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Effect of exercise intensity and training on antioxidants and cholesterol profile in cyclists

Antoni Aguiló[a], Pere Tauler[a], Maria Pilar Guix[b], Gerardo Villa[c], Alfredo Córdova[d], Josep A. Tur[a,*], Antoni Pons[a]

[a]*Laboratori de Ciències de l'Activitat Física, Univ. Illes Balears, Palma de Mallorca, Spain*
[b]*Laboratori del Carme, Hospital Son Dureta, Palma de Mallorca, Spain*
[c]*INEF Castilla y León, Universidad de León, León, Spain*
[d]*Escuela de Fisioterapia de Soria, Universidad de Valladolid, Soria, Spain*

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Abstract

The aim of this work was to evaluate the effect of different intensity of exercise and different training status on antioxidants and cholesterol profile in cyclists. 33 male cyclists (17 amateur and 16 professional cyclists) participated in this study. The amateurs all trained 14 ± 1 h each week, and their VO_2 max was 62.5 ± 1.8 ml/Kg.min; the professionals all trained 24 ± 1 h each week, and their VO_2 max was 80.2 ± 1.6 ml/Kg.min. Amateurs were submitted to the maximal and submaximal prolonged exercise tests. Professionals were submitted to a mountain stage (170 km) of cycling competition. Serum lipid and cholesterol profile (triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) and plasma antioxidant capacity (ascorbic acid, α-tocopherol, retinol, β-carotene and others) were measured before and after exercise tests. Hematological determinations (number of erythrocytes, hematocrit and hemoglobin concentration) and dietary intake were also measured. No significant differences were observed in basal values (before exercise tests) of amateur and professional cyclists. Negligible differences were found between dietary intake of amateur and professional cyclists, and also the results of hematological values showed there was no effect of degree of hydration or dietary intake on blood levels of studied antioxidant and lipid parameters. An increase in plasma levels of vitamin C, vitamin E, triglycerides and VLDL-cholesterol levels, and also a decrease of β-carotene and LDL-cholesterol. were observed in well-trained professional cyclists after the cycling stage - an endurance exercise - but not in amateur cyclists. Amateur cyclists showed only mild increases in total cholesterol after maximal and submaximal exercise, while a rise in HDL-cholesterol was only observed after maximal exercise; none of these changes were observed in professional cyclists. Plasma levels of antioxidant vitamins and carotenes, and also serum lipids, total cholesterol and lipoprotein-cholesterol showed an overall response to exercise, and their increase and/or decrease must be explained as a consequence of the different training status of sportsmen and intensity and duration of exercise tests.

Keywords: Antioxidants; Triglycerides; Lipoproteins; Cholesterol; Cyclists; Exercise

1. Introduction

Physical exercise is believed to have many beneficial effects, especially in preventing cardiovascular diseases (CVD) [1]; conversely, a lack of physical activity or a sedentary lifestyle has been recognized as a CVD risk factor [2-4]. However, the pro-oxidant nature of exercise has also been extensively recognized [5-7]. Although the precise mechanism by which exercise or regular physical activity may influence the progression of CVD is not known, one would expect that physical activity as a deterrent of CVD would be compatible with the oxidation hypothesis [8].

Exercise appears to result in an activation of neutrophils which is accompanied by an increased plasma level of granular enzymes [9,10]. Several studies reported exercise-induced increase in the plasma level of myeloperoxidase (MPO), an enzyme implicated in the oxidation of LDL in the artery [11], showing that exercise increases plasma levels of enzymes that have the potential to oxidize LDL.

Paradoxically, it has been found that beginning exercisers (amateur sportsmen) suffer oxidative stress and their LDL are more readily oxidized *in vitro* as compared to

* Corresponding author. Tel.: +34-971-173146; fax: +34-971-173184.
E-mail address: pep.tur@uib.es (J.A. Tur).

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sedentary subjects; whereas in contrast, LDL from chronic exercisers (professional sportsmen) are less readily oxidized as compared to sedentary subjects [12,13]. When there is oxidative stress (as in beginning exercisers) LDL may be oxidized by MPO. The oxidation of LDL might result in the induction of antioxidant enzymes in the artery and their enhanced clearance *via* the liver scavenger receptors. The increased antioxidant defense would prevent subsequent oxidative stress as seen in chronic exercisers. When there is inadequate defense or when there is overwhelming oxidative stress, further oxidation of LDL would result in atherosclerotic changes [8].

Few data are available on the handling of antioxidant vitamins C and E and carotenoids, such as β-carotene and lycopene, in exercise. Several studies have pointed out that exercise may decrease the tissue pool of antioxidants such as vitamin E and vitamin C, as well as postulating these antioxidants may be transferred from one body compartment to another as a result of exercise [14-18], while other authors have pointed out that tocopherol plasma and ascorbic acid plasma concentrations are increased following intense exercise [16,19,20]. It has also been suggested that an increase in serum ascorbate may have arisen not only from the tissue compartments, but also from the neutrophil compartment [21] according to the decreased ascorbic acid contents in leukocytes after intense exercise [22]. No data are available as to the relationship between the intensity of physical exercise and the effects produced on antioxidant vitamin plasma levels. Therefore, the knowledge of antioxidant nutrient handling in exercise may contribute to the establishment of physical exercise benefits and antioxidant nutrient diet supplementation in the prevention of exercise induced oxidative stress.

Atherosclerosis may be reduced through regular physical activity, which increases the turnover of lipid substrates, with effects on their transport and availability [23]. Very active middle aged men and women show higher plasma concentrations of HDL-cholesterol, lower levels of VLDL-cholesterol, cholesterol and triglycerides, and moderately lower levels of LDL-cholesterol than sedentary ones [3,24,25]. Acute effects of exercise on triglycerides and HDL-cholesterol appears to increase with overall energy expenditure, and prolonged exercise appears to be necessary for an acute effect of exercise on LDL-cholesterol levels [26]. Hence, considerable additional research will be required in order to clarify the beneficial effects of exercise, especially on CVD.

The aim of this work was to evaluate the effect of different intensity of exercise and different training status on antioxidants and cholesterol profile in cyclists. Details of the serum lipid and cholesterol profile (triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) and measurements of plasma antioxidant capacity (ascorbic acid, α-tocopherol, retinol, β-carotene and others) are provided.

2. Methods and materials

2.1. Subjects

Thirty-three male trained cyclists (amateurs or recreational weekend cyclists and professionals or those belonging to a professional cycling team) were invited to participate in this study. All the subjects were informed of the purpose of this study and the possible risks involved before giving their oral consent to participate in this study. The body weight and height of all cyclists were recorded, and then body mass index (BMI) was calculated as weight/height [2] and expressed as Kg/m^2. This work was approved by the Ethical Committee of 'Son Dureta' University Hospital (Palma de Mallorca, Balearic Islands, Spain).

2.2. Amateur cyclists and exercise

Seventeen well-trained amateur cyclists volunteered to participate in this study. Their mean ($\pm$ s.e.m.) age was 23.3 $\pm$ 2.0 years, height 168 $\pm$ 3 cm, weight 70.8 $\pm$ 1.2 Kg and body mass index (BMI) 24.5 $\pm$ 1.3 Kg/m^2. They all trained 14 $\pm$ 1 h each week, and their VO_2 max was 62.5 $\pm$ 1.8 ml/Kg.min. To measure VO_2 max two tests were performed on an electromagnetic reduction cycloergometer (Ergometrics 900, MedGraphics™, St. Paul MN, USA): the maximal exercise test and submaximal prolonged exercise test. In the maximal exercise test, subjects warmed up for three minutes at 30 Watts prior to starting the test. The test started at 50 Watts and the subjects' work rate was increased by 30 Watts every three minutes. The test ended when increased work did not increase or decrease the oxygen consumption; this value was the VO_2 max. During this exercise test the sportsmen did not drink anything. The submaximal prolonged exercise test was carried out one week after the maximal test. In this submaximal test the cycloergometer resistance was adjusted so the cyclists worked at 80% of their maximal capacity of oxygen consumption. This test was prolonged for 1 h 30 min and all subjects drank 500 ml of spring water. All the tests were performed on overnight fasted subjects.

2.2.1. Professional cyclists and exercise

Sixteen voluntary subjects participated in the study. They were all professional cyclists participating in the "Volta Ciclista a Mallorca" Challenge for professional cyclists. Their mean ($\pm$ s.e.m.) age was 23.8 $\pm$ 0.9 years, height 180 $\pm$ 2 cm, weight 70.0 $\pm$ 1.5 Kg and body mass index (BMI) 21.6 $\pm$ 1.7 $Kg/m^{2.}$ They all trained 24 $\pm$ 1 h each week, and their VO_2 max was 80.2 $\pm$ 1.6 ml/Kg.min. The exercise was a mountain stage (170 km) of the "Volta Ciclista a Mallorca" Challenge. The athletes' time consumption was a mean $\pm$ s.e.m. of 250 $\pm$ 10 min in this competition. VO_2 max of professional cyclists were measured by means of the same procedure used on the amateur cyclists. During the mountain stage, the cyclists usually used 80% of VO_2 max, as has been indicated previously [27].

2.2.2. Experimental procedure

Venous blood samples were collected from the antecubital vein with suitable vacutainers. The basal venous blood was obtained from all the subjects participating in this study on the morning of the competition or exercise test day, after 12 hr overnight fasted conditions and immediately after the exercise.

2.3. Hematological analysis

Hematological determinations (number of erythrocytes, hematocrit and hemoglobin concentration) were made using an automatic flow cytometer analyser Technicon H2 (Bayer) VCS system.

2.3.1. Plasma vitamin determinations

Plasma was obtained after centrifugation at 1000*g of the blood samples obtained as above and was stored at −80°C until use. The deep-frozen plasma was thawed and mixed to disperse possible precipitates.

The extraction of liposoluble vitamins was carried out using n-hexane after deproteinization with ethanol [28]. Liposoluble vitamins and carotenoid concentrations were determined by HPLC in the n-hexane extract of plasma after drying in a N_2 current and redissolving in methanol. The mobile phase consisted of 550:370:80 acetonitrile:tetrahydrofuran:H_2O. The HPLC was a Shimadzu with a diode array detector and the column was a Nova Pak, C18, 3.9 × 150 mm. retinol, α-tocopherol, vitamin D, β-carotene, lycopene and cryptoxanthin were determined at 330, 290, 260, 465, 460 and 460 nm respectively. Lycopene concentrations were calculated using a β-carotene standard, as indicated previously [29].

Plasma vitamin C was determined by an HPLC method with electrochemical detection [30,31]. Plasma samples were deproteinized with 30% trichloroacetic acid containing 2 mM EDTA. Appropriate volumes of deproteinized plasma, previously diluted with distilled water, were injected to HPLC system. The mobile phase consisted of 0.05 M sodium phosphate, 0.05 M sodium acetate, 189 μM dodecyltrimethylammonium chloride and 36.6 μM tetraoctylammonium bromide in 25/75 methanol/water (v/v), pH 4.8. The HPLC system was a Shimadzu with a Waters Inc. electrochemical detector and a Nova Pak, C18, 3.9x150 mm column. The potential of the chromatographic detector was set at 0.7 V versus an Ag/AgCl reference electrode. Previous work [21] showed this method to be useful to determine vitamin C plasma levels.

2.4. Determination of serum lipid and cholesterol parameters

Serum triglycerides, total cholesterol, and HDL-cholesterol were determined by colorimetric methods using autoanalyzer DAX-72 (Technicon, Bayer). Triglycerides were hydrolyzed to glycerol and fatty acids using lipoprotein lipase, and then glycerol mixed with glycerol phosphate oxidase and peroxidase to give chinoneimine. Serum samples were hydrolyzed with cholesterol esterase to obtain total cholesterol. 4-aminoantipirine and cholesterol oxidase reacts with cholesterol to form chinoneimine. The absorbance of chinoneimine was determined at 524 nm [32,33]. The determination of cholesterol bound to high density plasma lipoproteins (HDL-cholesterol) was developed after immuno-inhibihition of LDL, VLDL and chylomicrons with anti-β-lipoproteins, as an antibody to avoid the binding of lipoproteins to the enzymes used. Cholesterol esterase, cholesterol oxidase and peroxidase were afterwards mixed with serum HDL-cholesterol to give a blue compound, whose absorbance was measured at 600 nm [36].

VLDL-cholesterol and LDL-cholesterol were calculated according to the Friedewald [32] equations: [VLDL-cholesterol=triglycerides/5] and [Intermediate Density plus LDL-cholesterol = total cholesterol –(HDL-cholesterol + VLDL-cholesterol)].

2.4.1. Dietary Intake

To ensure that the observations represent differences due to the effect of different intensity of exercise and different training status instead of random changes, in particular due to aspects of the cyclist's dietary intake, the usual dietary habits of each amateur and professional cyclist were assessed using a 3-day 24-hr recall before the three months of diet supplementation was applied and blood samples were taken. A well trained dietitian verified and quantified the food records. All food items consumed were transformed into nutrients using a self-made computerized program based on the Spanish [34] and European Food Composition Tables [35]. The following food characteristics were used: total energy intake (relative to body mass), percentage of energy from carbohydrates, fats and proteins, and also the dietary intake (relative to body mass) of carbohydrates, fats, proteins, fatty acids (saturated fatty acids SFA, mono-unsaturated fatty acids MUFA, poly-unsaturated fatty acids PUFA), cholesterol, fiber, vitamins (A, B_1, B_2, B_6, B_{12}, C, D, E, niacin, folic acid) and minerals (sodium, potassium, calcium, phosphorus, magnesium, iron, zinc and iodine). The consumed food and beverage of professionals cyclists during the race were also considered and enclosed in their dietary data.

3. Statistics

Statistical analyses were performed on SPSS version 9.0.1. Mean values and s.e.m. are shown. The degree of significance of differences between means was calculated as follows: Student's paired test was used to find significant differences before v. after exercise test. Student's unpaired test was used to find significant differences between basal values of amateur and professional cyclists. Relationships between dietary components were investigated using Spearman's correlation coefficients (r).

Table 1
Daily caloric profile and dietary intake expressed per 1000 kcal and kg body weight (mean values ± s.e.m.) in amateur and professional cyclists

	Amateurs	Professionals[#]
Total energy intake		
kJ/kg bw	235.6 ± 38.5	349.9 ± 57.2*
kcal/kg bw	56.3 ± 4.1	94.0 ± 6.8*
Carbohydrate (% energy)	45.5 ± 2.2	54.5 ± 2.6*
Protein (% energy)	14.1 ± 0.7	12.1 ± 0.6
Fat (% energy)	40.2 ± 2.1	33.4 ± 1.7*
Dietary intake		
Protein (g/1000 kcal /kg bw)	8.49 ± 0.42	8.21 ± 0.41
Fat (g/1000 kcal /kg bw)	10.61 ± 0.85	10.06 ± 0.80
SFA (g/1000 kcal /kg bw)	3.82 ± 0.42	3.31 ± 0.37
MUFA (g/1000 kcal/kg bw)	1.27 ± 0.25	3.34 ± 0.67*
PUFA (g/1000 kcal /kg bw)	2.97 ± 0.30	0.80 ± 0.08*
Cholesterol (mg/1000 kcal /kg bw)	33.11 ± 2.12	32.39 ± 4.00
Carbohydrate (g/1000 kcal /kg bw)	29.29 ± 2.97	37.00 ± 3.75*
Fiber (g/1000 kcal /kg bw)	2.55 ± 0.42	2.02 ± 0.34
Vitamins		
Vitamin A (μg/1000 kcal /kg bw)	97.20 ± 11.88	84.74 ± 10.36
Thiamin (μg/1000 kcal /kg bw)	134.97 ± 19.95	134.61 ± 19.90
Riboflavin (μg/1000 kcal /kg bw)	179.97 ± 24.62	176.99 ± 24.21
Vitamin B6 (μg/1000 kcal /kg bw)	193.12 ± 37.78	222.82 ± 43.58
Vitamin B12 (μg/1000 kcal /kg bw)	0.85 ± 0.21	0.57 ± 0.14
Vitamin C (mg/1000 kcal /kg bw)	11.04 ± 2.97	8.89 ± 2.39
Vitamin D (μg/1000 kcal /kg bw)	0.47 ± 0.30	0.44 ± 0.28
Vitamin E (μg/1000 kcal /kg bw)	710.53 ± 13.58	677.95 ± 12.96
Niacin (m/1000 kcal /kg bw)	2.49 ± 0.23	1.84 ± 0.17
Folate (μg/1000 kcal /kg bw)	21.65 ± 4.24	34.47 ± 6.76*
Minerals		
Sodium (mg/1000 kcal /kg bw)	203.74 ± 16.98	247.48 ± 20.62
Potassium (mg/1000 kcal /kg bw)	263.16 ± 47.54	243.27 ± 43.94
Calcium (mg/1000 kcal /kg bw)	94.65 ± 5.52	57.69 ± 3.36
Phosphorus (mg/1000 kcal /kg bw)	106.96 ± 10.61	119.48 ± 11.85
Magnesium (mg/1000 kcal /kg bw)	28.01 ± 3.82	29.05 ± 3.96
Iron (mg/1000 kcal /kg bw)	1.70 ± 0.21	1.35 ± 0.17
Zinc (mg/1000 kcal /kg bw)	0.85 ± 0.08	1.75 ± 0.17
Iodine (μg/1000 kcal /kg bw)	4.67 ± 0.85	9.32 ± 1.69*

* Significant differences between professionals and amateurs ($p < 0.05$, Student's unpaired test).

[#] The consumed food and beverage of professionals cyclists during the race were also considered and enclosed in these data.

4. Results

The daily caloric profile and dietary intake expressed per 1000 kcal and kg body weight (mean values ± s.e.m.) in amateur and professional cyclists are shown in Table 1. Significant differences were not only obtained for total energy intake, and percentage of energy from carbohydrates and fats, but also for MUFA, PUFA, carbohydrate, folate and iodine daily intake.

A comparison of basal hematological parameters (mean values ± s.e.m.) between amateur sportsmen and professional cyclists (Table 2) showed that the number of erythrocytes, hematocrit, and hemoglobin concentration of professionals were similar to those of amateurs.

Table 3 shows plasma levels of vitamins (retinol, D, α-tocopherol and ascorbic acid) and carotenoids (β-carotene, cryptoxanthin, and lycopene) in amateur and professional cyclists before and after the exercise tests. No significant differences were observed between basal values (before exercise tests) of amateur and professional cyclists. Amateur cyclists did not change basal values of these parameters after maximal or submaximal exercise tests. Professional cyclists significantly increased plasma ascorbic acid (32.4%) and α-tocopherol (6.8%) contents and decreased plasma β-carotene (−15.2%) contents after a cycling stage - an endurance exercise. No significant correlation ($p = 0.124$) was found between vitamin C and vitamin E by Spearman's correlation test.

Table 2
Base hematological parameters (mean values ± s.e.m.) in amateur and professional cyclists

	Amateurs	Professionals
Erythrocytes ($10^6/\mu L$)	4.99 ± 0.08	4.99 ± 0.07
Hematocrit (%)	45.6 ± 0.6	45.8 ± 0.4
Hemoglobin (g/dL)	15.3 ± 0.2	15.5 ± 0.2

No significant differences were found between professionals and amateurs (Student's unpaired test).

Table 4 shows serum levels of triglycerides, total cholesterol and lipoproteins-cholesterol in amateur and professional cyclists before and after the exercise tests. Maximal and submaximal exercise tests significantly increased (5.8% and 6.0%, respectively) serum total cholesterol contents in amateur cyclists, but left them unchanged after the cycling stage in professional cyclists. However, the cyclist stage significantly increased serum triglycerides (42.0%) and VLDL-cholesterol (40.0%) in professionals, whereas in amateurs these parameters remained unchanged after maximal and submaximal exercise tests.

Serum LDL-cholesterol and HDL-cholesterol levels also showed different trends depending on the training and type of exercise test. The cyclist stage significantly decreased serum LDL-cholesterol (−5.3%) but left unchanged HDL-cholesterol contents in professional cyclists. The maximal exercise test significantly increased serum HDL-cholesterol (6.9%) but left unchanged LDL-cholesterol contents in amateur sportsmen. The submaximal exercise test left significantly unchanged serum HDL-cholesterol and LDL-cholesterol contents.

These results were also used to calculate the following relationships: a LDL-cholesterol/HDL-cholesterol quotient, and lipoprotein-cholesterol levels as a percentage of total cholesterol levels.

The LDL-cholesterol/HDL-cholesterol quotient significantly decreased after the cycling stage in professional cyclists and after the maximal but not the submaximal exercise test in amateurs. LDL-cholesterol percentage significantly decreased after the cycling stage (-3.6%) in professional cyclists and after the maximal exercise test (-1.1%) but remained unchanged by submaximal exercise test in amateurs. HDL-cholesterol percentage significantly increased (1.0%) only after the maximal exercise test in amateurs, and

Table 3
Plasma levels (mean values ± s.e.m.) of vitamins and carotenes in amateur and professional cyclists before and after exercise tests

	Amateurs				Professionals	
	Maximal test		Submaximal test		Cycling stage	
	Before	After	Before	After	Before	After
Vit C (μg/mL)	10.4 ± 0.1	10.6 ± 0.2	10.5 ± 0.1	10.6 ± 0.1	10.8 ± 0.8	14.3 ± 1.2*
Vit E (μg/mL)	26.4 ± 1.1	26.4 ± 1.0	26.0 ± 0.8	27.3 ± 1.1	27.8 ± 0.8	29.7 ± 1.1*
β-carotene (μg/L)	144 ± 14	159 ± 17	154 ± 15	145 ± 15	171 ± 17	145 ± 15*
Cryptoxanthin (μg/L)	511 ± 77	521 ± 82	528 ± 75	547 ± 82	471 ± 45	451 ± 46
Lycopene (μg/L)	112 ± 16	129 ± 20	139 ± 23	128 ± 24	110 ± 14	96 ± 16
Vit A (μg/mL)	0.53 ± 0.02	0.55 ± 0.02	0.56 ± 0.03	0.57 ± 0.02	0.57 ± 0.02	0.58 ± 0.03
Vit D (μg/mL)	1.04 ± 0.16	1.04 ± 0.15	1.03 ± 0.18	1.04 ± 0.13	1.10 ± 0.09	1.12 ± 0.15

* Significant differences before v. after exercise test ($p < 0.05$, Student's paired test). No significant differences were found between basal values of amateur and professional cyclists ($p < 0.05$, Student's unpaired test).

VLDL-cholesterol significantly only increased (3.5%) after the cycling stage in professional cyclists.

5. Discussion

This work shows that different intensity of exercise and different training status are able to modify plasma levels of antoxidant vitamins and carotenoids, and also serum lipids and cholesterol.

The negligible differences between the dietary intake of amateur and professional cyclists showed that the observed changes of plasma levels of antioxidant vitamins and carotenoids, and also serum lipids and cholesterol can not be attributed to aspects of the cyclists' usual diet, also comprising the professional cyclists' food and beverage consumed during the race. What is more, the results of the hematological values show there was no effect of degree of hydration on the blood levels of antioxidant and lipid parameters studied.

Well-trained professional cyclists increased ascorbic acid plasma levels after the cycling stage - an endurance exercise - but amateur cyclists maintained their initial levels after maximal and submaximal exercise tests. Controversial opinions have been given on the effects of exercise on ascorbic acid concentration, and several authors have pointed out no effect of exercise on ascorbic acid plasma levels [37] but we have shown it in amateur sportsmen after both maximal and submaximal exercise tests. It has been reported that endurance running increases serum ascorbic acid concentration and decreases ascorbate excretion in the urine compared to sedentary individuals [19]. Moreover, it has been found that plasma ascorbic acid increases about 27% after a 21 Km running race, suggesting that this increase is the result of a concomitant release of cortisol and ascorbic acid from adrenal glands [20]. After a 170 Km mountain cycling stage, it is easy to understand that vitamin C plasma levels had increased 32.4% in professional cyclists. This result also agrees with a previous work pointing out the increase of ascorbic acid plasma levels may be partially attributed to ascorbate recycling and efflux from neutrophils induced by exercise [21]. These adaptive changes happened in well-trained professional cyclists due to their participation in a cyclist stage - an endurance exer-

Table 4
Serum levels (mean values ± s.e.m.) of triglycerides, total cholesterol and lipoprotein-cholesterol in amateur and professional cyclists before and after exercise tests

	Amateurs				Professionals	
	Maximal test		Submaximal test		Cycling stage	
	Before	After	Before	After	Before	After
Cholesterol (mg/dL)	165 ± 4	174 ± 6*	166 ± 6	176 ± 6*	168 ± 6	169 ± 5
Triglycerides (mg/dL)	60.8 ± 7.5	66.2 ± 5.8	69.6 ± 7.1	78.8 ± 4.7	71.9 ± 4.9	102 ± 10*
VLDL-cholest. (mg/dL)	12.1 ± 1.6	12.5 ± 1.2	14.0 ± 1.7	14.6 ± 1.0	14.5 ± 1.0	20.3 ± 1.9*
LDL-cholest. (mg/dL)	85.1 ± 4.8	86.9 ± 6.1	86.6 ± 7.6	94.8 ± 8.9	93.5 ± 5.5	88.5 ± 6.2*
HDL-cholest. (mg/dL)	67.8 ± 2.7	72.5 ± 3.0*	71.7 ± 6.9	71.4 ± 3.7	59.6 ± 3.3	59.9 ± 3.1
LDL-chol./ /HDL-chol.	1.33 ± 0.1	1.29 ± 0.1*	1.24 ± 0.2	1.34 ± 0.1	1.65 ± 0.1	1.54 ± 0.1*
LDL-chol./ /total chol. (%)	51.5 ± 1.9	50.4 ± 2.1*	51.6 ± 2.6	51.0 ± 3.3	55.5 ± 1.9	51.9 ± 2.4*
HDL-chol./ /total chol. (%)	41.4 ± 1.9	42.4 ± 2.1*	44.1 ± 4.9	40.9 ± 2.2	35.7 ± 1.9	35.7 ± 1.8
VLDL-chol./ /total chol. (%)	7.1 ± 0.8	7.3 ± 0.5	8.1 ± 1.4	8.2 ± 0.8	8.8 ± 0.7	12.3 ± 1.4*

* Significant differences before v. after exercise test ($p < 0.05$, Student's paired test). No significant differences were found between basal values of amateur and professional cyclists ($p < 0.05$, Student's unpaired test).

cise - but did not happen in amateur sportsmen after maximal and submaximal exercise tests. Hence, the different ascorbic acid plasma levels of sportsmen found in our study must be explained as a consequence of the different training status and/or intensity exercise test (submaximal, maximal and mountain stage).

The increase in α-tocopherol plasma levels observed after the cycling stage in professional cyclists is coincident with the increase of triglycerides and VLDL-cholesterol levels, and the decrease of LDL-cholesterol levels; in medium-trained amateur cyclists, the intensity of exercise (maximal and submaximal tests) was not enough to show significant effects on these parameters. The mechanisms to produce this increase in α-tocopherol may be due to its mobilization from tissue stores (adipose tissue, liver, spleen, skeletal muscle, and other tissues) to the plasma circulation [15,16,38], reflecting the importance of vitamin E in protection against oxidative damage. Vitamin E is secreted from parenchymal cells of the liver in association with VLDL lipoproteins; some of the vitamin E associated with VLDL is transferred to peripheral cells and HDL lipoproteins, but also some vitamin E in VLDL ends up in LDL lipoproteins during VLDL metabolism, and this LDL-associated vitamin E follows the receptor-mediated uptake of LDL in parenchymal cells as well as peripheral cells [38].

The results obtained in this work point to the increase in plasma α-tocopherol induced by exercise being produced after the sportsmen have used and mobilized lipid reserves as a fuel to maintain physical activity, as has been evidenced by the increased triglyceride levels. The high mobilization of serum triglyceride levels observed after exercise is related to the release of free fatty acids from the periphery, mainly due to considerable energy expenditure during the physical performance in professional cyclists. Accordingly, we suspect that professional cyclists have higher abilities than amateurs to use lipids as a fuel. The exercise tests practiced by amateurs were not able to mobilize lipid reserves; plasma triglycerides and VLDL-cholesterol remained unchanged, and neither did the plasma levels of α-tocopherol increase. The oxidative stress associated to exercise arises when blood lactate concentration rises above basal values [39]; accordingly, we point out that vitamin E mobilization occurs after the utilization of fat reserves.

The lipophylic carotenoids, such as β-carotene, are mainly carried by LDL lipoproteins [40]. Thus, the decrease of LDL blood levels in professional cyclists may be the main cause of the decrease of β-carotene plasma levels observed in these sportsmen, pointed out by the disappearance of LDL-cholesterol levels. It is well known that *in vitro* synergistic effects have been observed between vitamin E and β-carotene, and also that LDL-Vitamin E is oxidized faster than LDL-β-carotene [40]. Therefore, the decrease in β-carotene and the increase in vitamin E after exercise and the appearance of oxidative stress may be understood as these two compounds are located in different compartments. The decrease in β-carotene may be understood as a loss of plasma LDL-cholesterol, but the observed decrease in LDL-cholesterol levels may also be explained as a part of the beneficial package of oxidative stress itself. Exercise-induced oxidative stress promotes antioxidant defense in the artery, i.e.: the vitamin E released from the liver will result in protection from oxidation of LDL newly generated from *de novo* plasma VLDL, but the "old" LDL will be oxidized and plasma carotenoids will decrease. Hence, the oxidized LDL will be cleared by the liver or the peripheral cells *via* the classical LDL receptor or non-receptor uptake [8].

The increase of total cholesterol observed in amateur cyclists after maximal and submaximal exercise tests, but not in professional cyclists, may be due to changes of LDL-cholesterol and/or HDL-cholesterol. Different mobilization of lipoprotein-cholesterol can be expected depending on exercise type. The lack of changes in HDL-cholesterol in professional cyclists after the cycling stage could be related to the low initial levels present in these sportsmen. The possibility that the training status of sportsmen may influence the magnitude of increase of HDL-cholesterol levels has also been pointed out [41]. An increase in HDL-cholesterol levels has been observed after acute exercise, but not after chronic exercise [26], which agrees with our results because maximal exercise is acute exercise (short term and high intensity), but submaximal exercise and a cycling stage are chronic exercises (long term and moderate or high intensity). In spite of the fact that the submaximal exercise needed less time than the cycling stage, it could also be considered chronic exercise, after the amateur cyclists used 80% VO_2 max, similarly to the professionals [27], but they also needed more time than the maximal test.

Moreover, the LDL-cholesterol/HDL-cholesterol quotient, and lipoprotein-cholesterol levels expressed as a percentage of total cholesterol also show that different exercises and training induce a different distribution of cholesterol between plasma lipoproteins. Acute exercise decreased LDL-cholesterol and increased HDL-cholesterol, whereas chronic exercise decreased LDL-cholesterol and increased VLDL-cholesterol in professionals, mainly due to the high turnover of VLDL [8]. Accordingly, it can be concluded that short term, high intensity exercise increases cholesterol mobilization from its reservoirs in moderately trained people, whereas long term, high intensity exercise does not mobilize cholesterol but eliminates LDL-cholesterol in well-trained people. Therefore, this is the type of exercise to be recommended in order to have a healthy cardiovascular lifestyle in amateur and professional sportsmen.

To sum up, we evidenced that blood levels of antioxidant vitamins and carotenes, and also serum lipids, total cholesterol and lipoprotein-cholesterol showed an overall response to exercise, and their increase and/or decrease must be explained as a consequence of the different training status of sportsmen and intensity and duration of exercise tests.

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