Effects of a Single Bout of Ultraendurance Exercise on Lipid Levels and Susceptibility of Lipids to Peroxidation in Triathletes

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Objective.—To determine the effects of a single bout of ultraendurance exercise, as a model for physiologic stress, on lipid and lipoprotein levels, and oxidative susceptibility of lipids in highly trained athletes.

Design.—Observational trial.

Population and Setting.—Thirty-nine volunteer subjects (26 men, 13 women; mean age, 38±10 years) who competed in and completed the 1994 Hawaii Ironman World Championship Triathlon consisting of a consecutive 3.9-km (2.4-mi) swim, 180.2-km (112-mi) bike ride, and a 42.2-km (26.2-mi) run. Subjects answered questionnaires and had blood samples obtained 2 days prior to and within 15 minutes of completion of the triathlon.

Main Outcome Measures.—Prerace vs postrace changes in lipid and lipoprotein levels, and susceptibility of lipids to peroxidation.

Results.—The mean duration of exercise was 753±128 minutes. With exercise, plasma volume—corrected levels of triglycerides decreased 39% from 1.58±0.83 to 0.97±0.68 mmol/L (139.6±73.6 to 85.8±60.5 mg/dL) (P<.001). Levels of total cholesterol decreased 9% from 4.94±0.88 to 4.50±0.75 mmol/L (190.6±33.8 to 173.9±30.6 mg/dL) (P<.001), low-density lipoprotein cholesterol decreased 11% from 2.59±0.77 to 2.30±0.86 mmol/L (100.1±29.9 to 88.7±33.3 mg/dL) (P=.02), and apolipoprotein B decreased 10% from 0.91±0.20 to 0.82±0.18 g/L (90.7±20.0 to 82.0±17.9 mg/dL) (P<.001). High-density lipoprotein cholesterol and apolipoprotein A-I increased with exercise but not significantly. The susceptibility of lipids to peroxidation decreased significantly (4.51±1.91 μmol/L, preexercise, vs 2.42±2.27 μmol/L, postexercise, P<.001), an effect that was not related to antioxidant use or levels of vitamins A, C, or E. Serum iron, a potential pro-oxidant, also decreased by 45% with exercise from 15.75±5.55 to 8.59±4.30 μmol/L (88±31 to 48±24 μg/dL) (P<.001), an effect that was weakly correlated with changes in lipid peroxidation (P=.05).

Conclusions.—These data suggest that a single bout of prolonged exercise can reduce lipid and lipoprotein risk factors for developing cardiovascular disease. Moreover, susceptibility of lipids to peroxidation is reduced by exercise, thereby adding to the benefits of physical activity. This effect appears to be independent of antioxidant supplement use and may be mediated by induction of endogenous antioxidants. These observations may explain in part the reduced risk of developing vascular and other diseases in individuals who are physically active.

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Reproductive processes provide little justification for their use in this population. To address this issue, we explored the effect of prolonged exercise on lipid and lipoprotein levels as well as susceptibility of lipids to peroxidation in highly trained athletes during their participation in the 1994 Hawaii Ironman World Championship Triathlon. The susceptibility of lipids to peroxidation provides an assessment of the relative strength of antioxidant and pro-oxidant factors in plasma and correlates with the risk of developing vascular disease.

METHODS

The subjects who volunteered for this investigation were exceptionally well-conditioned amateur athletes who were participating in an ultradistance triathlon. The details of their training regimen have been reported previously. Subjects trained an average of 21 hours per week. Average weekly training distances included 8 km (5 mi) of swimming, 330 km (205 mi) of cycling, and 75 km (47 mi) of running.

At baseline evaluation 2 days before the event, 25 mL of blood was collected in heparinized or sample tubes containing ethylenediaminetetraacetic acid (EDTA). A questionnaire was administered to obtain demographic, training, dietary, and cardiovascular risk factor data. Subjects had not exercised strenuously for at least 24 hours before the blood sample was taken and were not fasting. The samples were centrifuged without delay, and the heparinized plasma was immediately frozen on dry ice with subsequent storage at −70°C until assayed.

The event consisted of a 3.9-km (2.4 mi) swim, a 180.2-km (112 mi) bicycle race, and a standard 42.2-km (26.2 mi) marathon. Our previous data on dietary and fluid intake have shown that Ironman triathletes consume a prerace meal 1 to 4 hours prior to the event of 2772±378 kJ (men) and 1974±294 kJ (women). During the event, fluid replacement averaged 17.1±1.3 L for men and 15.6±1.4 L for women. Fluids consisted mainly of water, a sports beverage (Gatorade), soft drinks, and broth. Food supplements during the event consisted of potato chips, bananas, and carbohydrate-rich energy bars. Subjects did not differ in their use of antioxidant supplement use on the day of the event compared with the 2 months prior to it. Postrace samples were acquired within 15 minutes of completion of the race in a medical tent at the finish line, centrifuged, and stored at −70°C.

Levels of cholesterol, triglyceride, iron, and creatinine kinase (CK) were determined enzymatically on an auto-analyzer (Hitachi 911, Boehringer Mannheim Diagnostics, Indianapolis, Ind) according to the manufacturer’s recommendations. Triglyceride measurement was corrected for endogenous glycerol. High-density lipoprotein (HDL) cholesterol levels were determined following precipitation of the apolipoprotein B-containing particles by magnesium chloride and dextran sulfate (molecular weight, 50,000) as previously described. Low-density lipoprotein cholesterol levels were determined by β-quantification as described. Myoglobin and apolipoproteins A-I and B were determined by immunonephelometric techniques using an autoanalyzer (Behring BN-100, Behring Diagnostics Inc, Westwood, Mass). The apolipoprotein assays were standardized by calibrators newly approved by the International Federation of Clinical Chemistry/World Health Organization. Vitamins A (retinol) and E were simultaneously measured by a reversed-phase, high-performance liquid chromatographic method at wavelengths of 325 and 288 nm, respectively, after extraction with hexane. Ascorbic acid (vitamin C) was determined on an autoanalyzer (Hitachi 1100). Samples for plasma ascorbate were deproteinized with 10% metaphosphoric acid immediately after separation from blood cells, and the supernate was stored at −70°C until the analysis was performed. Ascorbic acid was then determined enzymatically using ascorbate oxidase.

For the determination of lipid peroxide concentrations, plasma specimens were collected in tubes containing EDTA as an antioxidant and stored at −70°C until analysis. Lipid peroxides were determined in duplicate both at the basal state and after incubation with hydrogen peroxide and ferrous salt to generate hydroxyl radicals by means of the Fenton reaction. A modification of the assay of Yagi, to eliminate interference from bilirubin and salic acid, was used for the determination of lipid peroxides. The intra-assay and interassay coefficients of variation for measured lipid peroxides were 4.0% and 4.2%, respectively, in our laboratory.

To account for fluid shifts known to occur with exercise and dehydration, all values were corrected for changes in plasma volume. The percentage change in plasma volume was calculated using hemoglobin and hematocrit measurements, according to the formula of Dill and Costill. Means and SDs were calculated for biochemical measurements using the prerase values, postrace values, and the difference between the postrace and prerace values. We performed paired t tests to test for differences between postrace and prerase values. To test for postrace differences in biochemical measurements by age, gender, or supplementation use we used least-squares multivariate linear regression. In each of these linear regression models, the postrace minus the prerase difference was modeled as a function of the prerase value, gender, age, and supplement use. All analyses were conducted using PC-SAS.

RESULTS

Of 44 competitors (27 men, 17 women) enrolled in the study, 39 completed the Ironman triathlon in a mean time of 753 minutes (±128 minutes). Of the finishers, 25 were men and 13 were women (mean age, 38±10 years; mean weight, 66.2±10.5 kg), and it is for these subjects that data are presented. None of the subjects had coronary artery disease, hypertension, or diabetes, or used tobacco. Two reported a family history of early coronary disease.

To correct for changes in plasma volume that might occur over the course of the triathlon, hemoglobin levels and hematocrit were measured both before and after the competition. Mean levels of plasma hemoglobin and hematocrit remained unchanged such that the mean percentage change in plasma volume was small (−2.14%±11.4%), but individual percentage changes ranged from −86% to +16%, making individual correction of plasma values important. The plasma volume changes are similar to those previously reported to occur during the course of an Ironman triathlon.

The effects of endurance exercise on lipid, lipoprotein, and apolipoprotein levels are shown in Table 1. Significant decreases in total cholesterol, triglyceride, LDL cholesterol, and apolipoprotein B levels were observed. The greatest change was observed in the plasma triglyceride levels, which decreased by 39% (P<0.001), whereas cholesterol, LDL cholesterol, and apolipoprotein B decreased by 9% (P<0.001), 11% (P=0.02), and 10% (P<0.001), respectively. When analyzed according to sex, similar results were obtained for men and women (data not shown); however, the change in triglyceride levels was greater in men than in women. High-density lipoprotein cholesterol level increased by 3%; however, this change was not significant.

Twenty-six individuals in our study cohort had routinely used 1 or more supplements of vitamins A, C, or E for at least 2 months prior to the competition. We used vitamin A, 21 used vitamin C, and 13 used vitamin E. The remaining group (n=13), which abstained completely from supplement use during the same time period, was used for comparison.
Aerobic exercise is coronary heart disease (CHD) and an additional risk factor for heart disease. The key point of the study was to determine the effect of aerobic exercise and the type of exercise on the occurrence of cardiovascular disease and heart disease. The study group consisted of 80 volunteers, divided into 4 groups: group 1 (in the control group), group 2 (in the aerobic exercise group), group 3 (in the CHD treatment group), and group 4 (in the CHD treatment and aerobic exercise group). The study patients were monitored for 6 months, with each group monitored for 3 months. The results showed that the occurrence of cardiovascular disease and heart disease was significantly lower in the aerobic exercise group and the CHD treatment and aerobic exercise group compared to the control group and the CHD treatment group. The study results suggest that aerobic exercise is beneficial for the prevention and treatment of cardiovascular disease and heart disease.
on well-established markers for cardiovascular disease risk and on lipid peroxidation—a process strongly implicated in the pathogenesis of atherosclerosis. The overall risk of cardiovascular disease in this group of men and women at baseline—reflecting intense training—was lower than average based on standard cardiovascular risk determinants. Prolonged exercise acutely induced marked and statistically significant reductions in total cholesterol, LDL cholesterol, triglyceride, and apolipoprotein B levels as well as a trend toward increased HDL cholesterol and apolipoprotein A-I, suggesting that cardiovascular risk-reducing changes in lipid profiles may potentially be achieved by extending the duration of regular physical activity. Susceptibility of lipids in plasma to peroxidation was also markedly reduced, an effect that was associated with but not necessarily caused by a concomitant reduction in plasma iron levels. Antioxidant use appeared to reduce exercise-induced skeletal muscle injury but had no bearing on the reduction in oxidative susceptibility induced by exercise. Thus, despite the already low cardiovascular risk profile of these athletes, changes associated with more favorable risk for developing cardiovascular disease were acutely induced by a single bout of prolonged exercise. Single bouts of exercise have been reported to result in increased free radical formation as exercise increases oxygen utilization and the formation of hydrogen peroxide in mitochondria. Furthermore, exercising-racing triathletes undergo increases in body temperature, catecholamine level, hemolysis, and skeletal muscle injury or ischemia, all of which can act as potent stimulators for free radicals. These effects of exercise may promote tissue and vascular injury and would suggest, paradoxically, that physical activity might actually be harmful. A major focus of this study was to determine the effects of exercise on plasma lipid peroxidation—a process implicated in the pathogenesis of vascular and other diseases and mediated by the balance of pro-oxidants and antioxidants in plasma. Preexercise baseline levels of peroxidation were lower in triathletes than those reported in other populations as well as in a group of randomly selected sedentary individuals (1.61 μmol/L vs 2.46 μmol/L, [Ironman group, n = 38] vs 1.95 μmol/L; [sedentary group, n = 38]; P < .01) (G. S. G. and N. R., unpublished data, 1995). These data may indicate that the cumulative effects of exercise or training effects tend to decrease peroxidation of lipids in plasma. We observed a further decrease with exercise suggesting that, despite regular training, the potential exists to further reduce oxidative susceptibility.

Few studies have addressed the effects of exercise on oxidant stress. Studies in mice have demonstrated reduced lipid peroxides and susceptibility to in vitro induced lipid peroxidation in skeletal muscle following exhaustive exercise, suggesting that training may reduce the free radical–induced skeletal muscle and tissue damage induced by exhaustive exercise. It is likely that along with increased numbers of mitochondria induced by training, a number of mitochondrial-based antioxidant enzymes are also induced: superoxide dismutase, catalase, and glutathione peroxidase. Most studies to date have demonstrated a net increase in activity of these enzymes with increased physical activity in rats, mice, and humans.

To understand how exercise might decrease susceptibility to lipid peroxidation in plasma, we measured the effects of exercise on plasma levels of antioxidant vitamins A, C, and E and of serum iron, a potential pro-oxidant. While other potential oxidants and antioxidants are present in serum, these vitamins and iron are readily and potentially modified by supplement use. Measured changes in antioxidant vitamins in our cohort could not explain the observed changes in susceptibility to peroxidation as levels of vitamins C and E did not change and vitamin A level decreased, which, if anything, would tend to increase susceptibility to lipid peroxidation.

Users of vitamin E had lower (but not statistically significant) susceptibility to peroxidation at baseline (Figure 3) consistent with their increased serum levels of vitamin E. Because susceptibility of lipids to peroxidation may be an important mechanism that contributes to...
coronary disease, decreased susceptibility of lipids to peroxidation in vitamin E users compared with nonusers at baseline supports the notion that vitamin E use may be protective against coronary disease development.22 Both users and nonusers of vitamin E had reduced susceptibility to lipid peroxidation after exercise, while nonusers tended toward larger decreases in oxidative susceptibility than users. Thus, any benefit from vitamin E use at baseline appears to be superseded by the effects of exercise.

Iron may be a modulator of lipid peroxidation. Increased plasma iron concentration has been associated with increased lipid peroxidation, decreased ascorbate level, and reduced superoxide dismutase activity.23 It has been shown that the redox-active form of iron catalyzes free radical formation, which in turn promotes lipid peroxidation. Decreases in total serum iron levels during prolonged exercise have been observed previously in triathletes and have been attributed to redistribution of iron to the reticuloendothelial system binding to ferritin as well as loss of iron through sweat.24 To be oxidatively active, iron must be liberated from transport proteins, such as ferritin.25 Thus, even redistributions of iron, as is hypothesized to occur with exercise, may reduce its oxidative potential. The effects of iron in vivo were demonstrated in one study that found diminished oxidizability of serum from a population that was nutritionally deficient in iron and copper—an effect that was reversible with iron supplementation.26 Human atherosclerotic lesions have been shown to contain redox-active iron that promotes lipid peroxidation.27 Thus, while the association of iron with vascular disease development is controversial,28,29 there is physiologic and biochemical evidence to suggest that reduction of serum iron may decrease susceptibility to lipid peroxidation. We observed a weak correlation between postexercise serum iron levels and the susceptibility to lipid peroxidation postexercise (peroxidation after exercise, r = 0.36, P = .05). Thus, changes in serum iron levels acutely associated with exercise may contribute to decreased susceptibility to lipid peroxidation following exercise, but clearly there must be other factors induced by exercise that contribute to our observations.

Several limitations are inherent in this study. Nonfasting samples were obtained postexercise, which would not affect the directly measured lipoproteins and cholesterol; however, triglyceride levels most likely would have been lower had the subjects been fasting. Quantitative dietary assessments were not performed, and thus accurate data on dietary (nonsupplement) antioxidant consumption are not available. To determine the mechanism and kinetics of reduced susceptibility to lipid peroxidation, we were limited in the types and numbers of assays performed on samples from this cohort. Exercise is a multifactorial event; protection derived from it is likely also multifaceted. This observational study only addresses a few of these factors and in doing so raises the prospects for observing subtle effects of exercise in the triathlete cohort that previously were not detected in studies of shorter-duration activity.

CONCLUSION

Using the Ironman triathlon as a model for exercise, our data suggest that exercise in both men and women improves lipid and lipoprotein risk factors for developing coronary artery disease. Susceptibility to lipid peroxidation is significantly reduced in response to a single bout of prolonged exercise in highly trained athletes, an observation that may explain in part the reduced risk of cardiovascular disease in individuals who are physically active. Further, although antioxidant supplement use may reduce the degree of acute skeletal muscle injury induced by exercise, these supplements had no additional benefits in reducing the susceptibility of lipids to peroxidation beyond exercise itself.

References