
	21	5.8±3	6.5±3	5.7±3	5.4±2	p=0.83
	28	6.5±3	5.1±3	4.3±2	5.4±2	p=0,30
Neutrophl %						
	7	54.2±7	40.1±8	46.5±9	37.1±5	p=0.0002
	14	53.1±5	47±7	43.6±7	40.5±6	p=0.0013
	21	53±5	45.8±7	44.6±7	38.8±4	p=0.0002
	28	53.3±7	47.4±8	44.6±5	37.7±5	p=0.0001

Table 2. Swimming time of the groups on the 28th day.

	CON	*C4*	*C9*	*C13*	*Main Effect*
Swim time (min)	1.6±0.3	4.6±1.2	6.3±1.7	8.8±1.5	p<0.001

DISCUSSION

It is well-known that vitamin C deficiency causes lack of appetite, exhaustion, a decrease in performance during physical activities, increased muscle pain, immune suppression, susceptibility to infections, and delays in the recovery from infection (1,8). Prior research has shown that infections of the upper respiratory tract (IURT) decreased 50% in a group of marathon runners that were supplemented with vitamin C, and similar results have been reported for untrained individuals (9). However, Marshall at al. reported that there is not any affect of vitamin C supplementation on antioxidant capacity in greyhound dogs (12).

De la Fuenta at al. reported that endotoxin shock decreased the chemotaxis of lymphocytes and increased free radical production. According De la Fuente's study, N-acetylcysteine and vitamin C prevented these changes in lymphocyte functions (11). In this study, it was also reported that lymphocytes were important targets of oxidative stress. Victor at al. reported that immune cells needed proper antioxidant levels in oxidative stress conditions. They also reported lymphocytes stored vitamin C, and that these stores were used by macrophage (4,5). Öztürk at al. reported that vitamin C prevented the entrance of Ca^{++} formed as a result of oxidative stress, and by this mechanism prevented intracellular damage (13).We did not investigate such an interaction by forming endotoxic shock. However, the higher number of lymphocytes observed in the groups supplemented with vitamin C (regarding control group) indicated a relationship between vitamin C and lymphocyte numbers. Also, the identification of the dose-response relation between vitamin C and lymphocytes is noteworthy.

The additional topic of investigation in our study was the effect of vitamin C on performance. There are several past studies on this topic (14-22). In fact it is known that intake of vitamin C in amounts lower than the necessary detrimentally affects exercise performance (7). It has also been reported that vitamin C intake must be increased with increases in physical exercises (19).

Several authors reported that there was no affect of excessive vitamin C supplementation on sportsmen or normal people (2,14,18). For example, no significant benefit of vitamin C supplementation was noted in several placebo-controlled studies (15-18). In fact, in the earlier noted study using greyhounds, vitamin C supplementation actually decreased performance (12).

The other result obtained in these studies was the increase in leukocytes (especially lymphocytes) and plasma C vitamin levels after exercises (7,23). It is thought that this result indicates the release of vitamin C from the organs as a defence because of the stress formed as a result of the exercise.

Senger and co-workers explained increasing plasma vitamin C levels as a result of increasing dehydration or the reflection of the vitamin excreted from the lymphocytes (23). Another explanation for increased plasma vitamin C after exercise is a release of vitamin C from the adrenal gland (24). In a study similar to ours, it was also reported mice fed with vitamin C and vitamin E increased growth and exercise performance (25).

All these results indicate that the effect of vitamin C on performance is debatable. Nevertheless, our data show clear benefits of vitamin C supplementation in mice. In addition, there were no differences in pathological examinations of the tissues obtained from liver, kidney, spleen, stomach, heart, tail, fury skin, brain and leg muscles both control group and experimental groups.

The results of the study must be evaluated by considering that mice are organisms that can synthesize vitamin C in their body in contrast to the human being.

CONCLUSIONS

Higher levels of lymphocyte were observed in the groups supplemented with vitamin C compared to control. By increasing the dose of supplemented vitamin C, lymphocyte levels and swimming time increased.

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