# รายงานวิจัยฉบับสมบูรณ์

ระดับวิตามินอีและบีตาแคโรทีนที่ตอบสนองต่อผลของการออกกำลังกายแบบฉับพลัน และแบบฝึกในผู้ที่ไม่ค่อยออกกำลังกาย

Effect of acute and trained exercise on vitamin E and β-carotene levels in sedentary people

โดย
นางสาวกุลธิดา กล้ารอด
สาขาวิชากายภาพบำบัด คณะสหเวชศาสตร์
มหาวิทยาลัยบูรพา

เดือน ปี ที่เสร็จโครงการ ๒๕ กุมภาพันธ์ พ.ศ. ๒๕๕๗

สัญญาเลขที่ ๑๑๓/๒๕๕๖

รายงานวิจัยฉบับสมบูรณ์

# โครงการ ระดับวิตามินอีและบีตาแคโรทีนที่ตอบสนองต่อผลของการออกกำลังกายแบบฉับพลัน และแบบฝึกในผู้ที่ไม่ค่อยออกกำลังกาย

# Effect of acute and trained exercise on vitamin E and β-carotene levels in sedentary people


| ผู้วิจัย | สังกัด |
|---|---|
| 1. นางสาวกุลธิดา กล้ารอด | สาขาวิชากายภาพบำบัด คณะสหเวชศาสตร์ มหาวิทยาลัยบูรพา |
| 2. นางสาวพัชรี บุญศิริ | ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น |
| 3. นางสาวรุ่งเพ็ชร ตั้งรัศมีประเสร็ฐ | ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น |
| 4. นางสาวประณิธิ หงสประภาส | ภาควิชาอายุศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น |
| 5. นางสาวกฤติกา หงษ์โต | สาขาวิชากายภาพบำบัด คณะสหเวชศาสตร์ มหาวิทยาลัยบูรพา |
| 6. นางสาวทิษฏยา เสมาเงิน | สาขาวิชาเทคนิคการแพทย์ คณะสหเวชศาสตร์ มหาวิทยาลัยบูรพา |



สนับสนุนโดยสำนักงานคณะกรรมการวิจัยแห่งชาติ (วช) ประจำปีงบประมาณ พ.ศ. ๒๕๕๖

**Manuscript: Low intensity endurance exercise influence on vitamin E and retinol levels in sedentary lifestyle**

Kultida Klarod, M.Sc.[a], Pranithi Hongsprabhas, M.D. FRCP(T)[b], Tistaya Samangeon, Ph.D.[c], Roongpet Tangrassameeprasert, M.Sc.[d], Krittika Hongto, M.Sc.[a], Patcharee Boonsiri, Ph.D.[d,*]

[a]Department of Physical Therapy, Faculty of Allied Health Science, Burapha University, Chonburi 20131, Thailand

[b]Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

[c]Department of Medical Technology, Faculty of Allied Health Science, Burapha University, Chonburi 20131, Thailand

[d]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

Short title: exercise and antioxidants in sedentary people

*Corresponding author:

Patcharee Boonsiri

Department of Biochemistry,

Faculty of Medicine,

Khon Kaen University,

Khon Kaen, 40002, Thailand

Tel & fax +66 43 348386

E-mail: patcha_b@kku.ac.th

**Abstract**

*Objective:* Exhaustive endurance exercise can induce reactive oxygen species; ROS. It was important for sedentary to adaptation in regular exercise. We aimed to determine the effect of acute and trained exercise on antioxidant levels and nutritional status in sedentary people performing low intensity endurance exercise.

*Methods:* Nutritional status was assessed by body mass index (BMI), body fat, lean body mass, and waist to hip ration (WHR). The C-reactive protein (CRP), creatinine phosphokinase (CPK), lactic acid, and serum vitamin E and retinol levels were determined.

*Results:* Twenty healthy sedentary volunteers with age range, 19-23 years old were enrolled. They were compared among pre-exercise, acute exercise, trained exercise at 4, and 8 weeks. There was significantly increased in vitamin E level after acute exercise ($p < 0.001$), trained exercise at 4weeks ($p < 0.001$) and 8 weeks ($p < 0.001$). Vitamin E level higher in trained exercise at 4 weeks than in acute exercise ($p = 0.004$). BMI was significantly lowered in acute exercise ($p < 0.001$) and trained exercise at 4 weeks ($p = 0.025$) compared to pre-exercise. Lean body mass was significantly declined in acute exercise ($p < 0.001$), trained exercise at 4 ($p = 0.001$) and 8 ($p = 0.002$) weeks compared to pre-exercise. There was significantly reduced in weight to hip ration in trained exercise at 8 weeks than in pre-exercise ($p = 0.013$).

*Conclusion:* Significant higher in vitamin E level were found in sedentary who performed low intensity exercise compared to pre-exercise. Levels of nutritional status was differed among acute and trained exercise bout. Thus, vitamin E might be the sensitive biomarker affected after sedentary attended in low intensity endurance training exercise. The protein supplementation should be taken into consideration.

*Key words*: Antioxidants, Exercise, Sedentary, Nutritional status

## 1. Introduction

Exercise was believed to have benefit for patients in rehabilitation program. It had remained recommendation for improving muscle mass, muscle strength, and muscle endurance in people with chronic disease (1, 2). During exercise session, there was an increase in consumption of oxygen, especially in endurance exercise. Exhaustive exercise can lead to muscle cell damage and inflammation reaction, demonstrated by the augmentation of sarcolemma disruption (3). Nevertheless, regular exercise can improve defense against ROS by influence adaptive effect in antioxidant and repair mechanism (4). Moreover, non-enzymatic antioxidants such as vitamin E (α-tocopherol) lives in membrane of mammalian cells, is the most potent scavenger of radical in phospholipid bilayers (5). Another is vitamin A, the mostly precursor of vitamin A is β-carotene. Vitamin A, in the retinol form, may act as a chain-breaking antioxidant with the consider mechanism like carotenoids that may interpose in lipid peroxidation phenomenon (6, 7).

Chronic exercise have been reported to expand life span in rodents (8, 9). Muscles cell have adaptation response to free radical during contraction in ROS exercising production, by increasing antioxidant protection mechanism. Then, the cells have capably organized with the regular ROS results (10). On the other hand, it is very crucial for sedentary people to acclimatize to exercise-induced detrimental effect from ROS during exercise. Thus, the intensity, time and the type of exercises must be considerable for these particular people.

The aim of our study was to determine the effect of acute and trained exercise on antioxidant levels (vitamin E and retinol), and nutritional status in sedentary people by low intensity endurance exercise. Additionally, the relationship between nutritional status, and antioxidant level were also assessed.

## 2. Materials and methods

### Participants

Twenty healthy sedentary volunteers (seventeen females and three males with an age range of 19-23 years old) were enrolled in this study. They were not did regularly exercise, 1 time/ day, 3 times/ week, for a least 3 months before participated. All of them were undergraduate students in Burapha University, Bangsean, Chonburi province. They were not tobacco smoking and alcohol drinking. Inclusion criteria were: (1) age range 18-25 years old and (2) healthy and sedentary lifestyle. Exclusion criteria were: (1) had been supplemented with retinol, α-tocopherol, β-carotene, lycopene, (2) had underlying diseases which were not safety for exercise, (3) tobacco smoking and alcohol drinking and (4) could not continue exercising untilled 8 weeks. The body weight, height, body fat, lean body mass, water levels and waist to hip ration of all subjects were recorded. The general characteristics of subjects were shown in Table 1.

### Physical exercise

Twenty healthy sedentary people were asked to conduct exercise by cycling. The subjects were warmed up for 2-3 minutes prior to exercise period, then increased the work load untilled the target heat rate is 40% heart rate reserve (HRR) for 20 minutes. During exercise, heart rate was recorded three times. Target heart rate was calculated based on Karvonen equation, [Target heart rate = (intensity fraction) x (maximal heart rate-resting heart rate) + resting heart rate] (11). The exercise was done for 1 time/ day, 3 days/ week for 8 weeks.

**Laboratory analysis**

The blood samples were collected 4 times; before exercise, immediately after first time of exercise (acute exercise), after trained exercise at 4 weeks, and after trained exercise at 8 weeks. The samples were transferred to sodium fluoride and clot blood tube. Then, they were separated by centrifugation. Plasma and serum were measured for lactic acid and biochemical data, respectively. Samples were obtained at Burapha University, transferred on ice and immediately measured by National Healthcare system (Samitivej Sriracha Hospital) and Chonburi Hospital. The blood serum were kept at -20 °C. Then, retinol and E were measured.

High-sensitivity C-reactive protein; hs CRP) (Olympus Automate chemistry analyzer SOP AU 400), creatinine phosphokinase; CPK (Olympus Automate chemistry analyzer SOP AU 400), Cholesterol, high density lipoprotein; HDL-C (Olympus Automate chemistry analyzer SOP AU 400), and triglyceride; TG (Olympus Automate chemistry analyzer SOP AU 400) levels were measured, and low-density lipoprotein (LDL-C) was calculated by Friedwald equation (12), lactic acid (Roche Cobas 6000 c501 module).

**Determination of vitamin E and retinol levels**

Serum retinol and E were determined by using reverse-phase high-performance liquid chromatography as modified from Boonsiri et al. (13) They were extracted into heptane layer by the following procedure. In a glass tube (75 x 100 mm), 120 µl of serum sample was added with 0.1% (w/v) of butylated hydroxytoluene (BHT) in ethanol containing 0.01 mg/ml of tocopherol acetate as internal standard. Then the mixture was mixed for 1 min. One hundred and twenty microliters of 10 mM sodium dodecyl sulfate (SDS) was added to the mixture and mixed briefly. Afterthat 3 ml of n-heptane was added, vortexed vigorously for 2 min and centrifuged at 3,000 rpm for 15 min at room temperature. The 2.8 ml of

supernatant in n-heptane layer were transferred to a 10 x 0.75 cm glass tube and evaporated under nitrogen gas at 45 °C. The residue was reconstituted with 120 µl of freshly prepare mobile phase and injected into a reverse phase C-18 Spherisorb ODS2 column (diameter 5 µm, 100 x 4.6 mm, Waters), protected by a 50-mm guard column with a frit (diameter 5 µM). Mobile phase was consisted of methanol/acetonitrile /dichloromethane at a ratio of 4:4:1 with flow rate 1 ml/min. A dual wavelength UV-visible detector (model 2847, Waters) was set at wavelength, 325 and 292 nm for the detection of retinol and E. Quantification was based on peak-height measurement by using a Clarity program.

**Nutritional status assessment**

Weight and height were measured and calculated as body mass index (BMI), weight (kilograms) is divided by the square of height in meters. BMI was classified according to the World Health Organization (WHO) criteria for Asian and Pacific populations (underweight <18.5, healthy 18.5-22.9, at risk 23-24.9, obese I 25-29.9, and obese II ≥30 kg/m$^2$) (14).

Body fat was evaluated by bioelectric impedance analysis (Tanita UM051, Tanita Corporation, Japan). Then, they were calculated to kilogram unit by proportion of body weight. Lean body mass was as protein, water, mineral. Lean body mass was received by body weight minus body fat (kgs).

Waist to hip ration (WHR) was used to a central obesity assessment. Waist circumference was measured by the measuring tape at the midpoint between the lower rib cage and the iliac crest in the centimeters. Hip circumference was measured at the widest level around the buttocks. The WHR was calculated by the waist circumference divided by hip circumference (15).

**Statistical analysis**

Data were shown as Mean ± standard deviation; SD, and frequencies (%) were used to describe subjects' characteristics. Paired *t* test or Wilcoxon was used to compare categorical variables or continuous variables between before and after exercise groups (acute exercise, trained exercise at 4 and 8 weeks) respectively. Pearson' correlation was used to calculate the relationship between groups.

A *p*-value $< 0.05$ was considered statistically significance. All statistical analyses were performed using 16.0 version of SPSS program (SPSS Inc., Chicago, IL). The study was reviewed and approved by the Burapha University institute review board.

## 3. Results

Twenty healthy sedentary volunteers were enrolled in the present study. Their age mean was 19.9 (range, 19-23) years old. They were not tobacco smoking and alcohol consumption. Most of them were students who were in undergraduate level, 17 (85%) were female and 3 (15%) were male. Their BMI was 20.90 ± 4.11 kg/ $m^2$. Their body fat and lean body mass were 13.76 ± 6.10 and 40.57 ± 7.50 kilograms. They had waist and hip ration which was 0.79 ± 0.052. The general characteristic of them was shown in Table 1.

Biochemical levels and biomarkers of antioxidants (vitamin E and retinol) were compared before and after exercise (acute exercise, trained exercise at 4 and 8 weeks) (Figure 1 and 2).

Acute exercise group was the most highest in lactic level when compared with pre-exercise ($p < 0.001$), trained exercise at 4 weeks ($p < 0.001$) and 8 ($p < 0.001$) weeks. After sedentary subjects were proceeded in exercising, there was a significantly decreased in lactic acid level at trained exercise at 4 ($p = 0.001$) and 8 ($p = 0.001$) weeks compared to acute exercise.

The CPK level showed the significantly lowered in acute exercise ($p = 0.003$), trained exercise at 4 ($p < 0.001$) and 8 ($p = 0.007$) weeks than in pre-exercise. There was no significant difference among group in CRP level.

The comparable with pre-exercise, there was a dramatic significantly increased in vitamin E level after acute exercise ($p < 0.001$), trained exercise at 4weeks ($p < 0.001$) and 8 weeks ($p < 0.001$). There was also a higher vitamin E level in trained exercise at 4 weeks than in acute exercise ($p = 0.004$). In contrast to retinol level, there was no significant different in retinol level among stage of exercises. But retinol level trended to drop in acute exercise, then returned to pre-exercise level after trained exercise at 4 and 8 weeks.

Lipid profile levels among stage of exercise were shown in Figure 3. There was no significantly differed in cholesterol, HDL-C, LDL-C among group. TG level was significantly higher in acute exercise ($p = 0.001$), trained at 4 ($p = 0.009$) and 8 ($p = 0.003$) weeks than in pre-exercise.

Nutritional parameters assessment were compared before and after exercise (acute exercise, trained exercise at 4 and 8 weeks), shown in Figure 4. BMI was significantly lowered in acute exercise ($p < 0.001$) and trained exercise at 4 weeks ($p = 0.025$) compared to pre-exercise. But BMI level was significantly increased in trained exercise at 4 weeks when compared to acute exercise ($p = 0.049$). When compared to pre-exercise, weight was significantly decreased in acute exercise ($p < 0.001$) and trained exercise at 4 weeks ($p = 0.022$). Lean body mass was significantly declined in acute exercise ($p < 0.001$), trained exercise at 4 ($p = 0.001$) and 8 ($p = 0.002$) weeks compared to pre-exercise. There was significantly reduced in weight to hip ration in trained exercise at 8 weeks than in pre-exercise ($p = 0.013$).

We assessed the relationship among vitamin E, retinol, lipid profiles, and nutritional parameter. We found not only the significant positively related between vitamin E in each stage and other parameters but also negatively correlation as shown in Table 2.

Positive and negative relationship between retinol and other parameters were expressed in Table 3.

## 4. Discussion

The present study shows that there was a inclining in vitamin E level after acute and trained exercise than pre-exercise group. After trained at 4 weeks was also expressed the increasing of these vitamin E than acute exercise group. Retinol level was shown trend to lower after acute exercise and trended to increase after trained exercise. However, no significant different were found in retinol level during each period of exercises. These results were contrast to Sacheck et al. (16) in associated with α-tocopherol level, but showed the similar to retinol level, which reported the lower in α-tocopherol and retinol level after trained exercise in eccentric exercise with 75% $VO_2$ max for 12 weeks. Thus, the intensity of exercise in Sacheck et al.'s study was stronger than present study. These suggest that this low intensity endurance exercise was appropriated for the first time sedentary people who was the first started to exercise. The results of the present study was similar to the study of Recep et al. (17), showed the increasing in enzymatic antioxidant levels after performing run submaximal trained exercise for 5 weeks. These might be the adaptive mechanism response to regular exercise training in ameliorated antioxidant effect and reduced the detrimental collapse from oxidative stress (4). Some reports also revealed that plasma vitamin E was higher during exercise (18, 19). Slattery et al. reported that swimmers who were during the training period consumed adequate dietary balance such as vitamin A, vitamin E and vitamin C, they might be performed excellent physical performance (20).

The study was showed the significant lowering in lactic acid after trained exercise when compared to acute exercise. The shift of using fatty acids instead of carbohydrate might be for conservation in muscle glycogen during exercise, resulting in reduction of lactic acid (21). Slow oxidative, type I muscle fiber worked at low intensity endurance exercise had high fat oxidation (22). In the pre-exercise group, retinol had negative correlation with lactic

acid production (r = -0.501, $p$ = 0.025). Kinnunen et al. reported antioxidant supplementation ($\alpha$-lipoic acid) decreased lactate level in blood during exercise the exercise in horses (23).

The muscle damage marker as CPK level was decreased after acute and trained exercise when compared to pre-exercise. Muscle cell damaging exercise has released a various types of skeletal muscle protein to the blood, it has been used CPK activity level as a marker of cell disruption which involved with increasing membrane permeability (24). Antioxidant has been associated with prevent membrane rupture. There was also had positive relationship between retinol in acute exercise and CPK level in acute exercise (r = 0.448, $p$ = 0.047). Retinol level may response to the various level of the change in CPK. The protocols, intensity and level of training exercise might be influenced on the level of CPK (25, 26).

There was no different of CRP level among the pre-exercise, acute exercise, and trained exercises. This result was consistent with Marcell et al. (27) and Fairey et al. (28) which reported no significant difference in CRP level between baseline and after trained exercise. Previous study showed high intensity exercise training had lowered CRP level (29). On the other hand, immediately after an ultramarathon race showed the increasing in CRP level (30, 31). These evidences suggested that trained exercise with high intensity had anti-inflammation effect (29) but the prolonged, strenuous bout exercise may be associated with inflammation process, muscle damage and myocardial infarction (30). Therefore, the low intensity of exercise in the present study may be not evoked the inflammation process which could be observed from no alteration in the CRP level after trained exercise. Additional, we also found the positive relationship between retinol in acute exercise and CRP levels in acute exercise period (r =0.448, $p$ = 0.047), and vitamin E in acute exercise and CRP in acute exercise (r =0.489, $p$ = 0.029). Both of the positive correlation was found in acute exercise bout. The acute exercise was followed by instant raise in producing ROS, which associated

with pro-inflammation response (32). According to the protection of balance in cells, it had induction to yielded antioxidant response opposed to ROS destruction.

Nutritional status assessment was indicated that BMI was decreased after participation in acute and trained exercise. Compared to pre-exercise, weight was declined after acute and trained exercise at 4 weeks, but not trained exercise at 8 weeks. No change in body fat was found after acute and trained exercise. Lean body mass was lowered after acute and trained exercise. This results were consistent with Melanie J. Bopp et al. (33) and Dumortier M et al. (34), which first authors reported lean body mass was declined in overweight and obese postmenopausal women with hypocaloric diet plus low-intensity exercise, therefore they also showed less lean body mass lost when higher protein intake (33). Another showed slightly decreased in lean body mass but not significantly on metabolic syndrome patients after exercise training for 2 months. On the other hand, Kemmler et al. (35) expressed the rise in lean body mass level on elderly women after conducting exercise program for 18 months. The proteolysis during exercise was showed the drop in whole-body protein synthesis and the increase in whole-body protein breakdown. After exercise, it appeared to have raised in the whole-protein synthesis (36). However, the rise in protein breakdown during fasting was through the degradation of contractile protein in skeletal muscle (37, 38). Our data suggest that the normal sedentary people who intended to start the first time in training exercise should consume adequate dietary protein. More protein in diets maintained lean body mass than less protein in diets in less protein diets (39). Feeding sufficient dietary protein, lower weight loss related lean body mass loss (40). Waist to hip ration was dropped after trained exercise at 8 weeks, when compared to pre-exercise group. Moreover, there was negatively correlation between vitamin E in trained exercise at 8 weeks and waist to hip ration in trained exercise at 4 weeks ($r = -0.477$, $p = 0.033$). These would imply that trained exercise at 8 weeks decreased central obesity. Obesity was related to hypertension, type II diabetes and

dyslipidemia (41). A large amount of ROS production and lowering of antioxidant protection, had revealed in many disease such as type II diabetes (42). Therefore, it could found that the waist to hip ration increased at trained exercise at 4 weeks, it would suggest vitamin E level would drop at trained exercise at 8 weeks.

Vitamin E and retinol were hydrophobic scavengers, they were established in lipoprotein and membrane. Their action can interfere the propagation in lipid peroxidation (5). Thus, there was the positively correlation between antioxidants (vitamin E and retinol) and lipid profiles in each exercise session in the present study.

## 5. Conclusion

The exercise training in low intensity exercise, had significantly increased vitamin E level. These low intensity exercise had decreased CPK level. No response in inflammation process (no different in CRP level). There was improvement in lactic acid after trained exercise. After exercise, there was a decreasing in lean body mass, but no different in body fat. Therefore, protein supplementation should be further studied in sedentary people with these low intensity exercise.

## 6. Acknowledgements

This work is supported by National research council of Thailand grant, and a Burapha University grant.

**7. References**


1. Nishitani M, Shimada K, Masaki M, Sunayama S, Kume A, Fukao K, et al. Effect of cardiac rehabilitation on muscle mass, muscle strength, and exercise tolerance in diabetic patients after coronary artery bypass grafting. J Cardiol.61(3):216-21.

2. Duran FS, Lugo L, Ramirez L, Eusse E. Effects of an exercise program on the rehabilitation of patients with spinal cord injury. Arch Phys Med Rehabil. 2001;82(10):1349-54.

3. Armstrong RB, Ogilvie RW, Schwane JA. Eccentric exercise-induced injury to rat skeletal muscle. J Appl Physiol Respir Environ Exerc Physiol. 1983;54(1):80-93.

4. Kim JD, Yu BP, McCarter RJ, Lee SY, Herlihy JT. Exercise and diet modulate cardiac lipid peroxidation and antioxidant defenses. Free Radic Biol Med. 1996;20(1):83-8.

5. Chaudière J, Ferrari-Iliou R. Intracellular Antioxidants: from Chemical to Biochemical Mechanisms. Food and Chemical Toxicology. 1999;37(9–10):949-62.

6. Burton GW, Ingold KU. beta-Carotene: an unusual type of lipid antioxidant. Science. 1984;224(4649):569-73.

7. Tesoriere L, Ciaccio M, Bongiorno A, Riccio A, Pintaudi AM, Livrea MA. Antioxidant activity of all-trans-retinol in homogeneous solution and in phosphatidylcholine liposomes. Arch Biochem Biophys. 1993;307(1):217-23.

8. Navarro A, Gomez C, Lopez-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. American journal of physiology Regulatory, integrative and comparative physiology. 2004;286(3):R505-11.

9. Holloszy JO, Smith EK, Vining M, Adams S. Effect of voluntary exercise on longevity of rats. J Appl Physiol (1985). 1985;59(3):826-31.

10. Gomez-Cabrera M-C, Domenech E, Viña J. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. Free Radical Biology and Medicine. 2008;44(2):126-31.

11. Karvonen MJ, Kentala E, Mustala O. The effects of training on heart rate; a longitudinal study. Ann Med Exp Biol Fenn. 1957;35(3):307-15.

12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

13. Boonsiri P, Pooart J, Tangrassameeprasert R, Hongsprabhas P. Serum beta-carotene, lycopene and alpha-tocopherol levels of healthy people in northeast Thailand. Asia Pacific journal of clinical nutrition. 2007;16 Suppl 1:47-51.

14. Inoue S, Zimmet P, Caterson I, Chen C, Ikeda Y. WHO Western Pacific Region. The Asia-Pacific perspective: redefining obesity and its treatment. Health Communications Australia, Australia Pty Ltd. 2000.

15. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253.

16. Sacheck JM, Milbury PE, Cannon JG, Roubenoff R, Blumberg JB. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. Free Radic Biol Med. 2003;34(12):1575-88.

17. Recep A, SEKEROGLU MR, Mehmet T, Fahri B, Ismail M. Effect of acute and regular exercise on antioxidative enzymes, tissue damage markers and membran lipid peroxidation of erythrocytes in sedentary students. Tr J of Medical Sciences. 1998;28:411-4.

18. Pincemail J, Deby C, Camus G, Pirnay F, Bouchez R, Massaux L, et al. Tocopherol mobilization during intensive exercise. Eur J Appl Physiol Occup Physiol. 1988;57(2):189-91.

19. Camus G, Pincemail J, Roesgen A, Dreezen E, Sluse FE, Deby C. Tocopherol mobilization during dynamic exercise after beta-adrenergic blockade. Arch Int Physiol Biochim. 1990;98(1):121-6.

20. Slattery KM, Coutts AJ, Wallace LK. Nutritional practices of elite swimmers during an intensified training camp: with particular reference to antioxidants. J Sports Med Phys Fitness. 2012;52(5):501-5.

21. Young AJ, Evans WJ, Cymerman A, Pandolf KB, Knapik JJ, Maher JT. Sparing effect of chronic high-altitude exposure on muscle glycogen utilization. J Appl Physiol Respir Environ Exerc Physiol. 1982;52(4):857-62.

22. Jackman MR, Willis WT. Characteristics of mitochondria isolated from type I and type IIb skeletal muscle. Am J Physiol. 1996;270(2 Pt 1):C673-8.

23. Kinnunen S, Hyyppä S, Oksala N, Laaksonen DE, Hannila ML, Sen CK, et al. α-Lipoic acid supplementation enhances heat shock protein production and decreases post exercise lactic acid concentrations in exercised standardbred trotters. Research in Veterinary Science. 2009;87(3):462-7.

24. Van der Meulen JH, Kuipers H, Drukker J. Relationship between exercise-induced muscle damage and enzyme release in rats. J Appl Physiol (1985). 1991;71(3):999-1004.

25. Klapcinska B, Iskra J, Poprzecki S, Grzesiok K. The effects of sprint (300 m) running on plasma lactate, uric acid, creatine kinase and lactate dehydrogenase in competitive hurdlers and untrained men. J Sports Med Phys Fitness. 2001;41(3):306-11.

26. Szabo A, Romvari R, Bogner P, Febel H, Szendro Z. Metabolic changes induced by regular submaximal aerobic exercise in meat-type rabbits. Acta Vet Hung. 2003;51(4):503-12.

27. Marcell TJ, McAuley KA, Traustadottir T, Reaven PD. Exercise training is not associated with improved levels of C-reactive protein or adiponectin. Metabolism. 2005;54(4):533-41.

28. Fairey AS, Courneya KS, Field CJ, Bell GJ, Jones LW, Martin BS, et al. Effect of exercise training on C-reactive protein in postmenopausal breast cancer survivors: a randomized controlled trial. Brain Behav Immun. 2005;19(5):381-8.

29. Mattusch F, Dufaux B, Heine O, Mertens I, Rost R. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. Int J Sports Med. 2000;21(1):21-4.

30. Strachan AF, Noakes TD, Kotzenberg G, Nel AE, de Beer FC. C reactive protein concentrations during long distance running. Br Med J (Clin Res Ed). 1984;289(6454):1249-51.

31. SJ Semple LS, AJ McKune, N Neveling, A Wadee. Alterations in acute-phase reactants (CRP, rheumatoid factor, complement, Factor B, and immune complexes) following an ultramarathon. South African Journal of Sports Medicine. 2004;16:17-21.

32. Fallon KE, Fallon SK, Boston T. The acute phase response and exercise: court and field sports. Br J Sports Med. 2001;35(3):170-3.

33. Bopp MJ, Houston DK, Lenchik L, Easter L, Kritchevsky SB, Nicklas BJ. Lean Mass Loss Is Associated with Low Protein Intake during Dietary-Induced Weight Loss in Postmenopausal Women. Journal of the American Dietetic Association. 2008;108(7):1216-20.

34. Dumortier M, Brandou F, Perez-Martin A, Fedou C, Mercier J, Brun JF. Low intensity endurance exercise targeted for lipid oxidation improves body composition and insulin sensitivity in patients with the metabolic syndrome. Diabetes & Metabolism. 2003;29(5):509-18.

35. Kemmler W, von Stengel S, Engelke K, Haberle L, Mayhew JL, Kalender WA. Exercise, Body Composition, and Functional Ability: A Randomized Controlled Trial. American Journal of Preventive Medicine. 2010;38(3):279-87.

36. Lowell BB, Ruderman NB, Goodman MN. Regulation of myofibrillar protein degradation in rat skeletal muscle during brief and prolonged starvation. Metabolism. 1986;35(12):1121-7.

37. Li JB, Goldberg AL. Effects of food deprivation on protein synthesis and degradation in rat skeletal muscles. Am J Physiol. 1976;231(2):441-8.

38. Dohm GL, Tapscott EB, Barakat HA, Kasperek GJ. Measurement of in vivo protein synthesis in rats during an exercise bout. Biochemical medicine. 1982;27(3):367-73.

39. Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. Am J Clin Nutr. 2006;83(2):260-74.

40. Pierson RN, Jr., Lin DH, Phillips RA. Total-body potassium in health: effects of age, sex, height, and fat. Am J Physiol. 1974;226(1):206-12.

41. Dalton M, Cameron AJ, Zimmet PZ, Shaw JE, Jolley D, Dunstan DW, et al. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. Journal of internal medicine. 2003;254(6):555-63.

42. Yang H, Jin X, Kei Lam CW, Yan SK. Oxidative stress and diabetes mellitus. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2011;49(11):1773-82.

Table 1 General characteristics

| Characteristics | N = 20 |
|---|---|
| Age (years) [median (IQR)] | 20 (19-23) |
| Gender, N (%) | |
| Male | 3 (15%) |
| Female | 17 (85%) |
| Occupation, N (%) | Student (100%) |
| Education, N (%) | Undergraduate (100%) |
| Tobacco smoking, N (%) | None |
| Alcohol drinking, N (%) | None |
| Body mass index ($kg/m^2$) (mean ± SD) | 20.90 ± 4.11 |
| Weight (kgs) (mean ± SD) | 54.33 ± 11.63 |
| Body fat (kgs) (mean ± SD) | 13.76 ± 6.10 |
| Lean body mass (kgs) (mean ± SD) | 40.57 ± 7.50 |
| Waist to hip ration (mean ± SD) | 0.79 ± 0.052 |

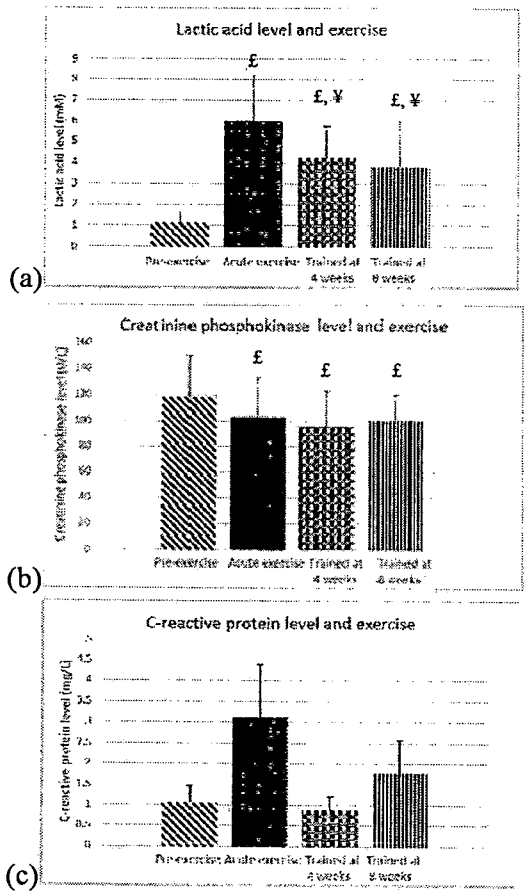


Figure 1 Biochemical levels among Pre-exercise, Acute exercise, and Trained exercise at 4 and 8 weeks, (a) lactic acid level, (b) creatinine phosphokinase level, (c) C-reactive protein level Value expressed as Mean ± SD, calculated the different between before and after exercise by Paired *t*-test

£ = Significant different from Pre-exercise; $p < 0.05$

¥ = Significant different from acute exercise; $p < 0.05$

* = Significant different from trained exercise at 4 weeks; $p < 0.05$

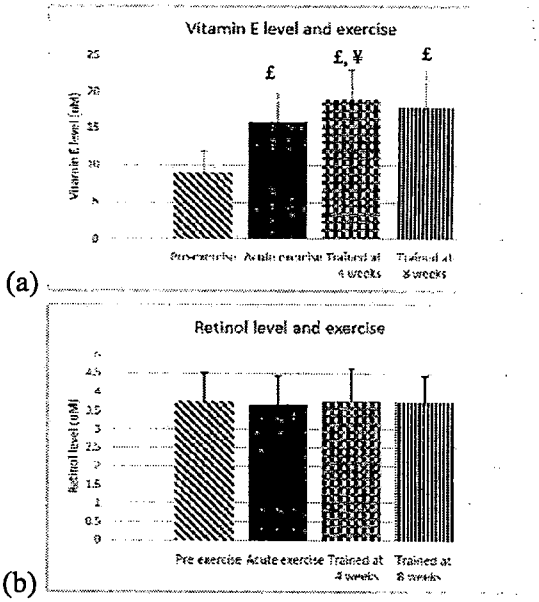


Figure 2 Biomarkers of serum antioxidants among Pre-exercise Acute exercise, and Trained exercise at 4 and 8 weeks, (a) vitamin E level, (b) retinol level, Value expressed as Mean ± SD, calculated the different between before and after exercise by Paired *t*-test

£ = Significant different from Pre-exercise; $p < 0.05$

¥ = Significant different from acute exercise; $p < 0.05$

* = Significant different from trained exercise at 4 weeks; $p < 0.05$

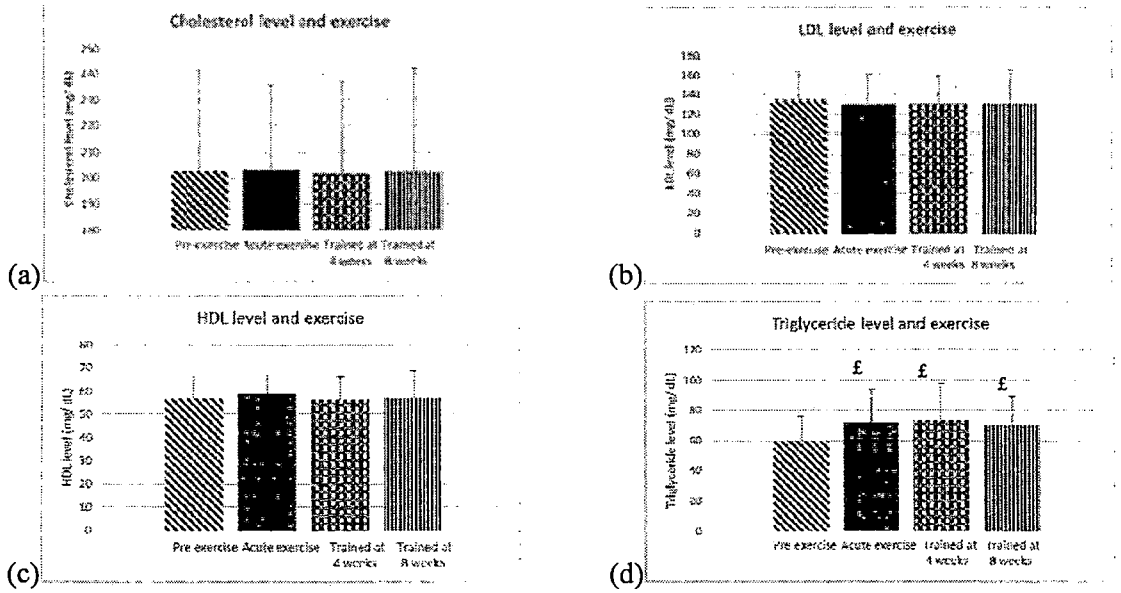


Figure 3 Lipid profile levels among Pre-exercise, Acute exercise, and trained exercise at 4 and 8 weeks, (a) cholesterol level, (b) LDL level, (c) HDL level, (d) triglyceride level, Value expressed as Mean ± SD, calculated the different between before and after exercise by Paired *t*-test, £ = Significant different from Pre-exercise; $p < 0.05$, ¥ = Significant different from acute exercise; $p < 0.05$, * = Significant different from trained exercise at 4 weeks; $p < 0.05$

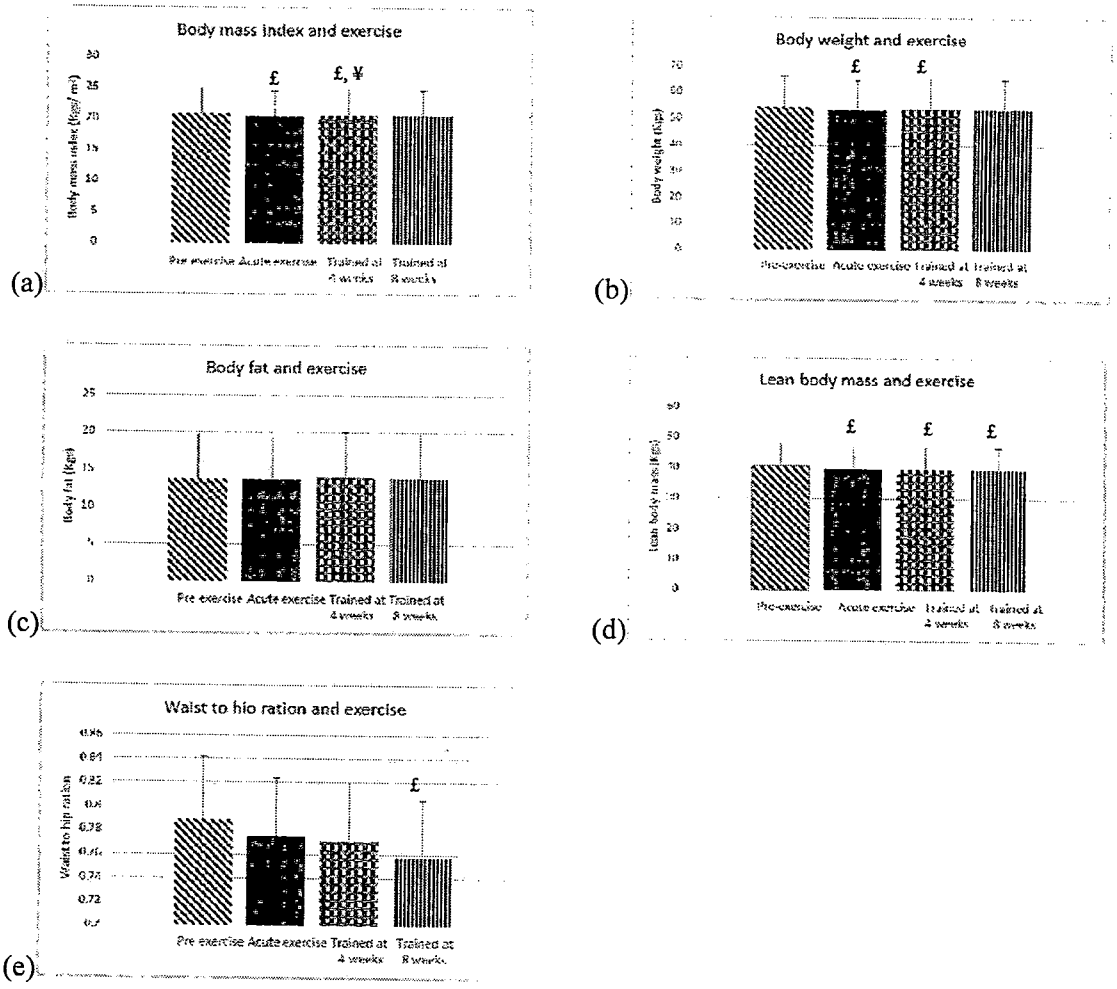


Figure 4 Nutritional parameter among Pre-exercise, Acute exercise, and trained exercise at 4 and 8 weeks, (a) body mass index, (b) body weight, (c) body fat, (d) lean body mass, (e) waist to hip ration, Value expressed as Mean ± SD, calculated the different between before and after exercise by Paired *t*-test, £ = Significant different from Pre-exercise; $p < 0.05$, ¥ = Significant different from acute exercise; $p < 0.05$, * = Significant different from trained exercise at 4 weeks; $p < 0.05$

Table 2 Correlation between vitamin E level in each stage of exercise and parameters

| Correlations | r | p |
|---|---|---|
| Vitamin E in acute exercise and cholesterol in pre-exercise | 0.462 | 0.040 |
| Vitamin E in acute exercise and cholesterol in acute exercise | 0.667 | 0.001 |
| Vitamin E in acute exercise and cholesterol in trained at 4 weeks | 0.463 | 0.040 |
| Vitamin E in acute exercise and CRP in acute exercise | 0.489 | 0.029 |
| Vitamin E in acute exercise and LDL in acute exercise | 0.622 | 0.003 |
| Vitamin E in acute exercise and LDL in trained at 4 weeks | 0.500 | 0.025 |
| Vitamin E in trained at 4 weeks and cholesterol in trained at 4 weeks | 0.548 | 0.012 |
| Vitamin E in trained at 4 weeks and cholesterol in trained at 8 weeks | 0.488 | 0.029 |
| Vitamin E in trained at 4 weeks and LDL in trained at 4 weeks | 0.476 | 0.034 |
| Vitamin E in trained at 4 weeks and LDL in trained at 8 weeks | 0.456 | 0.043 |
| Vitamin E in trained at 4 weeks and triglyceride in trained at 4 weeks | 0.464 | 0.039 |
| Vitamin E in trained at 4 weeks and retinol in trained at 4 weeks 0.527 | 0.017 | |
| Vitamin E in trained at 8 weeks and cholesterol in trained at 8 weeks | 0.567 | 0.009 |
| Vitamin E in trained at 8 weeks and HDL in trained at 8 weeks | 0.451 | 0.046 |
| Vitamin E in trained at 8 weeks and waist to hip ration in trained at 4 weeks | -0.477 | 0.033 |

Based on Pearson's correlation

Table 3 Correlation between retinol level in each stage of exercise and parameters

| Correlations | r | p |
|---|---|---|
| Retinol in pre-exercise and lactic acid in pre-exercise | -0.501 | 0.025 |
| Retinol in pre-exercise and cholesterol in pre-exercise | 0.488 | 0.029 |
| Retinol in pre-exercise and cholesterol in trained at 4 weeks | 0.515 | 0.020 |
| Retinol in pre-exercise and cholesterol in trained at 8 weeks | 0.485 | 0.030 |
| Retinol in pre-exercise and HDL in acute exercise | -0.467 | 0.038 |
| Retinol in pre-exercise and LDL in pre-exercise | 0.589 | 0.006 |
| Retinol in pre-exercise and LDL in trained at 4 weeks | 0.539 | 0.014 |
| Retinol in pre-exercise and LDL in trained at 8 weeks | 0.539 | 0.014 |
| Retinol in pre-exercise and triglyceride in pre-exercise | 0.537 | 0.015 |
| Retinol in pre-exercise and triglyceride in acute exercise | 0.464 | 0.039 |
| Retinol in pre-exercise and triglyceride in trained at 4 weeks | 0.650 | 0.002 |
| Retinol in pre-exercise and triglyceride in trained at 8 weeks | 0.616 | 0.004 |
| Retinol in pre-exercise and BMI in pre-exercise | 0.448 | 0.048 |
| Retinol in acute exercise and lactic acid in pre-exercise | -0.652 | 0.002 |
| Retinol in acute exercise and CPK in acute exercise | 0.448 | 0.047 |
| Retinol in acute exercise and cholesterol in acute exercise | 0.492 | 0.028 |
| Retinol in acute exercise and cholesterol in trained at 8 weeks | 0.461 | 0.041 |
| Retinol in acute exercise and CRP in trained at 8 weeks | 0.596 | 0.006 |
| Retinol in acute exercise and LDL in pre-exercise | 0.491 | 0.028 |
| Retinol in acute exercise and LDL in acute exercise | 0.544 | 0.013 |
| Retinol in acute exercise and LDL in trained at 8 weeks | 0.496 | 0.026 |
| Retinol in acute exercise and triglyceride in acute exercise | 0.520 | 0.019 |
| Retinol in acute exercise and triglyceride in trained at 4 weeks | 0.547 | 0.013 |
| Retinol in acute exercise and triglyceride in trained at 8 weeks | 0.684 | 0.001 |
| Retinol in acute exercise and waist to hip ration in trained at 8 weeks | 0.472 | 0.036 |
| Retinol in trained at 4 weeks and lactic acid in pre-exercise | -0.491 | 0.028 |
| Retinol in trained at 4 weeks and triglyceride in trained at 4 weeks | 0.527 | 0.017 |
| Retinol in trained at 4 weeks and triglyceride in trained at 8 weeks | 0.546 | 0.013 |
| Retinol in trained at 8 weeks and lactic acid in pre-exercise | -0.519 | 0.019 |
| Retinol in trained at 8 weeks and triglyceride in trained at 4 weeks | 0.548 | 0.012 |

Based on Pearson's correlation