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Stress Hormonal Factors, Fatigue, and Antioxidant Responses to Prolonged Speed Driving

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TSOPANAKIS, C. AND A. TSOPANAKIS. *Stress hormonal factors, fatigue, and antioxidant responses to prolonged speed driving.* PHARMACOL BIOCHEM BEHAV **60**(3) 747–751, 1998.—Oxygen free radicals have been implicated in exercise-induced cell and tissue injury, indicating an oxidative stress. Fatigue accompanied by a number of physiological and metabolic changes is an indication of overtraining. This study aimed to examine the influence of a continuous 24-h intermittent speed driving (1 h driving/1 h stop), on the response of hormones, antioxidative factors, lipid, and enzyme levels. Seven race car drivers of national level were examined before, during, and immediately after the trial of speed driving on a test designed to check endurance to stress. The parameters measured were: testosterone (Tes), cortisol (Cor), IgM, IgA, cholesterol, HDL, billirubin, ceruloplasmin, urea, uric acid, creatine kinase, and transaminases. Stress hormone Cor declined significantly ($p < 0.05$), while Tes did not change significantly. Fatigue enzyme, aspartate transaminase (GOT) increased significantly ($p < 0.05$), while alanine transaminase (GPT) did not change and urea declined. Muscle enzyme, creatine kinase (CK) increased to sixfold ($p < 0.01$). IgA, IgM and lipids did not change. The primary antioxidant ceruloplasmin increased significantly ($p < 0.001$), while antioxidants uric acid and glucose remained unchanged. Among the factors measured, ceruloplasmin, cortisol, urea, GOT, and CK seem to give a picture of the organism's alertness and defence capabilities in conditions of stress and fatigue. © 1998 Elsevier Science Inc.

Stress Fatigue Driving Exercise Testosterone Cortisol Lipids antioxidants Immunoglobulins

A variety of stress conditions can provoke important physiological changes, some of which can be of pathogenic significance (8,14).

Physical exercise could be considered as a physical stressor that can elicit physiological alterations (physiological stress) coupled with hormonal and biochemical changes in order for homeostasis to be preserved (17,20,27,28).

Speed driving is especially demanding for the driver athlete that conducts it. A speed driver has to simultaneously yield sprint and endurance abilities together with stress resistance, thus combining lower rates of glycolytic and oxidative muscle capacities together with higher rate of neurohormonal processes.

There is strong evidence that strenuous physical exercise is associated with increased free radical generation primarily due to an increase in oxygen uptake both at the whole body and local tissue levels (2,10,12). Oxygen free radical generation has been implicated to be a major cause for exercise-induced metabolic stress and concomittantly of cell and tissue injury (10,22,24).

The body is equipped with an efficient antioxidant defence system consisting of antioxidant vitamins, glutathione, and sulphydryls and antioxidant enzymic and nonenzymic factors (16); among the primary antioxidants are ceruloplasmin—a coper enzyme—while among the secondary are billirubin glucose and uric acid (3,9). Each of these plays a unique role, but they also complement each other functionally.

Fatigue is accompanied by a number of physiological and metabolic changes. Among the metabolic factors indicative of fatigue, are hematological and immunological parameters, blood glucose levels, lipid levels, enzyme activities, blood urea, uric acid, and other factors (uric acid plasma levels are

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also used as markers for ischemia and anaerobic stress) (9). These, combined with hormonal stress indicative factors such as cortisol and testosterone levels, may give an image of the bodys'response to a sustained stress of 24-h driving. Stress affected cortisol secretion has also been implicated in testosterone responce to stress.

In a previous study of ours we have reported that acute physical exercise, by provoking the mobilization of stress response mechanisms, can reveal the readiness of the organism to successfully respond to stress (27): a measurement of the ratio of plasma testosterone/cortisol levels (TES/COR) after a bout of acute physical exercise may give a picture of the physiological response ability to the stress produced. Other investigators have also been using the above ratio or similar ones as a marker of overtraining, monitoring fitness and recovery from overstrain (4,13,18).

The stress produced during acute physical exercise appears to influence the immune system. Behind these exercise induced changes are hyperthermia, cytokines, and stress hormones such as cortisol. Acute exercise has been reported to affect the levels of serum immunoglobulins IgA and IgM in elite athletes (19,29).

In this study we examined the influence of a continuous 24-h intermittent speed driving [(1 h driving/1 h stop), driving normal cars in an Autodrome], on the response of selected hormones, antioxidative and fatigue factors, lipid, and enzyme levels. The purpose of the study was to try and measure factors participating in the above processes in an attempt to isolate certain of them to be used as indices of physiological performance under extreme conditions.

TABLE 1
SOMATOMETRIC PARAMETERS OF THE DRIVERS

Parameters	$n = 7$	Mean ± SD
Age	(years)	38.37 ± 5.26
Body weight	(kg)	77.87 ±9.90
Height	(cm)	178.37 ± 3.46
BMI	(kg/m^2)	24.48 ± 3.08

Values are expressed as mean ± SDs. BMI = body mass index = $BW/H^{2.}$

METHOD

Athletes and Stress Conditions

Seven male national class race car drivers trained for at least 7 years, voluntarily participated in this study. They were aged 38.4 ± 5.3 years and were in healthy condition. Before they were selected they completed a questionnaire regarding their diet, medication, and smoking habits.

The drivers selected had all similar dietary habits, were nonsmokers, and did not receive any special medication. Seven days before the race and after a 12-h fast, they were

TABLE 2
LEVELS OF STRESS HORMONES, IMMUNOGLOBULINS, LIPIDS, ANTIOXIDANTS, AND ENZYME FATIGUE FACTORS

	Phases	Ph 1—Rest	Ph 2—Onset	Ph3—End
Parameters	Drivers	$n = 7$ (1000h)	$n = 7$ (1300h)	$n = 7$ (1200h)
Stress hormones				
Testosterone (TES)	nmol/l	16.22 ± 6.45	12.45 ± 4.82	12.38 ± 5.31
Cortisol (COR)	nmol/l	554.56 ± 109.26	376.05 ± 128.29*	311.49 ± 118.64*
Stress factor	[TES/COR $\times 10^2$]	3.01 ± 1.21	4.41 ± 2.56	4.93 ± 3.17
Immunoglobulins				
IgA	g/dl	0.23 ± 0.061	0.282 ± 0.115	0.277 ± 0.097
IgM	g/dl	0.168 ± 0.050	0.172 ± 0.052	0.208 ± 0.065
(IgM/IgA)	—	0.72 ± 0.20	0.67 ± 0.31	0.79 ± 0.25
Lipids				
Cholesterol (TC)	mmol/l	5.227 ± 0.803	4.995 ± 0.930	5.013 ± 0.772
HDL_c	mmol/l	1.245 ± 0.151	1.230 ± 0.111	1.212 ± 0.102
Risk factor [TC/HDL]	—	4.21 ± 0.53	4.04 ± .054	4.14 ± 0.62
Antioxidants				
Billirubin	μmol/l	13.17 ± 3.93	15.05 ± 4.28	16.07 ± 5.13
Uric acid	μmol/l	317.62 ± 101.7	285.50 ± 89.81	261.71 ± 117.17
Glucose	μmol/l	5.455 ± 0.359	5.297 ± 0.544	5.408 ± 0.547
Ceruloplasmin	mg/l	334.3 ± 58.6	300.0 ± 40.8‡	411.4 ± 19.5‡
Fatigue factors (enzymes)				
CK	μkat/l	1.759 ± 0.915	2.874 ± 1.445†	11.155 ± 5.298†
GOT	μkat/l	0.300 ± 0.076	0.328 ± 0.072*	0.457 ± 0.093*
GPT	μkat/l	0.352 ± 0.167	0.309 ± 0.084	0.402 ± 0.140
Urea	mmol/l	14.64 ± 2.95	9.89 ± 2.26*	10.50 ± 1.77*

MANOVA between phases (1), (2) and (3), where *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.
Values are expressed as mean ± SDs.
Where HDL_c = high density lipoproteins (cholesterol fraction), CK = creatine kinase and GOT/GPT = the transaminases aspartate (AST) and alanine (ALT).

measured for the following parameters—hormones: testosterone and cortisol; lipids: cholesterol and high-density lipoproteins (HDL); enzymes: creatine kinase (CK), the two transaminases (GOT, GPT) and also urea; immunological factors: IgA, IgM; antioxidants: ceruloplasmin, bilirubin, uric acid, and glucose.

These measurements were used as the drivers control values (phase 1 at rest). One hour after the race had started (phase 2), blood was drawn—at a resting position—and again the next day immediately after the end of the race—on the 24th hour (phase 3).

The Speed-Driving Test

The driving protocol included repeated alternating bouts of 1-h speed driving and 1-h stop without sleep (just sitting or standing), continuously for 24 h. The drivers during the race were permitted to take a small breakfast at 0800 h and a small dinner or lunch (sandwiches, etc.) after the analogous blood sampling was taken. Coffee during the 1-h breaks was at free will.

The driving test started at 1200 h. The drivers used four new normal cars, which ran for a total distance of 1600 km each with a mean velocity of 75 km/h. The autodrome route was 5 km long with many turns, and during the race it was necessary for 12 tyres (three sets) to be changed on each car. Lower and higher temperatures detected during night and day were 7 and 21°C, respectively.

Biochemical Methods

Serum for the first sampling was collected between 0930 to 1030 h, while for the second at 1300 h and the last at 1200 h. It was placed in an ice bath (4°C) and was centrifuged at 1500 × *g*.

Total cholesterol (TC) was determined enzymatically by PAP method (1) (Biomerieux France Cat. No. 61225), triglycerides (TG) enzymatically (30) (Biomerieux Cat. No. 61236), and HDL-C by $MgCl_2$–Na phosphotungstate precipitation (15) (Biomerieux Cat. No. 61531).

Testosterone (TES) and cortisol (COR) were assayed by radioimmunoassay (RIA) using Immunochem Kits (Cat. No. 07-289101 and 07-221102, respectively) ICN Biochemical, CA. IgM and IgA were determined by turbidimetry (Kone, Finland), billirubin chromatographically (Kone, Finland), ceruloplasmin by turbidimetry (Aptek, Belgium).

Transaminases by kinetic methods GPT (Boehringer M. Cat. No. 1087550), GOT (Cat. No. 1087576), uric acid by enzymatic PAP (Biomerieux Cat. No. 61923), urea by enzymatic colorimetric method (Boehringer M. Cat. No. 777510), Creatine kinase (CK) by kinetic method CK-NAC activated (Boehringer M. Cat. No. 1087533), and glucose by an enzymatic colorimetric test (Boehringer M. Cat. No. 676543).

Statistics

To analyze the data we used the SPSS/PS (1994) program. Group parameters (phases 1, 2, and 3) were compared for mean differences by repeated ANOVA (by parametric test—MANOVA and nonparametric Friedman two-way ANOVA, with the same results). Correlation tests were also performed.

RESULTS

Somatometric data are presented in Table 1. In Table 2 are presented the values of the various parameters during the three phases of driving: rest, begining of driving (first hour) and immediately after the end (24th hour).

At phase 2 (1 h after the onset of driving), cortisol declined significantly, $F(2, 18) = 5.66$, $p < 0.05$. The stress factor did not show any difference. Stress factor is an index derived from the ratio of TES/COR values with which in a previous study we found useful to express the degree of "stress resistance"(27). No significant difference was seen in serum testosterone (TES) values ($p = 0.06$). Creatine kinase (CK) increased significanlty, $F(2, 18) = 11.26$, $p < 0.01$, while urea decreased, $F(2, 18) = 8.39$, $p < 0.05$, and GOT increased, $F(2, 18) = 7.09$, $p < 0.05$. All other factors remained unchanged in this phase.

At the end of the race (phase 3) cortisol values declined further significantly, $F(2, 18) = 5.66$, $p < 0.05$. Testosterone values did not change significantly when MANOVA test was used ($p = 0.06$); However, when Wilcoxon text was used TES change was significant ($p < 0.05$).

Among the antioxidative factors ceruloplasmin increased significantly, $F(2, 18) = 58.19$, $p < 0.001$, while uric acid and glucose levels remained unchanged.

Fatigue factor creatine kinase (CK) increased to high levels, sixfold, $F(2, 18) = 11.27$, $p < 0.01$; CK values give also a picture of muscle mobilization during driving. Urea levels declined significantly, $F(2, 18) = 8.39$, $p < 0.05$, and the enzyme GOT activity increased, $F(2, 18) = 7.09$, $p < 0.05$; GPT activity values did not change.

Total cholesterol (TC) and HDL-C levels remained unchanged, as also the risk factor (TC/HDL). Unchanged remained also the immunological factors IgA and IgM. Table 3 depicts the different parameters that displayed among them high statistically significant correlation coefficients r ($p < 0.01$) at phase 3.

TABLE 3

CORRELATIONS AMONG VARIOUS PARAMETERS AFTER THE END OF DRIVING TEST (PHASE 3)

PARAMETERS	CORRELATION COEFFICIENT (r)
TES_3: COR_3	−0.94†
TES_3: Glu_3	−0.86†
COR_3: Bil_3	0.78*
COR_3: Glu_3	0.80*
Stress $factor_3$: Bil_3	−0.79*
Stress $factor_3$: Glu_3	−0.73*
IgA_3: $Uric_3$	0.71*
IgM_3: $Uric_3$	0.68*
IgM_3: CK_3	0.70*
RF_3: TES_3	−0.68*
RF_3: COR_3	0.68*
RF_3: $Uric_3$	0.73*
HDL_3: COR_3	−0.69*
CK_3: Cer_3	0.78*
CK_3: GOT_3	0.81*
GOT_3: GPT_3	0.83*

Correlation test; *$p < 0.05$, †$p < 0.01$. Values are expressed as mean ± SDs. Where TES = testosterone, COR = cortisol, Glu = glucose, Bil = billirubin, Uric = uric acid, CK = creatine kinase, RF = atherosclerosis risk factor TC/HDL, Cer = ceruloplasmin, IgA/IgM = Immunoglobulins, GOT/GPT = the two transaminases, aspartate (AST) and alanine (ALT).

Testosterone had a high correlation with cortisol and glucose ($p < 0.01$). Cortisol was correlated to billirubin and glucose, while stress factor was correlated to billirubin and glucose ($p < 0.05$).

Immunoglobulin A was correlated to uric acid, while IgM to uric acid and CPK ($p < 0.05$).

HDL-C was negatively correlated to cortisol, risk factor to uric acid and cortisol and negatively to testosterone ($p < 0.05$).

CK was correlated to ceruloplasmin and GOT and GOT correlated to GPT ($p < 0.05$).

DISCUSSION

Speed driving is a form of acute physical exercise that mobilizes the expression of diverse physiological functions. Both fast response and anaerobic capacities are drawn together to cover the metabolic needs, while endurance must prevail keeping up the whole system as long as the race lasts.

The conditions under which this study was performed were extreme and were considered stressful enough to mobilize many of the defense factors under investigation.

Cortisol concentration declined until the end of the race, suggesting a general stress. It is known that peak values of the hormone start at 0800 h and display a series of 5 to 10 peak pulses within the first 5 h from awakening. Because the last sampling was taken within the first 5 h, the measurements taken fall within the peak time range. The decline in Cor levels after stress has also been observed in a previous work of ours using the Wingate test, which, like driving, is a resistance–power exercise (27). This Cor decline may indicate a response to stress of the hypothalamus–pituitary–adrenocortical axis or might be explained by an increased elimination and/or turnover rate of the hormone as the athletes came to rest.

It has long been recognised that living organisms are capable of inducing the antioxidative defense system, by relatively rapid mechanisms, to cope with oxidative stress. A physical exercise bout can cause an increase in oxygen consumption and oxidative stress.

Among the primary antioxidants ceruloplasmin increased at the end of the race. Ceruloplasmin increases—perhaps due to an increased liver production in response to exercise stress—have been also reported in a 20-day road race (6). Ceruloplasmin acts as an acute phase protein to increase antioxidative defenses (3).

From the secondary antioxidants billirubin, uric acid, and glucose remained unchanged. Plasma uric acid levels are also used as markers for ischemia and anaerobic stress (11,23). It is expected that exposure to repeated exercise will induce cellular antioxidative systems, as a protection against oxidative damage (7,24). The discrepancy of the response of the above factors may be a response discrepancy of the kind of exercise employed, or of the differences along the metabolic pathways of the factors themselves—as the turnover or the clearance speed of each one of them. Other investigators have also found no glucose increase at the end of the race (21).

The physiological significance of an exercise induction of antioxidative factors is not clearly understood at present. It can be conceived that an upregulation of these factors may offer a greater protection to the various tissues during exercise (5).

The levels of muscle mobilization and fatigue (enzyme CK) increased continuously and remained high after the end of the race; this was expected because the drivers were in a continuous movement during the driving. It seems that our results are in agreement with those reported from endurance exercise (21), that were measured during and after a 24-h relay race. We measured a sixfold increase, while they found a 14-fold one.

Among the two transaminases, main indicators of liver function and fatigue the levels of GOT were increased at phase 3, showing an increase in liver metabolism to cope with stressful conditions (25).

From other fatigue factors urea declined signifying a metabolic "arrest" in energy stress liquidation rather, than a catabolic recycling, because a rise of stress levels and concomittantly of cortisol decline, shunts energy towards muscles during the race.

Lipids did not change significantly, and the lipid risk factor (TC/HDL) remained unchanged, indicating a moderate aerobic metabolism. This was expected because in a strong aerobic exercise lasting for 4 h, as we have reported (26), TC remained almost unchanged and HDL changed only slightly.

Acute exercise under the conditions of the driving test did not seem to influence the immunological factors we measured. Perhaps more time was necessary for differences to be observed.

It seems that certain factors we measured above may be more responsive to the conditions of exercise employed, in which case it may be useful to select the more prominently responsive ones. Furthermore, the high correlations observed in Table 3 among fatigue and antioxidative factors and also stress and antioxidative factors, may be indicative of an interrelation among these defense systems themselves.

For this reason we believe that among the factors measured, ceruloplasmin, GOT, Cortisol, urea, and CK are indicative, because they may show part of the body's responsiveness to stress and fatigue and could possibly be used as related indices.

As a conclusion it can be said that an extreme acute 24 h driving bout, as expected, initiates the mobilization of factors participating in the stress and antioxidative systems, suggesting the mobilization of at least part of the systems themselves.

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