Plasma Lipids and Urinary Excretion of Catecholamines in Man during Experimentally Induced Emotional Stress, and Their Modification by Nicotinic Acid

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ABSTRACT 33 male volunteers were studied in the morning after fasting overnight. 11 (the control group) were allowed to sit comfortably for three consecutive 2-hr periods, no stressors or treatment being introduced. The remaining 22 were divided into two groups, each being exposed to standardized, emotional stressors during the second of the three 2-hr periods. The subjects in one of these groups were each given a total dose of 3 g of nicotinic acid during the first 3 hr of the experiment, whereas the other group received no treatment.

Stress was accompanied and followed by increased levels of free fatty acids and triglycerides in arterial plasma, by an increase in catecholamine excretion, and a rise in heart rate and systolic and diastolic blood pressure. No such increases were seen in the control group.

The stress-induced rise in free fatty acids was inhibited by nicotinic acid, and the triglyceride rise was turned into a fall. The stressor-induced increase in catecholamine excretion was not significantly affected by nicotinic acid, neither were the increases in heart rate and blood pressure.

The hypothesis is discussed, from a qualitative as well as a quantitative viewpoint, that there is a direct relationship between the increased concentration of free fatty acids accompanying emotional stress in man and the eventual development of the stress hyperlipoproteinemia.

Received for publication 15 November 1967 and in revised form 29 February 1968.

INTRODUCTION

Chronic exposure of man to various emotional stressors leads to increased plasma concentration of cholesterol (1–4) and very low density lipoproteins (5). Little is known about the mechanism of this stress-induced hyperlipoproteinemia. The relevant data may be summarized as follows.

Emotional stress is accompanied by a considerable increase in the urinary excretion of catecholamines, probably reflecting a corresponding release of catecholamines from the sympathoadrenomedullary system (6–9). Catecholamines stimulate the mobilization of free fatty acids (FFA) from adipose tissue (cf. 10, 11). Short-term emotional arousal in man, such as fear, anxiety, and psychosexual stimulation, has also been found to increase plasma levels of FFA (12–14). Plasma FFA are rapidly incorporated into triglycerides of the liver and of the plasma lipoproteins (cf. 11). The liver is probably the main site of formation of endogenous plasma lipoproteins (cf. 11). In the fasting state, when lipogenesis is low, FFA are the main precursors of the hepatic and plasma triglyceride fatty acids. An increased mobilization of FFA from adipose tissue, e.g. by catecholamines, leads to a rise in the triglyceride content of the liver (15–18) and plasma (15, 18). Pro-

longed treatment with catecholamines also leads to hyperlipoproteinemia.

These data suggest the following hypothesis about the mechanism behind stress-induced hyperlipoproteinemia. Emotional stress is accompanied by an increase, mainly mediated by the sympathoadrenomedullary system, in the mobilization of FFA from adipose tissue. As a result, the amount of triglycerides in the liver increases, thereby, stimulating the secretion into plasma of triglycerides from this organ. Ultimately, plasma triglyceride levels rise, as do the levels of other lipoprotein constituents, e.g. cholesterol.

To test this hypothesis we have induced emotional stress of short duration and moderate intensity (6) and studied the effect on the concentration in arterial plasma of FFA, triglycerides, and cholesterol. To establish the role of FFA mobilization in this context, we also studied the effect of nicotinic acid on the stress-induced responses to the plasma lipids, as nicotinic acid is known to inhibit the stimulation of mobilization produced by exogenous catecholamines in vivo (20, 21) and in vitro (22, 23). The urinary excretion of catecholamines was followed in order to obtain a reflection of the sympathoadrenomedullary response to the procedures (7). Preliminary notes on some of the results have been published (8, 11, 24).

METHODS

The subjects were selected from patients consulting the medical outpatient clinic for minor complaints. With the exception of 10 subjects, who were hypertensive (during the prestress period of our study they had a mean diastolic blood pressure between 100 and 130 mm Hg), the 33 males finally selected were free from cardiovascular, endocrine, metabolic, or any other serious disease as judged from history, physical examination, and routine laboratory investigations of blood and urine. No subject was on any regular drug. The ECG at rest was normal in all subjects. Before consenting to participate the subjects had received standardized, detailed written and oral information and instructions about the nature and purpose of the study. The subjects were divided into three equal-sized groups, so that means and variations with respect to age, blood pressure, and level of plasma triglycerides and cholesterol were similar in all groups (Tables I and II). The 11 control subjects sat comfortably for the three 2-hr periods of the experiment; no stressors or treatments were introduced (*control group*). 11 subjects underwent the experimental stress procedure as described below, but did not receive nicotinic acid (*stress group*). The remaining 11 subjects (*nicotinic acid group*) also underwent the experimental stressor procedure. In addition, starting 30 min after the beginning of the prestress period, the subjects were given six doses of 0.5 g of nicotinic acid by mouth at intervals of 30 min, as indicated in Fig. 1. During the preexperimental week, the subjects in this group had been given nicotinic acid in increasing doses, up to a maximum of 1 g three times daily, reached after 4–5 days, in order to minimize and accustom them to the flush produced by the drug. This was considered necessary since the unpleasantness of the flush, if experienced during the actual experiment, might have an influence on stress-sensitive parameters included in this investigation. The last preexperimental dose was taken approximately 16 hr before the beginning of the experiment.

The subjects reported to the laboratory at 7 a.m. after fasting over night and refraining from food, tobacco, coffee, and tea in the morning. An arterial catheter was placed in the left brachial artery; heparin was not used. The subjects were then allowed to rest for at least 1 hr, i.e. until 9 a.m., when the experiment proper started. The experimental procedure has been described in detail

TABLE I

Age, Blood Pressure, Heart Rate, and FFA in the Three Groups at the Start of the First Control Period (0 hr). Mean Values (MV), Standard Errors of the Mean (SE), and Ranges

Group	Age	Blood pressure		Heart rate	FFA
		Systolic	Diastolic		
	yr	*mm Hg*		*beats/min*	*meq/liter*
Control					
MV ± SE	49 ± 2	153 ± 8	91 ± 4	66 ± 2	0.64 ± 0.08
Range	43 – 62	125 – 200	70 – 115	60 – 78	0.39 – 1.11
Stress					
MV ± SE	52 ± 2	164 ± 9	98 ± 5	61 ± 3	0.69 ± 0.09
Range	32 – 57	110 – 205	70 – 125	50 – 80	0.48 – 1.50
Nicotinic acid					
MV ± SE	48 ± 3	146 ± 6	95 ± 5	68 ± 4	0.62 ± 0.05
Range	32 – 59	120 – 190	70 – 120	54 – 96	0.47 – 0.94

TABLE II

Plasma Lipid Levels in the Three Groups Determined 3–8 wk before and at the Start of the Experiment (0 hr) Mean ± SE

Group	3–8 wk before the experiment		0 hr start of experiment		Change during the preexperimental period	
	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol
	mmoles/liter	*mg/100 ml*	*mmoles/liter*	*mg/100 ml*	*mmoles/liter*	*mg/100 ml*
Control						
MV ± SE	1.89 ± 0.17	293 ± 17	1.41 ± 0.18	307 ± 14	−0.49 ± 0.19‡	14 ± 22
Range	1.04 − 2.28	239 − 385	0.71 − 2.49	263 − 378	−1.28 − 0.30	−122 − 72
Stress*						
MV ± SE	2.09 ± 0.14	288 ± 14	1.85 ± 0.15	264 ± 14	−0.24 ± 0.17	− 24 ± 12
Range	1.49 − 2.80	252 − 386	1.31 − 2.65	208 − 342	−0.75 − 0.02	− 70 − 49
Nicotinic acid*						
MV ± SE	1.92 ± 0.18	300 ± 17	1.18 ± 0.15	198 ± 9	−0.73 ± 0.13‖	−102 ± 22§
Range	1.07 − 2.66	231 − 388	0.68 − 2.08	171 − 240	−1.52 − 0.19	−217 − 17

* n = 9 since no pretreatment values were available in two cases in each group.
‡ $P < 0.05$.
§ $P < 0.01$.
‖ $P < 0.001$.

previously (6). It involved three consecutive 2-hr periods, the second one was the *stress* period for two of the groups, as indicated above. The stressors included sorting small shiny steel balls of four very similar sizes in the presence of a loud industrial noise (97–104 dB–C), variations in the intensity of a dazzling light, rush due to lack of time, and standardized criticism. The criticism was presented in writing 13 and 28 min after the beginning of the stress period. During the *control* periods before and after this period the subjects relaxed by reading a weekly magazine and listening to soft music. All details including posture, time scheme, instructions, and stimuli were strictly standardized (9). No food was served. During each period the subjects ingested 300 ml of tap water. Urine samples were collected at the end of each 2-hr period.

Before the stress period, the subjects completed a simple questionnaire in which they were asked whether for some extraneous reason they had been more upset or less than usual. There were no significant differences in this respect between the groups. After the second 2-hr period, nine subjects of the control group, but none in the stress group, and three in the nicotinic acid group reported that they felt calm and unconcerned. This period was described as relatively laborious but not directly disagreeable by none, six, and seven subjects, respectively. in these three groups; two, three, and none of the subjects, respectively, reported it as being a relative strain, while two subjects in the stress group described the strain as being very considerable. Observations were also made every 10 min concerning the "level of general emotional arousal" manifested by the subjects during the second 2-hr period. This was rated on a five-point scale ranging from 1 (unconcerned) to 5 (extremely concerned). Both stressor-exposed groups were judged to be moderately emotionally aroused during the exposure (mean of all ratings close to three points in both groups), whereas the control subjects were left relatively unconcerned (mean ratings close to one point).

Serial blood samples were drawn into heparinized syringes every 15th or 30th min (Fig. 1 and Table III), immediately centrifuged, and the plasma lipids were extracted. FFA were determined by the method of Dole (25) as modified by Trout, Estes and Friedberg (26), total cholesterol according to Sperry and Webb (27, 28), and triglycerides by the method of Carlson (28, 29). Since arterial haematocrit did not change in a similar stressor exposure, nor did it change after administration of nicotinic acid, there was no need to correct for hemoconcentration or hemodilution. The urine samples were analyzed for adrenaline and noradrenaline by the method of Euler and Lishajko (30) as well as for creatinine (31). As a pilot study, 17-hydroxycorticosteroid assays (32, 33) were performed on some of the urine samples of the stressor-exposed groups. All statistical calculations were performed as recommended by Snedecor (34).

RESULTS

Plasma free fatty acids. In the *control* group the concentration of FFA remained largely unchanged throughout the experiment. In the *stress* group FFA remained constant during the prestress period and increased significantly during and after the emotional stress (Fig. 1, Table III). In the *nicotinic acid* group, the concentration of FFA fell during the prestress period, when the nicotinic acid treatment started (Fig. 1), reaching a mean level of 0.24 ± 0.07 meq/liter immediately before the start of the stressor exposure;

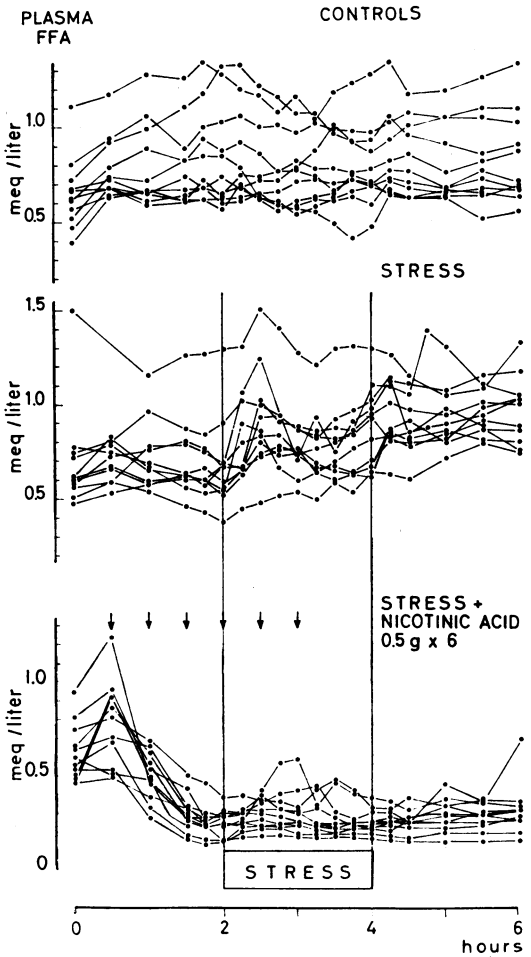

FIGURE 1 Individual values for the plasma levels of FFA during emotional stress and during control conditions in the three groups.

there was no significant mean rise during or after the stress (Table III). The absolute levels of FFA were significantly lower in the nicotinic acid group than in the other two groups during the last two periods.

Plasma triglycerides. As indicated earlier, the three groups originally had similar blood lipid levels, i.e., the levels found during the screening procedure 3–8 wk before the actual experiment (Table II). In the interval between this measurement and the actual experiment (*preexperimental period*), the triglyceride levels of the *control* group fell significantly but moderately. In the *stress* group not given nicotinic acid, there was no significant change. This difference in reaction pattern may have been due to the relative equanimity of the control group, in contrast to some probable apprehension in the stress group, as both groups knew the experimental procedure they were to undergo. The influence on the triglyceride levels may have been similar to that indicated below. Finally, the *nicotinic acid* group showed a significant and pronounced fall in plasma triglycerides during the preexperimental period, probably attributable to the lipid-lowering effect of the pretreatment with nicotinic acid (35).

During the experiment proper, the triglyceride levels of the *control* group remained unchanged, whereas those of the *stress* group increased significantly towards the end, the levels being significantly higher in the latter group throughout the experiment. In the *nicotinic acid* group, on the other hand, the triglyceride level decreased progressively and significantly. The contrasting behavior of the triglycerides in the *stress* group and the nicotinic acid group as summarized in Fig. 2, makes the group difference in respect of both the levels and the changes highly significant throughout the study (Table III).

Plasma cholesterol. At the start of the preexperimental period, the three groups had similar cholesterol levels (Table II). There were no significant changes during this period in either the control group or the stress group, whereas the nicotinic acid group exhibited a pronounced and significant fall (cf. Table II), probably a result of the pretreatment with nicotinic acid (35).

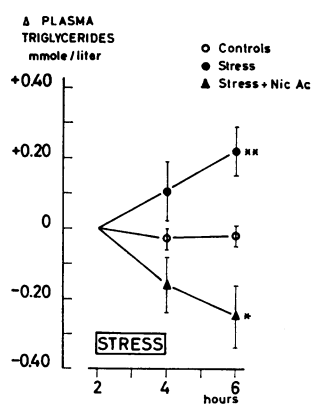

FIGURE 2 Mean ± SE for the changes in the plasma levels of triglycerides during and after the second two-hour period, designed to induce emotional stress in the stress group and the nicotinic acid group but not in the control group. + and ++ indicate that $P < 0.05$ and 0.01, respectively.

TABLE III

Statistical Comparisons of Means ± SE and Changes in Plasma FFA, Triglycerides and Cholesterol, and Urinary Adrenaline, Noradrenaline and 17-OHCS in the Three Groups

Group	Period*	Free fatty acid‡	Triglycerides§	Cholesterol§	Adrenaline	Noradrenaline	Corti-costeroids‖
		meq/liter ×100	*mmole/liter*	*mg/100 ml*	*ng/min*	*ng/min*	*μg/min*
Controls	A	78.6 ± 6.2	1.24 ± 0.19	308 ± 10	7.62 ± 1.10	23.4 ± 4.1	
	B	79.6 ± 6.2	1.21 ± 0.17	296 ± 11	5.59 ± 0.96	23.6 ± 2.9	
	C	82.2 ± 6.4	1.22 ± 0.17	292 ± 12	6.57 ± 0.90	24.3 ± 2.9	
	B–A	+ 1.0	− 0.02	− 12	− 2.04	+ 0.2	
	B–C	− 2.6	− 0.01	+ 5	− 0.98	− 0.7	
	A–C	− 3.6	+ 0.02	+ 16¶	+ 1.05	− 0.9	
Stress	A	71.0 ± 6.5	1.78 ± 0.15	271 ± 13	9.53 ± 2.37	23.8 ± 2.8	5.18 ± 0.52
	B	82.9 ± 6.2	1.88 ± 0.15	278 ± 12	13.31 ± 3.94	33.1 ± 3.6	5.41 ± 1.02
	C	93.2 ± 3.9	2.00 ± 0.18	268 ± 14	8.85 ± 1.72	28.2 ± 3.8	7.00 ± 1.25
	B–A	+ 11.9**	+ 0.10	+ 7	+ 3.78	+ 9.3‡‡	+ 0.23
	B–C	− 10.3¶	− 0.12	+ 10	+ 4.46	+ 4.9	− 1.59
	A–C	− 22.2‡‡	− 0.22¶	+ 3	+ 0.68	− 4.4	− 1.82
Nicotinic acid	A	45.2 ± 2.8	1.24 ± 0.15	199 ± 7	13.39 ± 1.39	29.0 ± 2.4	8.60 ± 1.80
	B	27.6 ± 2.3	1.07 ± 0.13	201 ± 9	19.52 ± 2.70	42.0 ± 3.9	8.19 ± 1.74
	C	27.4 ± 2.1	0.99 ± 0.12	200 ± 11	14.96 ± 1.73	32.0 ± 2.6	10.74 ± 2.44
	B–A	− 17.6‡‡	− 0.17	+ 2	+ 6.13**	+ 13.1‡‡	− 0.41
	B–C	+ 0.2	+ 0.08	+ 1	+ 4.57¶	+ 10.0**	− 2.55
	A–C	+ 17.7‡‡	+ 0.25¶	− 2	− 1.57	− 3.1	− 2.14
Difference between groups Stress minus Controls	A	− 7.6	+ 0.55¶	− 37¶	+ 1.91	+ 0.3	
	B	+ 3.4	+ 0.66**	− 18	+ 7.73	+ 9.5	
	C	+ 11.0	+ 0.78**	− 23	+ 2.28	+ 3.9	
	B–A	+ 10.9¶	+ 0.12	+ 19¶	+ 5.82¶	+ 9.2	
	B–C	− 7.6	− 0.12	+ 5	+ 5.45	+ 5.6¶	
	A–C	− 18.6**	− 0.23**	− 13	− 0.37	− 3.6	
Nicotinic acid minus Controls	A	− 33.4‡‡	± 0.0	− 109‡‡	+ 5.77**	+ 5.5	
	B	− 51.9‡‡	− 0.15	− 95‡‡	+ 13.94‡‡	+ 18.4**	
	C	− 54.7‡‡	− 0.23	− 91‡‡	+ 8.39‡‡	+ 7.7	
	B–A	− 18.6‡‡	− 0.15	+ 14	+ 8.17‡‡	+ 12.9¶	
	B–C	+ 2.8	+ 0.09	− 4	+ 5.55**	+ 10.7‡‡	
	A–C	+ 21.4‡‡	+ 0.23¶	− 18	− 2.62	− 2.2	
Stress minus Nicotinic acid	A	+ 25.8**	+ 0.54¶	+ 72‡‡	− 3.86	− 5.2	− 3.42
	B	+ 55.3‡‡	+ 0.81‡‡	+ 77‡‡	− 6.21	− 8.9	− 2.79
	C	+ 65.7‡‡	+ 1.01¶	+ 68**	− 6.11¶	− 3.8	− 3.75
	B–A	+ 29.4‡‡	+ 0.27¶	+ 5	− 2.35	− 3.7	+ 0.64
	B–C	− 10.4¶	− 0.20¶	+ 10	− 0.10	− 5.1	+ 0.96
	A–C	− 39.9‡‡	− 0.47‡‡	+ 5	+ 2.25	− 1.4	+ 0.32

* A stands for the period from 0–2 hr, B for 2–4 hr, and C for 4–6 hr.

‡ Mean values calculated on the basis of six measurements (period A), eight measurements (period B), or five measurements (period C) in each subject.

§ Mean values calculated on the basis of three measurements (Period A) or two measurements (periods B and C) in each subject.

‖ Pilot study. n = 6–9 subjects.

¶ $P < 0.05$.

** $P < 0.01$.

‡‡ $P < 0.001$.

The plasma cholesterol levels fell slightly towards the end of the actual experiment in the control group (Table III), but there were no significant changes in either the *stress* or the *nicotinic acid* group. The cholesterol levels of the nicotinic acid group were significantly lower than those of the other groups throughout the experiment proper. The difference between the decrease from the first to the second 2-hr period in the control group and the corresponding increase in the stress group is statistically significant ($P < 0.05$), cf. Table III.

Adrenaline excretion. In the *control* group, urinary adrenaline excretion decreased during the second 2-hr period, though the change is not significant (Table III). In the *stress* group there was an insignificant increase during the same period, while the increase in the *nicotinic acid* group was significant.

During the first control period, the adrenaline excretion was significantly higher in the *nicotinic acid* group than in the *control* group (Table III). Otherwise, there were no significant differences between the adrenaline levels of different groups during this period.

The catecholamine excretion was higher during all three periods in the nicotinic acid group compared with the stress group, but not significantly so. However, this lack of significance is caused by the very high catecholamine excretion of a single subject in the stress group throughout the experiment. If this subject is excluded (see Discussion), the difference in excretion levels between the two groups becomes significant in each of the three periods.

Comparing the changes in adrenaline excretion in the three groups with all subjects included, we found that there are no significant differences between the two stressor-exposed groups, whereas the reactions of both groups during the stress period are significantly different from that displayed by the control group (Table III).

Noradrenaline excretion. The noradrenaline excretion of the *control* group remained largely unchanged throughout the experiment (Table III), whereas in the other two groups it increased significantly and similarly during stress. During the prestress period the level of noradrenaline excretion was much the same in all three groups. However, see Discussion concerning the catecholamine hyperexcretor mentioned above.

Corticosteroid excretion. Our pilot study on corticosteroid excretion does not suggest that this excretion rate is influenced either by short-term stressor-exposure or by nicotinic acid treatment (Table III).

Urine volume, specific gravity. The urine volume in all three groups decreased significantly from the first to the third of the three 2-hr periods, probably caused by diurnal variation (36). Table IV lends some support to the hypothesis that both stress and nicotinic acid treatment affect urine flow. As to specific gravity, no significant changes occurred in any group, though the general trend was similar to that for urine flow, cf. Table IV.

Creatinine excretion. Creatinine excretion decreased significantly in the nicotinic acid group from the first to the third period of the experiment (Table IV). Apart from this, there were no significant changes in any of the groups. During the prestress period, when the nicotinic acid treatment was started, the nicotinic acid group exhibited a significantly higher creatinine excretion than the untreated stress group, but during the last two periods there were no differences between the groups.

Blood pressure. The blood pressure of the *stress* group increased significantly 15 min after the onset of the stressor exposure. Systolic blood pressure increased by 12 ± 2 mm Hg and diastolic blood pressure by 9 ± 2 mm Hg ($P < 0.001$ and $P < 0.01$, respectively) from the values found just before the exposure started. In the *nicotinic acid* group, the corresponding increases were 14 ± 4 mm Hg and 9 ± 2 mm Hg ($P < 0.01$ and $P < 0.001$, respectively). The control group exhibited no corresponding change.

The stressor-induced changes were of short duration. Comparing the *mean* blood pressure during *each* of the three 2-hr periods (Table IV), we found that the changes between periods were not significant in either of the two stressor-exposed groups. The *control* group showed a significant decrease of the mean systolic and diastolic blood pressure, see Table IV. The differences in levels and reactions between the two stressor-exposed groups were not statistically significant; neither were the corresponding differences between the stress group and the control group.

TABLE IV

Statistical Comparison of Means ± SE and Changes in Urine Volume, Specific Gravity, Urinary Creatinine, Systolic and Diastolic Blood Pressure, and Heart Rate in the Three Groups

Group	Period	Urine volume	Spec. gravity	Creatinine	Syst. blood pressure	Diast. blood pressure	Heart rate
		ml/min	*(N-1) × 1000*	*mg/min*	*mm Hg*	*mm Hg*	*beats/min*
Controls	A	3.10 ± 0.57	8.0 ± 1.3	1.14 ± 0.10	147.6 ± 2.0	90.3 ± 4.4	65.8 ± 2.0
	B	2.24 ± 0.26	7.8 ± 1.1	1.02 ± 0.05	138.4 ± 7.4	86.4 ± 4.5	65.1 ± 1.8
	C	1.79 ± 0.18	8.8 ± 1.4	1.08 ± 0.06	138.0 ± 8.2	84.3 ± 6.2	65.1 ± 2.8
	B–A	− 0.86	− 0.2	− 0.13	− 9.2*	− 3.9*	− 0.7
	B–C	+ 0.45	− 1.0	− 0.07	+ 0.4	+ 2.1	± 0.0
	A–C	+ 1.32‡	− 0.8	+ 0.06	+ 9.6‡	+ 6.0	+ 0.7
Stress	A	3.10 ± 0.41	8.6 ± 2.0	0.90 ± 0.06	158.8 ± 8.7	98.7 ± 5.2	62.7 ± 3.2
	B	1.53 ± 0.27	11.6 ± 1.8	0.86 ± 0.05	153.8 ± 8.3	99.1 ± 5.6	76.6 ± 5.1
	C	2.18 ± 0.29	8.4 ± 2.2	1.03 ± 0.15	156.3 ± 7.4	98.9 ± 4.7	64.9 ± 3.8
	B–A	− 1.56§	+ 3.0	− 0.04	− 5.0	+ 0.4	+ 13.8§
	B–C	− 0.65‡	+ 3.2	− 0.17	− 2.4	+ 0.2	+ 11.6§
	A–C	+ 0.91‡	+ 0.2	− 0.13	+ 2.6	− 0.2	− 2.2
Nicotinic acid	A	3.75 ± 0.40	7.9 ± 1.9	1.19 ± 0.06	143.4 ± 4.8	93.6 ± 2.9	69.4 ± 3.3
	B	2.61 ± 0.45	9.8 ± 2.0	1.04 ± 0.06	144.4 ± 4.4	95.9 ± 2.3	79.3 ± 3.1
	C	1.64 ± 0.30	12.4 ± 2.5	0.99 ± 0.06	141.6 ± 4.0	95.9 ± 2.9	69.2 ± 2.8
	B–A	− 1.15	+ 1.9	− 0.14	+ 1.0	+ 2.3	+ 9.8§
	B–C	+ 0.97‡	− 2.6	+ 0.05	+ 2.7	± 0.0	+ 10.1§
	A–C	+ 2.11§	− 4.4	+ 0.20‡	+ 1.7	− 2.3	+ 0.3
Difference between groups Stress minus Controls	A	± 0.00	+ 0.6	− 0.24	+ 11.2	+ 8.4	− 3.1
	B	− 0.71	+ 3.8	− 0.15	+ 15.4	+ 12.7	+ 11.4‡
	C	+ 0.40	− 0.4	− 0.05	+ 18.3	+ 14.6	− 0.2
	B–A	− 0.71	+ 3.2	+ 0.09	+ 4.2	+ 4.3	+ 14.6§
	B–C	− 1.00*	+ 4.2‡	− 0.11	− 2.9	− 1.9	+ 11.6§
	A–C	− 0.40	+ 1.0	− 0.19	− 7.1	− 6.2	− 2.9
Nicotinic acid minus Controls	A	+ 0.65	− 0.1	+ 0.04	− 4.3	+ 3.3	+ 3.6
	B	+ 0.37	+ 2.0	+ 0.03	+ 5.9	+ 9.6	+ 14.2§
	C	− 0.14	+ 3.6	− 0.09	+ 3.6	+ 11.6	+ 4.1
	B–A	− 0.29	+ 2.1	− 0.02	+ 10.2*	+ 6.2	+ 10.6§
	B–C	+ 0.51	− 1.6	+ 0.12	+ 12.3	− 2.1	+ 10.1§
	A–C	+ 0.80	− 3.6	+ 0.14	− 7.9	− 8.3‡	− 0.4
Stress minus Nicotinic acid	A	− 0.66	+ 0.7	− 0.29*	+ 15.4	+ 5.1	− 6.7
	B	− 1.08	+ 1.8	− 0.18	+ 9.4	+ 3.2	− 2.7
	C	+ 0.54	− 3.9	+ 0.05	+ 14.6	+ 3.0	− 4.3
	B–A	− 0.42	+ 1.12	+ 0.10	− 6.0	− 1.9	+ 4.0
	B–C	− 1.62*	+ 5.7‡	− 0.23	− 5.2	+ 0.2	+ 1.6
	A–C	− 1.20‡	+ 4.6	− 0.33‡	+ 0.8	+ 2.1	− 2.4

See explanations in Table III.

* $P < 0.01$.

‡ $P < 0.05$.

§ $P < 0.001$.

Heart rate. Stressor exposure was accompanied by significant accelerations in heart rate, the levels and responses being of the same magnitude in both exposed groups. The heart rate of the *control* group remained on a constant level throughout the experiment (Table IV), signficantly below that during the stress period for the two stressor-exposed groups.

DISCUSSION

Lipid metabolism, emotional stress, and nicotinic acid

Free fatty acids and sympathoadrenomedullary activity. The standardized stressor exposure used (6) caused a significant increase in the concentration of FFA both during and after the stress

period in the *stress* group but not in the nicotinic acid group. The FFA increase was probably caused by an increased mobilization from adipose tissue.

Increased activity of the sympathetic nervous system may be important for the increase of FFA concentration seen after emotional stimuli (37). Thus, ganglionic blockade with Arfonad inhibits the rise in FFA during anxiety (37). In the *stress* group there was a significant, positive correlation between the changes in adrenaline excretion and the changes in FFA levels between the prestress and the stress period ($r = 0.61$, $P < 0.05$). The corresponding correlations in the control group and in the nicotinic acid group were, as expected, not significant ($r = 0.23$ and $r = 0.11$, respectively). The low correlation between these two variables in the nicotinic acid group may have been caused by a selective blockade of FFA without a corresponding influence on adrenal medullary function. There was no significant correlation between the corresponding changes in noradrenaline excretion and in FFA levels in any group. However, nothing is known about the relationship, if any, between increased sympathetic activity *locally* in adipose tissue and the urinary excretion of catecholamines. Furthermore, the urinary catecholamines provide an index of the *mean* sympathoadrenomedullary activity during the period of urine collection, whereas the blood samples inform about the *momentary* situation as regards lipid metabolism. Finally, other FFA mobilizing hormones too may have been released during and particularly after the stress period and contributed to the FFA level changes.

The effect of nicotinic acid on the concentration of FFA during the resting period is in accordance with previously reported results in man (20, 21). The decreased response of FFA to emotionally charged stimuli during nicotinic acid treatment suggests an inhibition of the stress-induced enhancement of lipid mobilization (21) due to inhibition of lipolysis in adipose tissue (22, 23).

Triglycerides and cholesterol. The emotional stress was accompanied by an acute rise in the plasma triglyceride concentration of the non-treated stress group, without inducing concomitant changes in the cholesterol level, which indicates that there was an increase in the amount of triglyceride-rich but cholesterol-poor very low density lipoproteins. An increase in these lipoproteins that raises the plasma triglyceride content by 0.20 mmole/liter would increase the cholesterol concentration by only about 5 mg/100 ml. Such small changes in cholesterol concentration would not be detected with the technique used. Measurements of the concentration of plasma lipoproteins in medical students during stressful examination periods have shown (5) that the concentration of the cholesterol rich low density lipoproteins (S_f0–12) was unaffected, while that of the triglyceride-rich very low density lipoproteins (S_f12–400) increased by 50%. This is in accordance with our findings during short-term emotional stress.

Plasma triglycerides and FFA. Our working hypothesis was that there is an increased mobilization of FFA from adipose tissue during emotional stress, resulting in an increase in triglyceride concentration in plasma. Our data support this hypothesis since the plasma triglycerides increased in the *stress* group, in which the concentration of FFA increased, and decreased in the *nicotinic acid* group, in which the concentration of FFA decreased. This decrease occurred before the actual stress and the FFA then remained at a low level throughout the study. The following rough calculations have been made in order to evaluate the quantitative aspects of our hypothesis.

The increase in plasma triglycerides was approximately 0.20 mmole/liter from the start of the stress period (2 hr) to the end of the experiment (6 hr). If we assume that the plasma volume was 3 liters, the mean increase of triglycerides in plasma works out as $3 \times 0.2 = 0.6$ mmole, which corresponds to $3 \times 0.6 = 1.8$ meq of fatty acids. Similarly, the plasma pool of triglyceride fatty acids is calculated to have decreased by 2.2 meq in the nicotinic acid group. If our hypothesis is correct, the amount of FFA taken up by the liver must thus have changed by at least 2 meq in both the stress group and the nicotinic acid group. To calculate the changes in the influx of FFA to the liver, let us assume (*a*) that 25% of the FFA turnover is taken up by the liver (38), and (*b*) that the fractional turnover rate of FFA at rest and during emotional stress in the stress group was 0.30/min (39), rising to 0.40/min during administration of nicotinic acid when the FFA level was around 0.30 meq/liter (21). The amount of FFA taken up by the liver at rest was thus $0.25 \times 0.30 \times 0.7 \times 3 \times 60 \approx 10$ meq/hr.

In the stress group the FFA level increased to

about 0.9 meq/liter for 4 hr. The increase above the resting state of the influx of FFA to the liver was thus: $(0.25 \times 0.30 \times 0.9 \times 3 \times 60 \times 4) - (10 \times 4) \approx 10$ meq/liter. In the nicotinic acid group, the FFA level was reduced to 0.3 meq/liter for approximately 4.5 hr. As above, it can be estimated that the hepatic uptake of FFA during this time was reduced from the basal uptake by $(4.5 \times 10) - (0.25 \times 0.40 \times 0.3 \times 0.3 \times 60 \times 4.5) \approx$ 20 meq. The calculated changes in the amount of triglycerides in plasma, 1.8 and 2.2 meq, respectively, are thus much lower than the figures for the changes in the hepatic uptake of FFA, 10 and 20 meq, respectively. The changes in the hepatic uptake of FFA are thus great enough to cause changes 5–10 times greater than those seen in the plasma-triglyceride pools.

Thus, our results support the hypothesis but cannot establish it as other mechanisms than those involved in our hypothesis may also have contributed to the observed changes in plasma-triglyceride levels. Hepatic triglyceride output could have been stimulated in other ways than by increased flux of FFA, while during nicotinic acid treatment its output could have been reduced by other mechanisms, e.g. by direct interference of nicotinic acid on lipoprotein secretion in the liver. Furthermore, both emotional stress and nicotinic acid may have influenced the fractional turnover rate of very low density lipoproteins.

Urinary catecholamines, emotional stress, and nicotinic acid

One subject in the stress group excreted 27.6, 49.7, and 21.6 ng of adrenaline per min and 47.5, 64.0, and 59.5 ng of noradrenaline per min during control period I, the stress period, and control period II, respectively. These values are among the highest ever recorded in series of studies comprising some 4000 separate catecholamine determinations (8, 36). It thus appears justifiable to question the "normality" of this case from the catecholamine point of view and to suppose that these extreme values are attributable to some intrinsic cause not relevant in this context. If this subject is excluded, the two stressor-exposed groups differ significantly from each other in catecholamine excretion during all three periods, that of the nicotinic acid group being higher. The addition of nicotinic acid, nicotinamide, nicotinuric acid, and *N*-methyl-nicotinic acid in a concentration of 2 g/liter to separate urine samples did not influence the determinations of catecholamine levels performed in this study. Consequently the elevated values of the nicotinic acid group cannot be ascribed to any interference from nicotinic acid or its main metabolites with the procedure for estimation of catecholamines. The *nicotinic acid* group exhibited a significantly higher creatinine excretion than did the *stress* group only in the prestress period. This might have been accompanied by an increased renal clearance of catecholamines. While it is true that the effects of nicotinic acid on the sympathoadrenomedullary system require further study, it is of major importance in our context that nicotinic acid certainly did not inhibit the stressor-induced rise in catecholamine excretion.

Cardiovascular reactions

Stressor exposure was accompanied by a short-lived rise in systolic and diastolic blood pressure, whereas rest under control conditions was accompanied by a fall. Nicotinic acid treatment did not modify the blood pressure responses. This is in accordance with previous findings that nicotinic acid does not inhibit the blood pressure response to injected noradrenaline (20). Similarly, our results indicate that emotional stress is accompanied by an acceleration in heart rate, which is not modified by nicotinic acid treatment. This lends some support to our assumption that the nicotinic acid blockade of the rise in plasma lipoproteins that accompanies emotional stress is explained by an inhibition of the sympathoadrenomedullary-mediated mobilization of FFA from adipose tissue, and not by an effect of the sympathoadrenomedullary activity per se.

Clinical aspects

This study has clearly shown that increased plasma-triglyceride levels, and probably an increased sympathoadrenomedullary activity, are readily induced even by a work situation which is not real but simulated, of short duration and moderate intensity. It is tempting to speculate about the effects of the socioeconomic or other real-life stressors, which often are repeated over months and years and surely may have a threatening significance to the individual far exceeding that implied in our laboratory situation (1, 9, 36,

40). The frequent findings of elevated levels of triglycerides in plasma from patients with coronary heart disease are worth considering in this context (cf. 11).

ACKNOWLEDGMENTS

This paper was supported by grants from the Swedish Medical Research Council (Project 19X-204-02), Svenska Nationalföreningen mot Hjärt-Kärlsjukdomar, Stiftelsen Clas Groschinskys Minnesfond, and Syskonen Wesséns Stiftelse.

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