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The influence of psychological stress on total serum protein and patterns obtained in serum protein electrophoresis

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ABSTRACT

Background. Significant alterations in total serum protein (TSP) patterns obtained in serum protein electrophoresis and serum proteins have been reported in patients with major depression and in subjects submitted to a combination of psychological and physical stress. The aim of the present study was to examine the effects of academic examination stress, on TSP and patterns obtained in serum protein electrophoresis.

Methods. TSP and the concentrations and percentages of the major electrophoretically separated serum proteins were measured in 41 healthy biomedical students the day before a difficult academic examination (i.e. the stressful condition), as well as a few weeks before and after the stressful condition (i.e. two baseline conditions).

Results. Academic examination stress increased TSP and the $\alpha 1$, $\alpha 2$, β and γ concentrations in stress-reactors, but not in stress non-reactors (as defined by changes in the Perceived Stress Scale). Academic examination stress reduced the percentage of albumin in the stress-reactors, but not in stress non-reactors. There were significant positive relationships between the stress-induced changes in TSP and serum $\alpha 2$, β and γ concentrations and the stress-induced changes in the Perceived Stress Scale.

Conclusions. The results show that even mild psychological stress of short duration can lead to measurable changes in TSP and in patterns obtained in serum protein electrophoresis.

INTRODUCTION

There are some indications that psychological stress may be accompanied by changes in total serum protein (TSP), serum concentrations of the electrophoretically-separated proteins and acute phase proteins (APPs). First, major depression, an illness accompanied by chronic stress and increased stressor perception and triggered by subacute or chronic psychosocial stressors (Brown & Harris, 1989) is characterized by alterations in electrophoretically-separated serum protein fractions and an APP response. Song *et al*. (1994) and Maes *et al*. (1995, 1996) found lower TSP and serum albumin (Alb) concentrations and higher $\alpha 1$ and $\alpha 2$ globulin concentrations or percentages in depressed patients than in normal controls. We observed significantly lower TSP and serum Alb concentrations in patients with treatment resistant depression than in normal volunteers (Van hunsel *et al*. 1996). Major depression is also accompanied by increased plasma concen-

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trations of positive APPs, e.g. haptoglobin (Hp), α_1-antitrypsin (α_1 AT), ceruloplasmin (Cp) and α_1-acid glycoprotein (α_1 S), and lower levels of negative AP proteins, e.g. Alb and transferrin (Maes *et al*. 1991, 1992*a*, *b*, 1993; Joyce *et al*. 1992; Song *et al*. 1994). Thus, the changes in the patterns obtained in serum electrophoresis in depression may be explained by the migration of α_1 AT, α_1 S and α_1 AC in the α_1 zone, and of Hp and Cp in the α_2 zone (Putnam, 1984*a*, *b*).

Secondly, a combination of psychological and physical stress can induce changes in APPs similar to those observed during infection. Singh *et al*. (1991) examined a study group before and after a 5 day period of sustained physical and psychological stress, called the 'hell week'. It was found that this 'hell week' produced changes in APPs that are characteristic of an AP response, such as increased serum ferritin, Hp, Cp and creatinine kinase concentrations, and lower serum Alb concentrations.

Thirdly, there is evidence that psychological stress is accompanied by changes in TSP and the production of immunoglobulins (Igs). Muldoon *et al*. (1992) and Patterson *et al*. (1993) found that a brief experimental stressor increased TSP. McClelland *et al*. (1985) found that examination stress increased salivary IgA concentrations. Kiecolt-Glaser *et al*. (1984) found a significant increase in plasma IgA and a trend toward a significant increase in plasma IgM and IgG concentrations.

However, to the best of our knowledge, no papers have reported the effect of academic examination stress on patterns obtained in serum protein electrophoresis. The aim of the present study was to examine the effects of psychological stress, i.e. academic examination stress, on patterns obtained in serum protein electrophoresis. Based on the above results, it was hypothesized that academic examination stress could be accompanied by lower Alb and increased α1, α2, β and γ fractions.

METHOD

Subjects

The subjects taking part in this study were undergraduate students ($N = 41$, age: 19–22 years) attending the second year of biomedical sciences at the Rijks Universitair Centrum Antwerpen (RUCA), University of Antwerp, Belgium. The students were recruited by written advertisements. Our study population consisted of 14 females who were not taking contraceptive drugs, 14 females who had been taking contraceptive drugs (monophasic oestro-progestativa) for at least 3 consecutive months and 13 male students. Inducements for the participation included 3000 BF ($\pm$\$100). The study protocol was approved by the institutional review board of the RUCA, Antwerp, Belgium. All subjects gave informed consent after the study design was fully explained.

Exclusion criteria were: (*i*) medical, e.g. endocrine, immune, metabolic disorders, such as diabetes, autoimmune disorders, inflammatory bowel disease; (*ii*) currently (i.e. 2 weeks prior to the first blood sample) having suffered from an infectious, allergic or inflammatory response; (*iii*) ever having taken major psychotropic medications (e.g. antidepressants, anti-psychotics); (*iv*) drug abuse (alcohol and any other drug of dependence) or acquired immuno-deficiency syndrome; (*v*) a past or present history of psychiatric disorder; (*vi*) fainting due to blood drawing (consequently one subject was excluded); or (*vii*) tobacco use of more than 15 cigarettes/day (of the 41 participants there were only 6 subjects with tobacco use, i.e. 1·5, 2, 10, 10, 15 and 15 cigarettes/daily). Moreover, subjects were excluded in case they: (*i*) had abnormal baseline blood tests, such as liver tests (SGPT, SGOT, γGT), thyroid function tests (free thyroxine, free triiodothyronine, basal thyroid secreting hormone), BUN, WBC differentials and C-reactive protein concentrations; (*ii*) had a new major medical illness occurring during the study span; (*iii*) reported ingestion of new medical drugs during the study span, including psychostimulants or over the counter drugs, such as aspirin, the days before the blood samplings. The subjects abstained from caffeine and nicotine for at least 8 h before each session.

Experimental design

The students have two examination blocks during the academic year, i.e. the first in January–February (lasting 3 weeks) and the second in June–July (lasting 5 weeks). The students were examined the day before a difficult, oral exam during the first examination period,

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i.e. 15 January 1995–2 February 1995. All subjects had repeated measurements for serum proteins: blood was collected few weeks before (NEUTRAL PRE) and after (NEUTRAL POST) as well as the day before the examination (STRESS). In order to minimize sources of pre-analytical variation, blood collections were always performed in standardized conditions (Maes *et al*. 1994). The students came to the test room at the RUCA, Antwerp, after an overnight fast. At 9.00 a.m. ($\pm$45 min), in a 22–23 °C room, peripheral venous blood was collected by venepuncture through a catheter inserted in the antecubital vein. During the NEUTRAL PRE, STRESS and NEUTRAL POST periods, there were 2, 5 and 3 days, respectively, on which blood was sampled. On each occasion 55 ml of blood was taken for diverse biological assays. Serum (250 μl) for the assay of TSP and serum protein electrophoresis was stored at -70 °C. All blood specimens of all subjects were assayed in a single run with a single batch of reagents and consumables used by a single operator (K. B.).

All subjects completed the original 14-item Perceived Stress Scale (PSS) (Cohen *et al*. 1983) in the NEUTRAL PRE, STRESS and NEUTRAL POST conditions. The PSS is assumed to assess the perception of stress and is not merely a measure of psychological distress or symptomatology (Cohen *et al*. 1983). Although the questions in the PSS ask about feelings and thoughts during the last month, we adapted the PSS for the perceived stress during the last week. The subjects were divided in two groups on the basis of a higher increase on the PSS score, i.e. STRESS PSS scores minus the mean of the NEUTRAL PRE + POST PSS scores > 5. This method allows the subjects to be divided into stress reactors (with a Δ change in the PSS from NEUTRAL PRE to the STRESS condition of 12·3) *versus* stress non-reactors (with a Δ PSS change from NEUTRAL PRE to the STRESS condition of 0·1).

The students were asked a number of health-behaviour-related questions: (*i*) the number of alcoholic drinks during the last week (expressed in ml daily); (*ii*) the mean number of cigarettes/day the week before the assessments; (*iii*) use of psychostimulants; (*iv*) use of any medications; and (*v*) symptoms of infectious disease, allergic or inflammatory reactions. Since it was impossible to examine all female students during the NEUTRAL PRE/POST as well as STRESS conditions in a same phase of their menstrual cycle, we decided to control our results for the possible effects of the sex-hormonal state by entering follicle stimulating hormone (FSH), luteinizing hormone (LH) and oestradiol as covariates in repeated measures ANCOVAs. Since it is possible that stress-induced changes in serum protein concentrations may be influenced by stress-induced changes in blood viscosity, we decided to control our results for the possible effects of the haematocrit (Ht) by entering the latter as a covariate in repeated measure design ANCOVAs. The blood concentrations of caffeine were assayed during the three conditions and used as covariates in ANCOVAs.

Biochemical analysis

TSP and the electrophoretically separated serum protein fractions were determined as explained previously (Maes *et al*. 1995). TSP was assayed using a Vitros 750XRC analyzer (Johnson and Johnson Clinical Diagnostics, Beerse, Belgium). The inter-assay and intra-assay coefficients of variation were 2·01 % and 0·83 %, respectively. Electrophoresis was carried out with the Beckman Paragon SPE agarose system (Analis, Namur, Belgium) with densitometric quantitation of the protein fractions. The inter-assay and intra-assay CV values for the percentages of the protein fractions were: Alb, 3·05 % and 0·63 %; α1, 5·29 % and 1·84 %; α2, 7·05 % and 3·64 %; β, 9·03 % and 3·12 %; and γ, 8·87 % and 2·20 %, respectively. The Ht was determined by means of the Technicon H2 system (Brussels, Belgium). The inter-assay CV value obtained during the study period was 1·3 %. LH and FSH were determined by means of chemoluminescent immunoassays (ACS, Ciba-Corning). The analytical intra-assay CV values for LH and FSH were 3·8 % and 2·6 %, respectively. Oestradiol was determined by means of an enzyme-linked fluorescent immunoassay (Vidas, Bio-Merieux). The intra-assay CV value was 7·3 %. Both caffeine and amphetamines were determined by gas-chromatography with NPD detection after an extraction at alkaline pH (Demedts *et al*. 1996). The intra-assay CV value for caffeine was 3·2 %. Samples were checked for the presence of

amphetamines and analogues: amphetamine, methamphetamine, MDMA, MDA and MDE, ephedrine, pseudoephedrine, norephedrine, diethylproprion (+metabolites), fenfluramine+ norfenfluramine, and phentermine.

Statistics

Repeated measures analyses of variance (ANOVAs) were used to investigate: (*i*) the intra-individual variability with the NEUTRAL PRE/POST and STRESS effects; (*ii*) inter-individual variability with effects of gender/hormonal state (i.e. three groups: males, females without oral contraceptives, females with oral contraceptives), and stress reactor status (i.e. stress reactors *versus* non-reactors); and (*iii*) two-way or three-way interactions between time × gender/hormonal state, and time × stress responsivity; time × stress responsivity × gender/hormonal state. Repeated measures analyses of covariance (ANCOVAs) were employed in order to control for possible effects of alcohol use, blood concentrations of caffeine, the sex-hormonal state (FSH, LH, oestradiol) and blood viscosity (Ht). Multiple *post hoc* differences among group means were ascertained by means of Fisher's least significant difference (LSD). Group mean differences were checked by means of ANOVAs. The independence of classification systems was assessed by means of analysis of contingence (χ^2 test). Relationships between variables were assessed by means of Pearson's product moment correlation coefficients or through multiple regression analysis. Time-relationships were computed by means of intraclass regression analyses, pooled over the repeated measurements in the 41 subjects (this method eliminates the inter-individual variability).

RESULTS

Serum protein assays in the stress condition

Table 1 shows the measurements of the PSS, TSP and the serum protein percentages and concentrations in the NEUTRAL PRE/POST and STRESS conditions. If there were significant interaction patterns between time × stress responsivity, the measurements are shown both in stress reactors and non-reactors. If there was no significant interaction between time × stress responsivity, both above groups were combined. The PSS score was significantly higher in the STRESS condition than in the NEUTRAL PRE and POST conditions. The NEUTRAL POST PSS scores were significantly lower than the NEUTRAL PRE values. According to the criteria of stress responsivity, 15 subjects were stress reactors, whereas 26 were stress non-reactors.

We found higher TSP and serum Alb, $\alpha 1$, β and γ concentrations in the STRESS than in the NEUTRAL PRE and POST conditions. The stress-induced responses in TSP and serum $\alpha 1$, $\alpha 2$, β and γ concentrations were significantly higher in stress reactors than in non-reactors. There were also significant interactions between time × stress responsivity in the case of the percentage of Alb (lower in the STRESS condition in stress reactors), and the $\alpha 2$, β and γ percentages (all higher in the STRESS condition in stress reactors than in non-reactors).

Relationships between PSS and serum proteins

Intraclass regression analysis (pooled over the subjects and taking into account the three conditions) showed significant time-relationships between the PSS and TSP ($r = 0{\cdot}37$, $P = 0{\cdot}001$) and serum Alb ($r = 0{\cdot}24$, $P = 0{\cdot}03$), $\alpha 2$ ($r = 0{\cdot}28$, $P = 0{\cdot}01$), β ($r = 0{\cdot}35$, $P = 0{\cdot}002$) and γ ($r = 0{\cdot}39$, $P = 0{\cdot}0007$), but not $\alpha 1$ ($r = 0{\cdot}19$, $P = 0{\cdot}09$), concentrations. There were no significant time-relationships between the PSS and the Alb ($r = -0{\cdot}19$, $P = 0{\cdot}1$), $\alpha 1$ ($r = -0{\cdot}01$, $P = 0{\cdot}9$), $\alpha 2$ ($r = 0{\cdot}09$, $P = 0{\cdot}6$) and β ($r = 0{\cdot}13$, $P = 0{\cdot}2$) percentages. There was a positive time-relationship between the PSS and the γ percentage ($r = 0{\cdot}25$, $P = 0{\cdot}03$). The above time-relationships, however, may underestimate the actual relationships between stress-induced perceived stress and serum proteins, since the latter return to pre-stress levels, while the NEUTRAL POST PSS scores are significantly lower than the NEUTRAL PRE PSS scores. Therefore, we have recomputed the time-relationships between the PSS and serum proteins by taking into account the NEUTRAL PRE and STRESS conditions only. The time-relationships between the PSS and the serum protein concentrations, which take into account these two conditions, were similar or stronger than those described above, i.e. TSP ($r = 0{\cdot}52$, $P = 0{\cdot}0008$), Alb ($r = 0{\cdot}49$, $P = 0{\cdot}001$), $\alpha 1$ ($r = 0{\cdot}29$, $P = 0{\cdot}06$), $\alpha 2$ ($r =$

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Table 1. *Measurements of the Perceived Stress Scale* (*PSS*), *serum total protein* (*TSP*) *and the electrophoretically separated protein concentrations and percentages in* 41 *university students the day before a difficult examination* (*STRESS*), *a few weeks earlier* (*NEUTRAL PRE*) *and later* (*NEUTRAL POST*); *the subjects were divided into stress reactors* (*R*) *and non-reactors* (*NR*)

Variables	NR/R	NEUTRAL PRE	STRESS	NEUTRAL POST	*F*1	df	*P*	*F*2	*P*
PSS	NR	25·6 (6·9)	25·7 (5·4)	18·9 (8·0)	91	2,75	$< 10^{-4}$	27·7	$< 10^{-4}$
	R	24·2 (7·2)	36·5 (6·4)	16·3 (6·4)	—	—	—	—	—
TSP (g l^{-1})	NR	78·4 (5·0)	79·3 (4·4)	78·2 (4·6)	7·9	2,73	0·001	3·6	0·03
	R	76·5 (5·2)	81·3 (6·8)	76·8 (3·3)	—	—	—	—	—
Alb (%)	NR	60·9 (3·3)	61·6 (3·1)	60·3 (3·4)	0·3	2,75	0·8	7·1	0·002
	R	59·1 (3·0)	58·3 (3·3)	60·3 (3·0)	—	—	—	—	—
Alb (g l^{-1})	NR + R	46·8 (3·6)	48·3 (3·5)	46·8 (3·5)	4·9	2,75	0·009	—	—
α1 (%)	NR + R	3·38 (0·62)	3·45 (0·62)	3·43 (0·63)	0·4	2,77	0·7	—	—
α1 (g l^{-1})	NR	2·65 (0·44)	2·69 (0·48)	2·69 (0·46)	4·7	2,73	0·01	3·7	0·03
	R	2·60 (0·64)	2·88 (0·59)	2·61 (0·52)	—	—	—	—	—
α2 (%)	NR	9·45 (1·62)	9·58 (1·41)	9·89 (1·24)	0·6	2,75	0·6	8·7	0·0007
	R	10·21 (1·19)	10·12 (1·37)	9·58 (1·48)	—	—	—	—	—
α2 (g l^{-1})	NR	7·42 (1·40)	7·28 (1·14)	7·72 (0·96)	1·6	2,73	0·2	9·5	0·0004
	R	7·79 (1·02)	8·26 (1·49)	7·34 (1·08)	—	—	—	—	—
β (%)	NR	12·7 (1·2)	12·4 (1·3)	12·8 (1·2)	1·3	2,75	0·3	5·1	0·009
	R	13·5 (1·2)	13·4 (1·2)	13·1 (1·3)	—	—	—	—	—
β (g l^{-1})	NR	9·93 (1·07)	9·82 (1·15)	9·96 (0·95)	3·8	2,73	0·03	6·6	0·003
	R	10·26 (1·13)	10·92 (1·38)	10·03 (0·98)	—	—	—	—	—
γ (%)	NR	13·5 (2·3)	13·4 (2·3)	13·6 (2·4)	1.6	2,75	0·2	4·3	0·02
	R	13·8 (2·0)	14·6 (2·3)	13·7 (1·3)	—	—	—	—	—
γ (g l^{-1})	NR	10·67 (2·27)	10·66 (2·14)	10·68 (2·27)	7·9	2,73	0·001	8·3	0·0009
	R	10·65 (1·98)	11·91 (2·46)	10·53 (1·27)	—	—	—	—	—

All results are given as mean (±S.D.).

*F*1, *F*2, all results of repeated measures ANOVAs, with effects of time, i.e. all subjects being analysed together (*F*1) and time × stress-reactor status (*F*2).

0·27, $P = 0·08$), β ($r = 0·37$, $P = 0·01$) and γ ($r = 0·49$, $P = 0·001$).

Effects of background variables

Effects of blood viscosity

Ht was significantly higher in the STRESS (40·5 ± 2·7 %) than in the NEUTRAL PRE (38·5 ± 2·8 %) and NEUTRAL POST (39·8 ± 2·9 %) conditions ($F = 17·1$, df = 2, 77, $P < 10^{-4}$). Intraclass regression analysis, showed significant time-relationship between Ht and TSP ($r = 0·52$, $P < 10^{-4}$) and serum Alb ($r = 0·48$, $P < 10^{-4}$), α2 ($r = 0·28$, $P = 0·01$), β ($r = 0·37$, $P = 0·001$) and γ ($r = 0·36$, $P = 0·001$), but not α1 ($r = 0·20$, $P = 0·07$), concentrations. In the STRESS condition, there were significant relationships between Ht and TSP ($r = 0·32$, $P = 0·04$) and serum Alb ($r = 0·49$, $P = 0·001$), but not between Ht and serum α1 ($r = 0·08$, $P = 0·6$), α2 ($r = -0·19$, $P = 0·2$), β ($r = 0·11$, $P = 0·5$) and γ ($r = 0·05$, $P = 0·7$) concentrations. Since it is possible that the increased concentrations of TSP and protein concentrations are a result of changes in blood viscosity, we have adjusted the results of Table 1 for possible effects of blood viscosity, by introducing Ht as a covariate in repeated measures ANCOVAs.

After considering the effect of Ht in an ANCOVA, the time effect on TSP ($F = 4·8$, df = 2,73, $P = 0·01$) remained significant, while there was a trend toward a significant time × stress responsivity interaction ($F = 2·6$, df = 2, 73, $P = 0·08$). After considering the effects of stress-induced increments in Ht, we found that the significant time-effects in serum Alb concentrations had disappeared ($F = 1·7$, df = 2,76, $P = 0·2$). After considering the effects of stress-induced increments in Ht, the significant effects of time (*F*1) and time × stress reactor status (*F*2) remained significant for serum α1 ($F1 = 6·3$, df = 2,73, $P = 0·003$; $F2 = 4·4$, $P = 0·01$), α2 ($F1 = 4·5$, df = 2,73, $P = 0·01$; $F2 = 12·5$, $P < 10^{-4}$), β ($F1 = 4·2$, df = 2,73, $P = 0·02$; $F2 = 7·0$, $P = 0·002$) and γ ($F = 13·0$, df = 2,73, $P < 10^{-4}$; $F2 = 11·2$, $P < 10^{-4}$) concentrations. After considering the effects of Ht (as an additional explanatory variable in multiple regression analyses), we found significant time-relation-

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ships between the PSS and TSP ($F = 5{\cdot}8$, $P = 0{\cdot}01$) and serum $\alpha 2$ ($F = 3{\cdot}8$, $P = 0{\cdot}05$), β ($F = 6{\cdot}1$, $P = 0{\cdot}01$) and γ ($F = 8{\cdot}5$, $P = 0{\cdot}005$), but not with serum Alb ($F = 0{\cdot}9$, $P = 0{\cdot}7$) or $\alpha 1$ ($F = 1{\cdot}5$, $P = 0{\cdot}2$) concentrations.

Caffeine and alcohol

There were no significant time-relationships between serum proteins and use of alcohol and caffeine (results of multiple regression analyses pooled over the subjects and taking into account the three conditions), i.e. TSP ($F = 1{\cdot}6$, df = 2, 74, $P = 0{\cdot}2$), serum Alb ($F = 1{\cdot}3$, df = 2,74, $P = 0{\cdot}3$), $\alpha 1$ ($F = 0{\cdot}7$, df = 2,74, $P = 0{\cdot}5$), $\alpha 2$ ($F = 0{\cdot}6$, df = 2,74, $P = 0{\cdot}5$), β ($F = 0{\cdot}6$, df = 2, 74, $P = 0{\cdot}6$) and γ ($F = 1{\cdot}4$, df = 2,74, $P = 0{\cdot}3$) concentrations. Nevertheless, since the use of alcohol was significantly lower ($F = 12{\cdot}3$, df = 2,75, $P = 0{\cdot}0001$) in the STRESS ($32{\cdot}5 \pm 70$ ml/day) than in the NEUTRAL PRE ($120 \pm 187{\cdot}5$ ml/day) and POST (125 ± 315 ml/day) conditions and since there was a trend ($F = 2{\cdot}5$, df = 2, 74, $P = 0{\cdot}09$) toward increased serum caffeine concentrations in the STRESS ($1{\cdot}61 \pm 2{\cdot}53$ mg/l) than in the NEUTRAL PRE ($1{\cdot}08 \pm 1{\cdot}93$ mg/l) and POST ($1{\cdot}00 \pm 1{\cdot}65$ mg/l) conditions, possible effects of these variables were ruled out by means of ANCOVAS. All significant effects of time ($F1$) and time × stress-reactor status ($F2$) established in Table 1 remained significant after considering possible effects of alcohol use and serum caffeine concentrations (entered as covariates in repeated measures ANCOVAs), i.e. TSP ($F1 = 6{\cdot}8$, df = 2,72, $P = 0{\cdot}002$; $F2 = 3{\cdot}2$, $P = 0{\cdot}04$), $\alpha 1$ ($F = 1{\cdot}1$, df = 2,72, $P = 0{\cdot}3$; $F2 = 3{\cdot}3$, $P = 0{\cdot}04$), $\alpha 2$ ($F2 = 10{\cdot}4$, df = 2,72, $P = 0{\cdot}0003$), β ($F1 = 0{\cdot}8$, df = 2,72, $P = 0{\cdot}6$; $F2 = 5{\cdot}0$, $P = 0{\cdot}009$) and γ ($F1 = 4{\cdot}2$, df = 2,72, $P = 0{\cdot}003$) concentrations.

Sex-hormonal state

There were no significant interactions between time × sex-hormonal states (i.e. males and females with and without anticontraceptive drugs) in the following serum proteins: TSP ($F = 1{\cdot}3$, df = 4,69, $P = 0{\cdot}3$), serum Alb ($F = 0{\cdot}5$, df = 4,71, $P = 0{\cdot}7$), $\alpha 1$ ($F = 0{\cdot}8$, df = 4,69, $P = 0{\cdot}05$), $\alpha 2$ ($F = 0{\cdot}8$, df = 4, 69, $P = 0{\cdot}5$) and β ($F = 1{\cdot}7$, df = 4,69, $P = 0{\cdot}2$) concentrations. There was a significant interaction between time and the sex-hormonal status for the γ concentrations ($F = 3{\cdot}6$, df = 4,69, $P = 0{\cdot}01$), although the three-way interaction time × sex-hormonal state × stress reactor status was not significant ($F = 0{\cdot}6$, df = 4,65, $P = 0{\cdot}6$).

All above significant effects of time ($F1$) and time × stress reactor status ($F2$) remained significant after controlling for the putative effects of the sex-hormonal state (by entering FSH, LH and estradiol serum levels as covariates in ANCOVAs), i.e. TSP ($F1 = 8{\cdot}0$, df = 2,71, $P = 0{\cdot}001$; $F2 = 4{\cdot}0$, $P = 0{\cdot}02$), $\alpha 1$ ($F1 = 6{\cdot}6$, df = 2, 71, $P = 0{\cdot}003$; $F2 = 4{\cdot}6$, $P = 0{\cdot}01$), $\alpha 2$ ($F2 = 13{\cdot}5$, df = 2,71, $P < 10^{-4}$), β ($F = 3{\cdot}9$, df = 2, 71, $P = 0{\cdot}02$; $F2 = 8{\cdot}1$, $P = 0{\cdot}001$) and γ ($F = 7{\cdot}2$, df = 2,71, $P = 0{\cdot}002$; $F2 = 7{\cdot}3$, $P = 0{\cdot}002$) concentrations.

DISCUSSION

The results of the present study show that: (*i*) academic examination stress significantly increased TSP and serum $\alpha 1$, $\alpha 2$, β, and γ concentrations and decreased the percentage of Alb in students with high-stress perception, but not in these with low-stress perception; and (*ii*) that there were significant and positive correlations between the stress-induced increases in TSP and serum $\alpha 2$, β, and γ concentrations and the changes in perceived stress. These findings support the contention that TSP and patterns obtained in serum protein electrophoresis are sensitive to graded differences in the perception of stressor severity.

The findings of the present study extent those of previous reports showing that psychological stress significantly increased TSP, some AP proteins migrating in the $\alpha 1$ and $\alpha 2$ zones, and serum Ig concentrations. Thus, Singh *et al.* (1991) found that a week of sustained psychological and physical stress may induce increases in plasma AP proteins, such as Hp, ferritin, Cp, and CRP (migrating in the $\alpha 1$, $\alpha 2$ and β zones) and lowered those of Alb. However, it was not clear from that paper (Singh *et al.* 1991) whether these changes in plasma proteins should be ascribed to the effects of physical stress rather than to psychological stress. Indeed, it is known that physical stress due to exertion may induce an APR in humans (Liesen *et al.* 1977; Dufaux *et al.* 1983). Our results are also in accordance with those of Muldoon *et al.* (1992) and Patterson *et al.* (1993) who found that a brief experimental stressor increased TSP. Our

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findings that academic examination stress increases the percentage and concentration of serum γ globulin are in agreement with previous papers, showing that psychological stress may increase plasma levels of Igs. Kiecolt-Glaser *et al.* (1984) found that psychological stress significantly increased plasma IgA and caused a non-significant increase in IgM and IgG. Indeed, Igs migrate electrophoretically in the γ electrophoretical zone, i.e. the γ fraction consists of IgA, IgM and, in the main, IgG (Ritzmann & Daniels, 1976; Putnam, 1984*a*, *b*). McClelland *et al.* (1985) found that academic examination stress increased salivary IgA concentrations, which are a measure of B-cell immune function. Therefore, our results are in agreement with previous papers showing an increased number of B lymphocytes during psychological stress (Dorian *et al.* 1982). Jemmott *et al.* (1983), on the other hand, found that the secretion of salivary IgA was significantly lower during periods of examination stress than at low stress periods.

There is now some evidence that acute experimental stressors and academic examination stress may increase Ht and blood viscosity (Ibister, 1987; Muldoon *et al.* 1992; Patterson *et al.* 1993). Matthews *et al.* (1995) and Muldoon *et al.* (1995) explained these findings by stress-induced changes in cell-volume distribution of red blood cells, through a fluid shift out of the intravascular space, concentrating non-diffusible blood constituents. In this respect, we found that the stress-induced changes in Ht were significantly and positively correlated with the stress-induced changes in TSP and serum Alb, $\alpha 2$, β and γ concentrations. Moreover, the present study showed that stress-induced increases in serum Alb may be attributed to stress-induced increases in blood viscosity. Another factor that could have influenced the serum proteins measured here is the effect of repeated blood samplings. However, because of the pattern of changes (e.g. increase in stress reactors only; and normalization some weeks after the stressful condition) it seems unlikely that this is a significant factor.

The stress-induced alterations in the serum proteins measured here may be related to a cytokine-induced AP response, increased secretion of catecholamines and/or increased activity of the hypothalamic–pituitary–adrenal axis. An AP response is the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, e.g. infection, trauma, inflammation, neoplastic growth, immunological disturbances, exertion or affective and psychotic disorders (Kushner, 1982; Heinrich *et al.* 1990; Khansari *et al.* 1990; Baumann & Gauldie, 1994; Maes *et al.* 1997). An APR is accompanied by increased and decreased plasma concentrations of positive and negative AP proteins, respectively, and by increased plasma concentrations of $\alpha 1$ and $\alpha 2$ proteins. Cytokines, in particular interleukin-1 (IL-1) and IL-6, stimulate APP gen-expression and production in hepatocytes (Heinrich *et al.* 1990; Baumann & Gauldie, 1994). Psychological stress may increase plasma IL-6 in the rodent (LeMay *et al.* 1990). A significant positive correlation between plasma CRP and IL-6 has been reported following acute psychological stress in normal volunteers (Dugué *et al.* 1993). These studies suggest that an increased production of pro-inflammatory cytokines, such as IL-6, may mediate the effects of psychological stress on serum proteins. Van Gool *et al.* (1984) reported that a single injection of adrenalin in the rodent elicits high levels of $\alpha 2$ macroglobulin, another APP. Van Gool *et al.* (1990) hypothesized that since a single injection of adrenalin in the rat caused a pattern similar to that of an APR, some of the effects of adrenalin can be explained by its effect on IL-6. Moreover, glucocorticoids are known to potentiate cytokine-induced APP production in hepatocytes, while corticotrophin-releasing hormone is another possible mediator of the AP response in experimental animals (Hagan *et al.* 1993). It is known that during an AP response, cytokine and adrenocortical secretion is stimulated, with consequent increases in serum glucocorticoids and activation of the sympathetic nervous system, followed by a release of catecholamines (Khansari *et al.* 1990). Therefore, it may be hypothesized that academic examination stress induces the production/secretion of pro-inflammatory cytokines, glucocorticoids and catecholamines and, consequently, induces a moderate APP response.

In conclusion, academic examination stress increased serum concentrations of TSP, and serum $\alpha 1$, $\alpha 2$, β, and γ concentrations and induced a significant decrease in the percentage of Alb. These findings may suggest that psycho-

logical stress induces a moderate AP protein response in normal humans.

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