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研究成果報告(精簡版)

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Introduction

The benefits of regular endurance exercise on the cardiovascular system and general health have been well-established,[2,20] however, there are still concerns on the possible elevated free radical production during exercise. In deed, it has been shown in vivo, with electron spin resonance[3] and dichlorofluorescin,[4] that free radical production is elevated after strenuous physical activities in humans and animal models. In addition, markers of free radical damage, such as exhaled ethane and pentane,[12] thiobarbituric acid reactive substances,[19] conjugated diene,[16] LDL susceptibility to oxidation,[26] protein carbonyl concentration,[30] and urinary 8-hydroxy-deoxyguanine secretion[23] were all elevated after a single bout of strenuous aerobic exercises in trained and untrained subjects. However, the elevated oxidative stress appeared to be transient as most lipid peroxidation byproducts were removed shortly after exercise.[12]

It has been generally accepted that regular exercise would reduce the risk of cardiovascular disease by inducing the favorable lipid profile and metabolism.[5,7] Most studies suggested that acute or long-term exercise with sufficient intensity and energy expenditure could result in lower total cholesterol (TC) and LDL-C and higher HDL-C levels in subjects with broad age range[8,32].

Bicycling has been gaining significant popularity in Taiwan and many other countries as an activity of sport, recreation, and communication. Its benefits on a variety of aspects in health and nontraumatic nature make bicycle touring and recreational riding increasingly popular. [6] Multi-day endurance exercise events have been gaining significant popularity in recent years. Plenty of bicycle touring events have been held to promote the activity in Taiwan. Organized tours offer recreational bicyclists the opportunity to participate in multi-day rides with preplanned support and the companionship of other bicyclists. The number of recreational participants in such tours has been increasing dramatically in recent years.

The physiological and biomechanical responses during multi-day ultraendurance cycling competitions in professional athletes have been investigated extensively.[14,15,22] In addition, the physiological characters of highly trained cyclists have also been reported.[21] However, such responses have not been examined in untrained recreational participants. In addition, most studies on exercise effects only measured the parameters before and after exercise with little or no information on the changes during the training period. Therefore, the aims of the present study were to investigate the changes in oxidative stress markers, plasma malondialdehyde (MDA) and oxidized LDL (oxLDL) levels, and plasma lipid profile, including TC, LDL-C, HDL-C, and triglyceride (TG), during a 15-day 1100-km cycling tour in untrained healthy subjects.

Method

Subjects

Eighteen healthy subjects aged 19-38 years old, including 9 males and 9 females, who participated in Cycling Taiwan 1000 km were recruited. The tour covered approximately 1100 km in 15 days with 80-120 km per day in most of the days. All subjects were in a free feeding style during the tour. All subjects have never received any form of regular physical training. All subjects signed an informed consent after all procedures and risks of the study are thoroughly explained.

Blood sampling

Fasting blood samples were collected in the early morning on day 1, 4, 8, 12, and 15 during the tour. Twenty ml blood was collected with venupuncture into tubes containing EDTA as anticoagulant. Plasma were separated from erythrocytes by centrifugation at 1500 g for 10 min. Plasma samples were sent by frozen delivery to the laboratory and stored at –70℃ for further analysis.

Biochemical measurements

Plasma MDA concentration was measured as thiobarbituric acid (TBA) adduct by HPLC.[13,31] Briefly, after incubation with TBA for 60 min at 100℃, the mixture was extracted with butanol. MDA-TBA adduct was analyzed by HPLC equipped with a μBonapak C18 column (Waters Corporation, Millford, Massachusetts, USA). The fluorescence detector was set at 532 nm excitation and 553 nm emission. The values of plasma samples were compared to the known amounts of 1,1,3,3-tetraethoxypropane standard.

Plasma oxLDL level was measured with an ELISA kit according to the manufacturer's recommendations (Mercodia AB, Uppsale, Sweden). Plasma TC, LDL-C, HDL-C, and TG concentrations were measured with an automatic analyzer (Hitachi 7010, Tokyo, Japan) using standard commercial kits.

Statistical analysis

The differences among the 5 sampling points throughout the 15-day tour were originally compared by two-way repeated measurement analysis of variance (ANOVA) with gender and time as main effects. However, gender effect was not significant in any of the variables investigated. Therefore, data from males and females were pooled together and analyzed by one-way repeated measurement ANOVA. If a significant effect was found, Bonferroni test was performed for post-hoc comparison. All analyses were performed using SPSS 11.0 for Windows (Chicago, IL, USA). The statistical significance level was set at $p<0.05$. Data were expressed as mean±SD.

Results

The basic characteristics of subjects are presented in Table 1. The males were significantly higher and heavier than the female subjects.

Plasma MDA levels during the tour are shown in Figure 1. Plasma MDA concentrations were significantly lower in day 8 (0.47±0.20 μM) and 12 (0.54±0.16 μM) compared to day 1 (0.93±0.39 μM), then returned to the baseline level at the end of the tour. Plasma oxLDL concentrations were shown in Figure 2. OxLDL level increased slightly until day 12, then dropped to the level significantly lower than the baseline (53.69±17.45 U/L) on day 15 (43.35 ±12.61 U/L).

Plasma TC, LDL-C, HDL-C, and TG concentrations during the tour are depicted in Figure 3. TC levels were significantly decreased on day 4 and 8 (4.16±0.68 and 3.83±0.58 mM, respectively), then returned to the baseline level on day 12 and 15 (4.43±0.59 and 4.19± 0.51 mM, respectively). The changes in TC concentrations resulted mostly from the changes in LDL-C as these 2 variables showed the similar trend during the tour. Plasma HDL-C level was significantly higher in day 12 (1.76±0.19 mM) than that in day 1 (1.53±0.28 mM), then returned to the baseline level at the end of the tour. Plasma TG levels reduced significantly in day 4 and 8 (0.55±0.17 and 0.55±0.20 mM, respectively), compared to that in day 1 (0.91± 0.42 mM). TG concentrations returned to the basal value in the last 2 sampling periods of the tour.

Discussion

To our knowledge, this is the first study to investigate the changes in oxidative stress and blood lipid profile during a multi-day strenuous endurance exercise in untrained subjects. The current study suggested that this type of endurance exercise did not result in higher oxidative stress at the end of the tour.

It has been shown that plasma MDA level and autoantibodies against oxLDL were elevated in professional athletes and after 10 months of exercise training in middle-aged overweight subjects.[11,17,24,29] On the other hand, MDA level was lower in soccer players compared to sedentary control,[18] suggesting an adaptation to regular training on antioxidant defense system. However, most available studies on the long-term effect of training on oxidative stress used professional athletes as subjects. The training effect on oxidative stress in previously untrained subjects was unclear. The current study showed that, although MDA and oxLDL levels significantly changed during the first half of the tour, both markers of oxidative stress returned to or became lower than the basal values at the end of the tour. It suggested that the antioxidant defense system may be able to adapt to the exercise load after 15 days of strenuous training in previously untrained male and female subjects.

Our results showed that the change in MDA concentrations was not correlated with that in oxLDL levels. MDA is a relatively nonspecific marker of lipid peroxidation, while oxLDL measured the oxidation of unsaturated fatty acids and proteins in the specific lipoprotein particle. The peroxidation of lipid and protein may involve different mechanisms.[25]

It has been generally accepted that regular exercise would reduce the risk of atherosclerosis by inducing the favorable lipid profile and metabolism.[5,7] TC, TG, and HDL-C levels were significantly reduced after 6 months of regular exercise, 3-4 times a week, in elderly subjects [27]. Hubinger and Mackinnon[9] showed that HDL-C level and HDL-C/LDL-C ratio increased significantly after 30 min of exercise at 60% maximal heart rate, while TC or LDL-C levels remained unchanged. The acute effect of exercise on HDL-C and TG appears to increase with overall energy expenditure possibly because the effect maybe mediated by reductions in intramuscular triglycerides.[28] It has been suggested that the exercise-induced change in plasma lipid profile may also be influenced by the previous training status.[10] A single bout of exercise increases cholesterol mobilization from its reservoirs in moderately trained people, whereas regular training does not mobilize cholesterol but eliminates LDL-cholesterol in well-trained people.[1] Our subjects had little or no history of regular physical activity prior to the tour. However, after approximately 1 week after daily strenuous exercise, most of them appeared to be adapted. The results of this study suggested that, despite initial drops in TC and TG concentrations in the first week, these 2 parameters returned to the basal levels after the strenuous tour in untrained subjects. In addition, LDL-C level was decreased after the tour, while HDL-C level stayed relatively constant during the tour. This study indicated that the effect of a 15-day strenuous exercise on plasma lipid profile was minor in previously untrained young subjects.

Conclusion

The current study suggested that 15 days of endurance exercise did not result in higher oxidative stress at the end of the tour in untrained male and female subjects. In addition, the tour only had minor effect on plasma lipid profile in these subjects. Further research on subjects with different training experience, age, and gender are warranted as the participants of this type of activity are very diversified.

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TABLE 1. BASIC CHARACTERISTICS OF SUBJECTS (MEAN±SD).

	Male	Female	Total	Range
N	9	9	18	
Age (year)	29.3±11.1	23.1±10.1	26.2±10.8	19-52
Height (m)	1.72±0.06	1.58±0.06	1.65±0.10	1.45-1.81
Weight (kg)	68.0±8.6	48.8±4.7	58.4±11.9	40.0-79.8

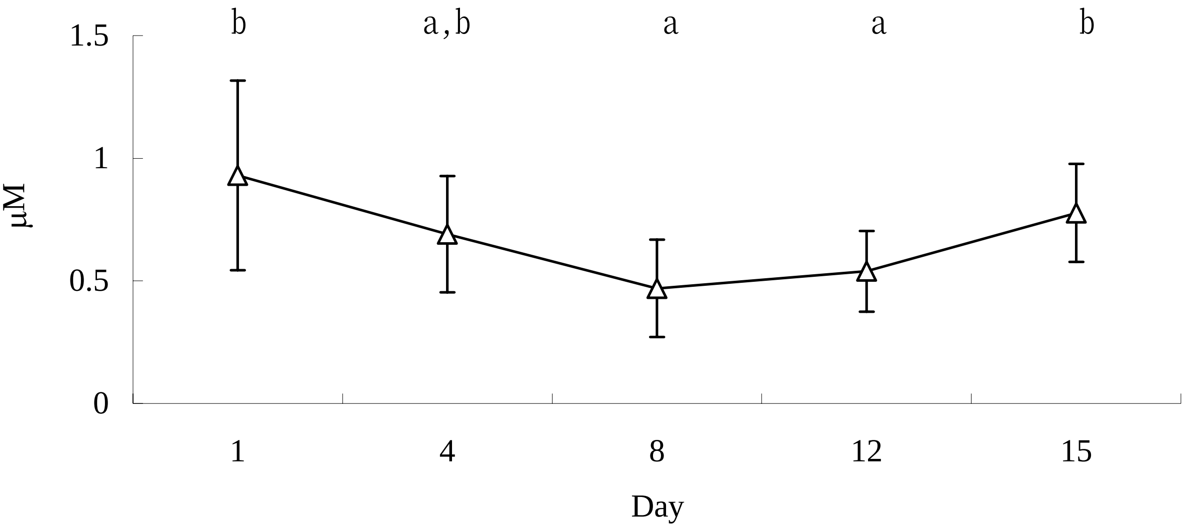

Figure 1. Plasma MDA concentrations during the 15-day cycling tour. Data were presented as mean±SD. Points with different letters were significantly different ($p<0.05$).

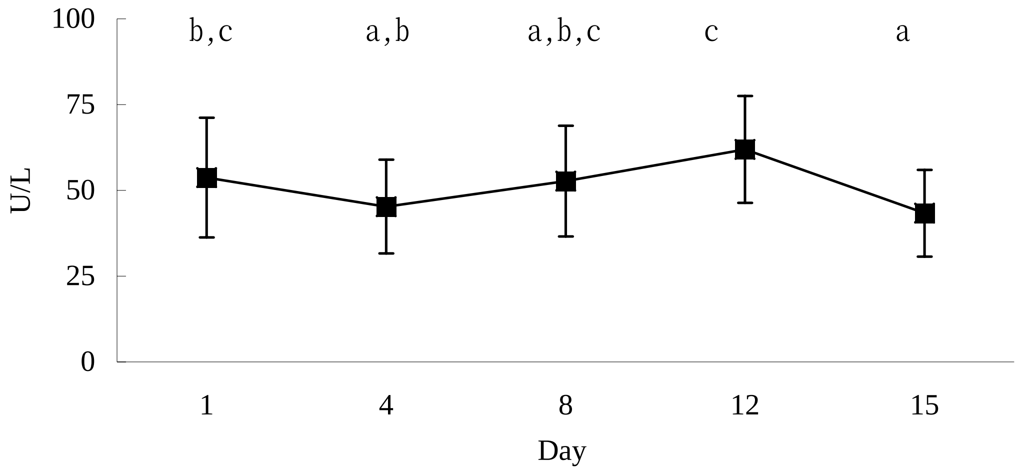

Figure 2. Plasma oxLDL concentrations during the 15-day cycling tour. Data were presented as mean±SD. Points with different letters were significantly different ($p<0.05$).

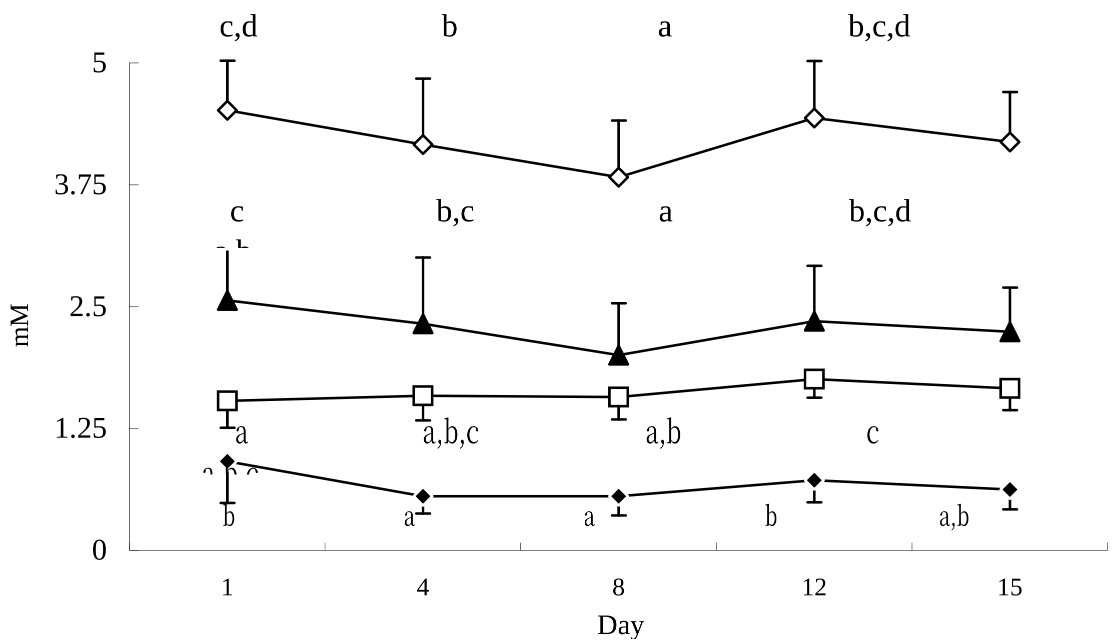

Figure 3. Plasma TC (◇), LDL-C (▲), HDL-C (□), and TG (◆) concentrations during the 15-day cycling tour. Data were presented as mean±SD. Points in the same line with different letters were significantly different ($p<0.05$).