RELATIONSHIP OF EXAMINATION STRESS TO SERUM LIPID PROFILE

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Summary : Although mental stress as well as hypercholesterolaemia have been individually linked with atherosclerosis, the relationship between mental stress and hypercholesterolaemia is poorly understood. Serum lipid profile was studied in eight male medical student volunteers before, near and after examinations. Identical observations were also made on seven well-matched control volunteers. As compared to pre-exam levels, total serum cholesterol (T-C) increased significantly ($P<0.05$) near exams, and so did low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). The HDL-C/T-C and HDL-C/LDL-C ratios remained essentially constant throughout the study. Control subjects did not show any significant change in serum lipid profile. Further serial measurement in five of the subjects revealed that examination-related changes were transient. Moreover, a second examination after about 40 days did not evoke any change in the lipid profile. The response to examination stress may be related to the enhanced utilisation of cholesterol in the adrenal cortex for steroidogenesis.

Key words : stress lipoproteins cholesterol

INTRODUCTION

Mental stress has been widely incriminated in the aetiology of hypertension and atherosclerosis. Individuals with a restless and achievement-oriented personality (type A) are more prone to atherosclerosis (17). The biochemical feature which has attracted the most sustained and widespread attention in relation to aetiology, prevention as well as treatment of atherosclerosis is serum cholesterol or its fractions (7, 9, 17), and hypercholesterolaemia is now recognized as a major risk factor for coronary artery disease

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(10). There are, however, relatively few studies seeking a connection between mental stress and serum lipid profile (5, 11, 12, 14.) In the course of our long term nutritional studies on medical student volunteers, we observed that blood samples drawn towards the end of the academic term showed higher serum cholesterol levels in volunteers who were close to a final examination. Careful analysis of the data revealed that there was a significant rise in total serum cholesterol, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) 1-3 wk before the examination as compared to observations made 5-8 wk before or 6-12 wk after the exams. However, there was no significant change in HDL-C/Cholesterol ratio associated with examination stress (1). We have continued to make observations from this angle in our subsequent nutritional studies, the pooled results of which form the subject of the present report.

MATERIAL AND METHODS

Examination stress was studied in eight healthy young male medical student volunteers (age 19-22 yr, weight 47-66 *kg*). Two of these volunteers were studied during August-September 1982, and six during August-September 1983. The volunteers had their pre-professional examinations in October and professional examinations in December. The observations were commenced in August and the effect of stress was investigated in September, 3 wk before the pre-professional examination. In five of the volunteers, the observations were also made in November 1983, 1 wk before the professional examination, and in January 1984, 4-6 wk after the professional examination. As controls, seven volunteers (age 18-22 yr, weight 47-61 *kg*) were studied during August-September 1982. The controls were comparable to the exam-going volunteers not only in age and weight but also in terms of their lipid profiles; in fact two of the volunteers who served as controls in August 1982, became the exam-going volunteers in August 1983. During the 4-wk interval between August and September, exam-going as well as control volunteers were on the same nutritional study, and were thus on identical diets. The diet was consumed in the hostel and was reasonably constant from day to day. Its components and average composition were as follows : breakfast, 4 slices of bread, 10 g butter, 1 egg, 1 tea; lunch, 2 buckwheat *paronthas*, 1 helping of *dal* 1 helping of vegetable, 1 helping of curd, a small quantity of salad; evening, 2 slices of bread with 10 g butter or a snack, 1 tea; dinner, 2 chapaties, 1 helping of rice, 2 helpings of dal, 1 helping of vegetable, 1 helping of sweetdish, a small quantity of salad; total energy content, about 3,000 kcal. Our experimental as well as control subjects indulged in only mild to moderate physical activity, and they were requested not to alter it during the study. In spite of the request, however, three of the volunteers reported a decline in physical activity close to the examination. Only one of the volunteers smoked, and reported an increase in smoking from his usual

1 cigarette per day to 6-7 cigarettes per day when under examination stress. None of our volunteers consumed alcohol on a regular basis.

The parameters studied were serum lipid profile and arterial blood pressure. The lipid levels reported here are the average of two samples taken on consecutive days. Lipoprotein fractions were separated by the dual precipitation method (16) and cholesterol was determined by the ferric chloride method (3). The normal lipoprotein profile of an Indian population, as determined by these techniques in our laboratory, has been reported earlier (6).

All the volunteers gave their informed written consent for participation in the study. The experimental design and the procedures followed were in accord with the ethical standards laid down for human studies by our Institute and the Indian Council of Medical Research.

RESULTS

The serum lipid profile changes in exam-going volunteers and controls at corresponding periods of the year while on an identical diet in the intervening period are shown in Fig. 1. For exam-going subjects, the August observations were made well before the exams (about 7 wk before), and September observations were close to the exams (3 wk before the pre-professional exams). Total serum cholesterol (T-C) increased significantly near the exams, and so did low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). Since T-C, HDL-C as well as LDL-C were elevated near the exams, there was no significant change in HDL-C/T-C or HDL-C/LDL-C ratio. Control subjects did not show any significant change in serum lipid profile.

Serial measurement of serum lipids on 5 subjects (Fig. 2) revealed a return to essentially control levels in November, and no further change was observed in January the following year.

Among our volunteers, there were two subjects who participated in the August 1982 as well as August 1983 study. In 1982, they had no professional examination and served as controls, whereas in 1983, they had a professional examination. In view of their small number, looking at their results in isolation may not have much value from a statistical

angle but doing so is interesting and instructive since these subjects provide the ideal situation of being their own controls. These subjects also showed marked elevations in T-C, LDL-C and HDL-C only during the examination phase (Table I), as did the other volunteers, thus strengthening the validity of the observations.

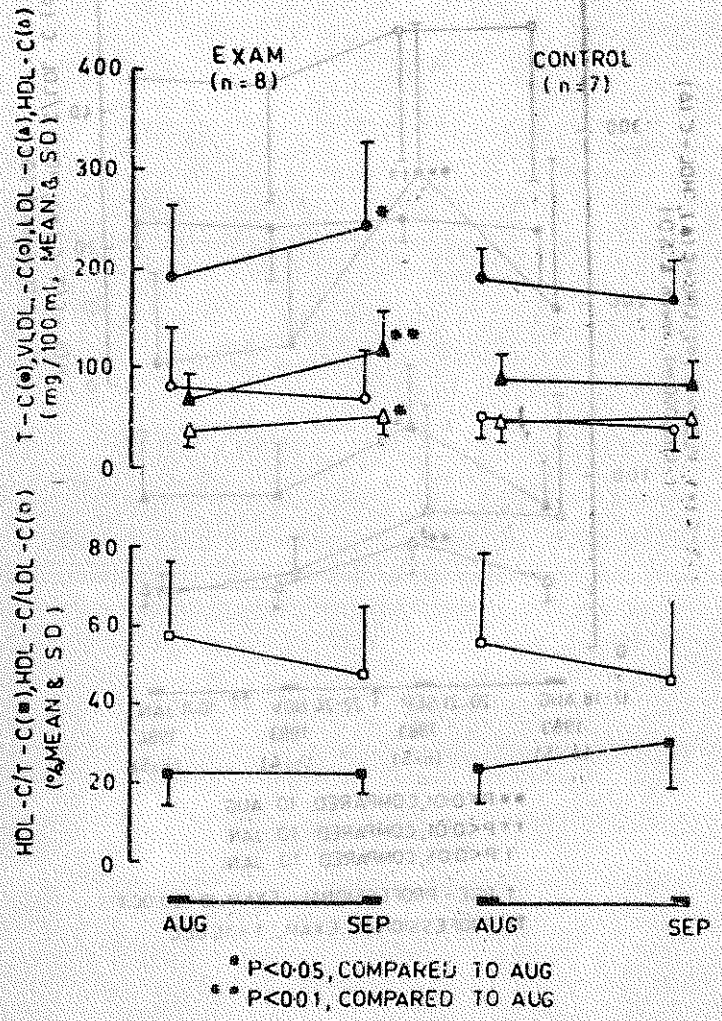

Fig. 1 : Lipid profile in exam-going and control volunteers at identical periods of the year on identical diets. For exam-going volunteers, the August sample was taken 7 wk, and the September sample 3 wk before the examinations.

There were no appreciable or consistent changes in arterial blood pressure or body weight associated with examinations.

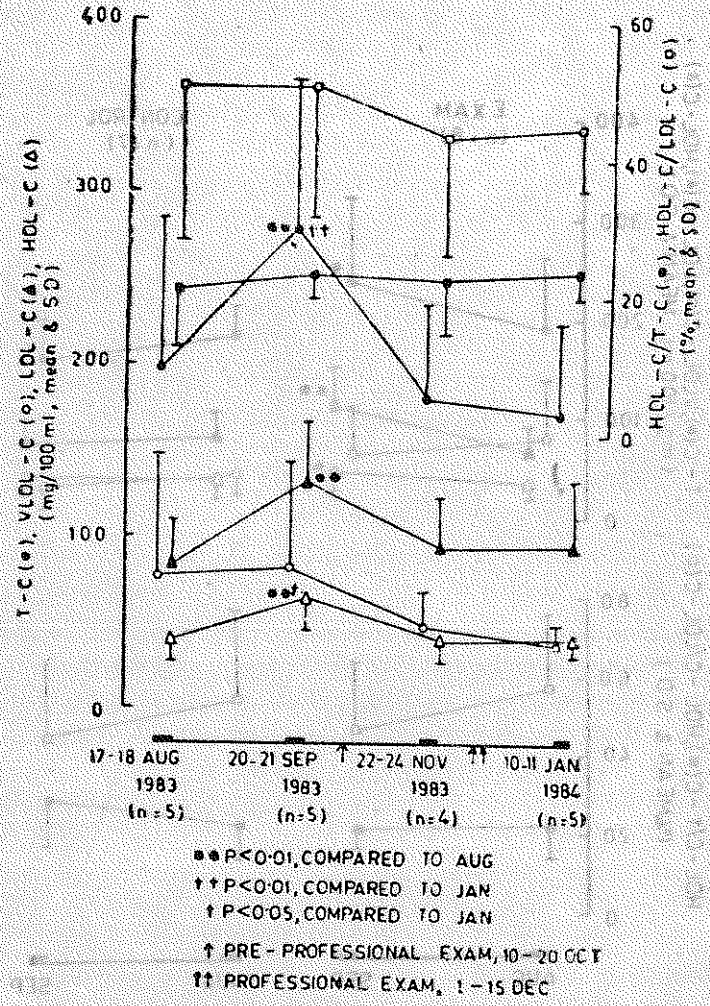

Fig. 2 : Serial estimation of lipid profile in five exam-going volunteers. The rise in T-C, LDL-C and HDL-C in September seems to be related to the examination in October. Note that the lipoprotein levels are back to control values in November, and have not gone up in spite of a second examination due in December.

TABLE I: Examination-related changes in lipid profile in two volunteers who served as their own control.

Parameter (mg/100 ml)	Subject	Control phase (1982)		Examination phase (1983)	
		August	September	August	September
T-C	A N	207	154	161	268
	A B	240	235	335	420
VLDL-C	A N	52	32	53	59
	A B	64	58	204	139
LDL-C	A N	125	85	76	146
	A B	126	128	74	134
HDL-C	A N	30	38	31	63
	A B	50	49	57	96

T-C, total cholesterol; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

DISCUSSION

There is a rise in total serum cholesterol as well as LDL-C and HDL-C in a sample collected 3 wk before the commencement of the pre-professional examination. Another sample taken 4 wk after the pre-professional examination but 1 wk before the professional examination shows that the serum lipoprotein profile has returned to control levels. This does not necessarily mean that the professional examination is less stressful than the pre-professional examination. The stress of the professional examination would depend partly on how the candidate has fared in the pre-professional examination. What the results indicate, however, is that serum lipid changes induced by stress are transient, and that a second stress within a short period fails to evoke the lipoprotein response. The control level is maintained in a sample taken about 4 wk after the end of the professional examination. Rise in serum cholesterol in association with examination stress (1, 4, 14, 15) and other forms of acute stress (11, 12) has been reported earlier. The time relations of the serum cholesterol changes in various studies have been compared in Table II. There is general agreement that rise in serum cholesterol may be detected 3 wk before the commencement of examinations, and may disappear as early as a week after the examinations. The discrepancy in the study by Francis (4) appears to be because he has related the lipoprotein changes to an objective measurement of psychological stress rather

than the date of the examination. Stress could start weeks before the examination, and serum cholesterol may rise only 10 days after the tension builds up to a reasonably high level. These studies also indicate that if a second stressful event follows within 40 days of the first one, the rise in cholesterol may be much less or absent.

TABLE II : Time-relations of examination-related changes in plasma cholesterol.

Study	*Time before exams when rise in serum cholesterol observed*	*Time after exams when fall in serum cholesterol to control level observed*	*Effect of second stress within a short time*
Thomas and Murphy (14)	16 days	3 wk	Comparable rise in cholesterol (2nd stress 70 days after 1st stress)
Wertlake *et al.* (15)	1 wk	1 wk	Not studied
Francis (4)	10 days after peak stress	20 days after peak stress	Rise in cholesterol much less than with 1st stress (2nd stress 40 days after 1st stress)
Bijlani *et al.* (1)	3 wk	6-12 wk	Not studied
Present study	3 wk	4 wk	No response (2nd stress 40 days after 1st stress)

The mechanism whereby stress raises serum cholesterol is uncertain. It is likely to be intimately related to the fact that during prolonged stress, ACTH and adrenal glucocorticoid secretion is increased (13). That this happens also during examination stress is suggested by the fall in eosinophil count observed by Thomas and Murphy (14). Cholesterol is the precursor for glucocorticoid synthesis, and, both at rest and under ACTH stimulation, about 80% of it is derived from plasma and only about 20% from endogenous synthesis in adrenal cortical cells (2). ACTH depletes adrenal cholesterol, making steroidogenesis critically dependent on plasma cholesterol. Depending upon the experimental preparation used, LDL or both LDL and HDL cholesterol is used by the adrenal cortex for steroidogenesis (8). How LDL and HDL cholesterol get mobilised in stress to perform this important function is a question which does not seem to have been addressed by any experimental study so far. Closely related to this mechanism would be

the explanation for the observation that if a second stressful period closely follows the first, the cholesterol response to it is weak or absent. This observation is consistent with the fact that if an individual is repeatedly exposed to the same stressful stimulus, the adrenal soon shows no response (13). This may represent either an exhaustion of the metabolic response, or an adaptation to the stress.

Regarding the effect of stress on individual lipoprotein fractions, Francis (4) found that while LDL-C values parallel T-C values, HDL-C values stay essentially constant. In contrast, in our earlier study (1) as well as the present one, HDL-C values have also changed in the same direction and to the same extent as total cholesterol and LDL-C. Therefore, while Francis (4) found a fall in HDL-C/T-C ratio during stress, we have found no sig nificant change in the ratio. The reason for this discrepancy is not clear, but could reflect genetic differences. It may be related to the fact whether the adrenal cortex uses cholesterol from LDL-C, or both LDL-C and HDL-C, as in different experimental preparations (8).

LDL-C is now well recognized as a risk factor for, and HDL-C as a protective factor against atherosclerosis (7). In our studies, we have consistently found a rise in both these fractions in relation with examinations. Since the ratios HDL-C/T-C and HDL-C/LDL-C have not changed, the lipoprotein changes *per se* do not predict any change in susceptibility of an individual to atherosclerosis as a result of examination-related stress. The transient nature of the changes in spite of continuing stress further diminishes the possibility of any change in susceptibility to a disease that depends on the cumulative effect of multiple factors. The two type of responses to stress, viz. a fall in HDL-C/T-C (4) or no change in the ratio (our studies) may also explain part of the individual difference in vulnerability to stress.

No change was observed in blood pressure during the stressful period. Thomas & Murphy (14), however, observed an increase in the blood pressure in relation to examinations. The discrepancy may be because Thomas & Murphy made their observations on the days of the examinations immediately after the examination, while our observations were made 3 wk before the pre-professional, and 1 wk before the professional examination. Thus the change in blood pressure seems to be an acute change coinciding closely with the examination itself.

There are thus reasonably consistent changes in lipid profile associated with examination stress. The mechanisms underlying these changes and their pathophysiological implications, if any, need further exploration.

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