Effects of Hemoconcentration and Sympathetic Activation on Serum Lipid Responses to Brief Mental Stress

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Objective: Previous studies suggest that hemoconcentration may be one mechanism by which acute psychological stress causes elevations of serum total cholesterol and its subfractions. Alternatively, such elevations may result from sympathetically mediated changes in lipid metabolism. This study evaluated these two hypotheses by manipulation of sympathetically mediated responses to stress using a nonselective adrenoceptor antagonist, labetalol. **Method:** In a 2 × 2 factorial design, 52 healthy male participants were randomly assigned to a stress or no-stress condition and, within each condition, were administered either labetalol or saline. Participants assigned to stress completed three cognitive and evaluative tasks lasting a total of 18 minutes. Indices of hemoconcentration (hematocrit and hemoglobin), heart rate, blood pressure, and serum lipids (total, high-density lipoprotein (HDL), low-density lipoprotein (LDL), free fatty acids, and triglycerides) were assessed at preinfusion and infusion baselines and after mental stress (or rest). **Results:** Labetalol reduced sympathetic activation, as shown by a substantial reduction in heart rate elevation during stress, but did not alter changes in blood pressure or in hemoconcentration, as indicated by equivalent increases in hematocrit and hemoglobin in the two stressed groups. Labetalol blocked stress-induced increases in free fatty acid concentrations and lowered triglyceride levels but did not influence rises in total, HDL, or LDL cholesterol among stressed subjects. However, arithmetic correction for hemoconcentration eliminated the increases in total, HDL, and LDL cholesterol. **Conclusions:** These findings suggest that elevations in total cholesterol and its HDL and LDL subfractions during acute stress are caused by accompanying hemoconcentration, whereas concomitant rises in free fatty acids and triglycerides result from the direct metabolic effects of sympathetic activation. **Key words:** cholesterol, lipids, stress, hemoconcentration, sympathetic nervous system.

HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; DBP = diastolic blood pressure; SBP = systolic blood pressure; HR = heart rate.

INTRODUCTION

Substantial evidence indicates that risk for developing coronary heart disease rises with elevated levels of total cholesterol, triglycerides, and LDL cholesterol and with lower levels of HDL cholesterol (1, 2). Additionally, there has been much interest in possible influences of behavioral factors on serum lipids and lipoproteins. Research of the past four decades has shown that many stressful life events, such as important academic examinations or threats of unemployment, are associated with elevations in total cholesterol and related lipid fractions (3, 4). Studies have also found that acute stressors, such as those presented in laboratory settings, are also capable of eliciting rises in total cholesterol and its fractions. Despite these observations, the mechanism(s) underlying relationships between stress exposure and elevated concentrations of blood lipids remain poorly understood (4).

With respect to potential mechanisms, one possibility is that activation of the sympathetic nervous system during psychological stress increases the production of serum lipids and lipoproteins by altering lipid metabolic processes (5). It is well known, for example, that catecholamines induce lipolysis and release free fatty acids into the circulation; free fatty acids, in turn, serve as substrate for the resynthesis of triglycerides and, subsequently, VLDL production by the liver (6–8). An alternative pathway for stress-related changes in serum lipids involves altered hemoconcentration, whereby acute loss of plasma volume within the intravascular space concentrates nondiffusable blood constituents. By this hypothesis, rises in serum lipid and lipoprotein levels reflect a filtration of fluid out of the intravascular space (ie, a passive increase in cholesterol) rather than an increase in the synthesis of these compounds per se. Several studies have suggested that hemoconcentration accounts for rises in total and HDL cholesterol during brief exposure to experimental stressors, such as challenging mental arithmetic and word tasks (9–12). However, Stoney and colleagues found that adjusting for hemoconcentration did not eliminate increases in lipid concentrations evoked by an especially provocative public-speaking task (13–

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Received for publication March 23, 2001; revision received August 20, 2001.

DOI: 10.1097/01.PSY.0000021943.35402.8A

0033-3174/02/6404-0587

15). If hemoconcentration serves as a mechanism for stress-induced rises in serum lipids, lipid changes may be transient, just as hemoconcentration is often reversible. Furthermore, pharmacologic interventions for clinical or experimental purposes would need to inhibit stress-induced blood pressure elevations, which are understood to drive hemoconcentration.

Earlier studies in both laboratory animals (16) and humans (17) indicated that stressor-induced lipid responses may be blocked by pretreatment with a beta-adrenoceptor antagonist. Brennan et al. (16) found that elevations in serum cholesterol following daily sessions of uncontrollable shock in rats were inhibited by the beta-antagonist, atenolol. Taggart et al. (17) also found that oxprenolol attenuated increases in serum free fatty acids during a public-speaking task in men. Such findings suggest that sympathetic activation may directly mediate lipid responses to brief mental stress. However, because neither study measured hemoconcentration, it is unclear if adrenergic blockade ameliorated lipid responses through this alternative mechanism.

The purpose of this experiment was to compare these two hypothesized mechanisms for change in lipid and lipoprotein concentrations during brief psychological stress. By employing an adrenoceptor antagonist, our strategy was to block sympathetically mediated responses evoked by stress while monitoring stress effects on serum lipid fractions and hemoconcentration. Labetalol, which is a nonselective beta- and weak alpha-adrenoceptor antagonist, effectively blocks or attenuates the physiologic effects of sympathetic activation (eg, heart rate elevations) during mental stress (18–20). However, because of its weaker alpha-adrenoceptor properties (four to eight times more potent at beta- than alpha-adrenoceptors; 21), previous studies have found that labetalol does not diminish the magnitude of blood pressure response to acute mental stress (22).

Previous research has shown that the increase in free fatty acids during acute stress, fasting, or exercise is due to beta-adrenoceptor-mediated stimulation of adipose tissue hormone-sensitive lipase (23, 24). The mechanism underlying the increases in serum triglycerides, which can also occur with acute stress, is less clear but is also presumably due to metabolic sequelae of beta-adrenoceptor activation. Because hemoconcentration is likely to be driven by increases in blood pressure, causing changes in intravascular pressure (10, 25, 26), we predicted that labetalol would block the effects of sympathetic activation on heart rate and lipid metabolism without preventing stress-induced hemoconcentration. Lipid elevations in the presence of hemoconcentration in labetalol-treated stressed subjects would suggest that such changes are a function of hemoconcentration. However, if lipid alterations were prevented with adrenergic blockade (in the presence of hemoconcentration), this would support sympathetic mediation of altered synthesis or catabolism of lipids during acute stress.

Serum lipids and lipoprotein fractions may be differentially influenced by brief episodes of sympathetic activation. For example, the release of free fatty acids from adipose tissue by catecholamines is known to occur rapidly in humans, whereas lipoprotein particles take several hours or days to synthesize (5, 27). Therefore, this study examined the effects of brief psychological stress on a panel of lipid measures and the roles that the two mechanisms play in accounting for their changes. The serum lipid measures included total, HDL, and LDL cholesterol, triglycerides, and free fatty acids. Changes in hematocrit and hemoglobin were also measured as indices of hemoconcentration. It was predicted that labetalol would attenuate or block stress-induced increases in free fatty acids and triglyceride concentrations but not total, HDL, or LDL cholesterol concentrations or hemoglobin and hematocrit.

METHODS

Subjects and Design Overview

Participants were 52 healthy male volunteers (aged 18–30 years), recruited from university advertisements. All subjects had a body mass index below 30, were nonsmokers, and reported taking no medications and consuming fewer than 10 alcoholic beverages per week. In a 2×2 factorial design, subjects were randomly assigned to either a stress or no-stress condition, and within each of these conditions, received either intravenous labetalol or saline (placebo). The laboratory sessions had three periods: a) a 30-minute resting preinfusion baseline; b) a 15-minute infusion baseline period, during which participants continued to rest; and c) an 18-minute task period. Depending on their experimental condition, subjects received either labetalol or placebo during the infusion baseline period and were either stressed or instructed to rest during the task period. Blood samples for the measurements of hematocrit, hemoglobin, total and HDL cholesterol, triglycerides, and free fatty acids were obtained at the end of each period. Heart rate and blood pressure (BP) were also measured at intervals throughout each period. Identical measurements were obtained in all participants, regardless of experimental condition. Each participant gave written informed consent, as approved by the Biomedical IRB of the University of Pittsburgh. The data collected for this study were obtained as part of a previously published study examining immunologic responses to acute stress; the heart rate and blood pressure data presented here are the same as reported previously (22).

Procedures

Subjects abstained from food, caffeine, and exercise for 12 hours before participation. On their arrival at the laboratory, subjects were seated in a semirecumbent position, and an intravenous catheter

was inserted into the antecubital fossa of the subject's nondominant arm. A curtain was drawn across the subject's arm to prevent him from viewing the phlebotomy and infusion procedures. A blood pressure cuff was attached to the subject's dominant arm and connected to a vital-signs monitor (Critikon Dinamap 8100, Tampa, FL) for automated serial measurement of heart rate (in beats per minute (bpm) and systolic and diastolic BP (SBP, DBP). Subjects then rested during the 30-minute preinfusion baseline and 15-minute infusion baseline periods.

During the infusion baseline period, each subject received either 0.75 mg/kg labetalol or a similar volume of saline intravenously. Half of the labetalol was administered during the first 2 minutes and the remainder during minutes 5 and 6. Following the infusion baseline, unstressed controls were instructed to continue to rest quietly for the 18-minute task period, while the stress groups performed a task consisting of a) a modified Stroop color-word interference test for 8 minutes, followed by b) mental arithmetic for 5 minutes, and finally, c) simulated public speaking for 5 minutes. The Stroop test, which is a cognitive task, was comprised of a series of color words that were printed in incongruent colors and presented on a computer screen. Participants were instructed to identify the color of the print while ignoring the word itself, in the presence of time pressure and a voice distractor (22). During mental arithmetic, participants solved consecutive one- to three-digit addition and subtraction problems, which increased in complexity when their task performance improved and decreased in complexity when their performance declined. The public-speaking task consisted of 2 minutes of preparation for a speech in defense of a hypothetical shoplifting charge, followed by 3 minutes of videotaped speech delivery (22).

Heart rate and BP were recorded at 2-minute intervals during the last 2 minutes of the preinfusion baseline period, the last 4 minutes of the infusion baseline period, and throughout the task period. Blood samples (10 ml) were collected at the end of each period for determination of hematocrit, hemoglobin, and serum lipid concentrations.

Blood-Level Measurements

Serum samples were separated after centrifugation and stored at −70°C until assayed. Serum lipid concentrations were determined by the Heinz Nutrition Lipid Laboratory of the University of Pittsburgh, which has met the accuracy and precision standards of the Centers for Disease Control and Prevention. Total serum cholesterol level was determined enzymatically by use of a bichromatic autoanalyzer. High-density lipoprotein (HDL) cholesterol level was determined by removing very low-density and low-density lipoproteins (LDLs) through the selective precipitation of these compounds by heparin/manganese chloride and centrifugation. Triglyceride concentrations were determined enzymatically according to the procedure of Bucolo and David (28). This involved hydrolysis of triglycerides and subsequent quantification of glycerol content. Free fatty acids were determined enzymatically (Wako Chemicals USA Inc., Richmond, VA) with acylation of coenzyme A by fatty acids and subsequent generation of hydrogen peroxide in the presence of acyl-CoA synthetase and oxidase. Hydrogen peroxide, in the presence of peroxidase, permits the oxidative condensation of 3-methyl-N-ethyl-N-(B-hydroxy-xyethyl)-aniline with 4-aminoantipyrine to produce a purple-colored adduct measured colorimetrically at 550/650 nm on the Abbott VP Spectrophotometer (Abbott Laboratories, Chicago, IL).

Hematocrit and hemoglobin concentrations were determined in edetic acid anticoagulated samples with an automatic cell counter (STKR Coulter Counter, Coulter Electroniks Inc, Hialeah, FL). Hematocrit was calculated automatically from the red blood cell concentration and the impedance-determined mean corpuscular volume; hemoglobin concentrations were measured by the cyanomethemoglobin method. Baseline plasma volume was calculated as follows: 1 − hematocrit. Task plasma volume was calculated from changes in hemoglobin level and hematocrit according to the method of Dill and Costill (29). As described below, estimated changes in plasma volume were used for the adjustment of plasma lipid concentrations in instances where hemoconcentration occurred.

Statistical Analyses

Before statistical analysis, HR and BP data were reduced by calculating mean values for the preinfusion baseline, infusion baseline, and task periods. Due to blood sample clotting, one subject assigned to the saline-rest condition was eliminated from investigation, leaving 51 subjects for analysis.

To investigate baseline equivalency of the four groups at the beginning of the experiment, 2×2 ($\text{group}_{\text{stressed, control}} \times \text{drug}_{\text{saline, labetalol}}$) analyses of variance (ANOVA) were performed on the preinfusion baseline values of HR, BP, hematocrit, hemoglobin, and serum lipids. The effects of drug and saline (placebo) infusion on these baseline levels were then tested with $2 \times 2 \times 2$ ($\text{group}_{\text{stressed, control}} \times \text{drug}_{\text{saline, labetalol}} \times \text{period}_{\text{preinfusion baseline, infusion baseline}}$) repeated measures ANOVAs. Any alteration in baseline values following labetalol administration would be reflected in a significant drug × period interaction.

To assess whether labetalol and mental stress or continued rest affected cardiovascular and blood measures between the infusion baseline and task periods, 2×2 ($\text{drug}_{\text{saline, labetalol}} \times \text{period}_{\text{infusion baseline, task}}$) repeated measures ANOVAs were conducted on each measure. These analyses were conducted separately on the stress and no-stress conditions because subsequent arithmetic correction of plasma lipids for stress-induced hemoconcentration (ie, rises in hemoglobin and hematocrit) was relevant only to subjects exposed to the stressor. In these analyses, modification of stress effects by labetalol would be reflected in the drug × period interaction term.

In instances where hemoconcentration occurred, serum lipid analyses were repeated after adjusting for hemoconcentration, according to methods previously described (9, 10). Briefly, acute changes in plasma volume can occur with exercise, rising from lying to standing, and from acute stress. Increased intravascular pressure forces movement of fluid out of the intravascular space, leaving nondiffusable blood constituents more concentrated. This reduction in plasma volume is estimated from changes in hemoglobin and hematocrit according to the method of Dill and Costill (29), and the change in plasma volume is used to arithmetically adjust concentrations of blood constituents for hemoconcentration (9, 10). In all analyses, post hoc comparisons among means were performed using Newman Keul's procedure. For all analyses, alpha level = .05.

RESULTS

Baseline Comparisons Between Groups, Immediate Effects of Labetalol or Saline Infusion, and Stability of Cardiovascular and Blood Measures in Nonstressed Controls

Table 1 depicts the means and standard deviations of the cardiovascular, lipid, and hematologic variables for each experimental group at the three study periods.

At preinfusion baseline, there were no significant

TABLE 1. Means (SD) of Cardiovascular, Lipid, and Hematologic Measures in the Four Experimental Groups at Each Experimental Period[a]

	Saline-Rest (N = 12)			Labetalol-Rest (N = 13)			Saline-Stress (N = 13)			Labetalol-Stress (N = 13)		
	Preinfusion Baseline	Infusion Baseline	Task	Preinfusion Baseline	Infusion Baseline	Task	Preinfusion Baseline	Infusion Baseline	Task	Preinfusion Baseline	Infusion Baseline	Task
Cardiovascular												
SBP (mm Hg)	122.3 (7.5)	121.0 (8.3)	122.7 (7.2)	122.1 (8.0)	114.7 (5.5)	114.4 (4.9)	118.5 (6.9)	117.6 (6.6)	132.8 (8.5)	119.9 (7.0)	113.7 (6.5)	128.1 (7.5)
DBP (mm Hg)	66.1 (7.0)	64.0 (7.9)	65.4 (7.6)	71.2 (7.1)	64.2 (7.9)	63.7 (6.7)	66.1 (5.0)	65.5 (4.4)	76.2 (5.8)	64.5 (4.7)	58.3 (5.7)	69.9 (7.7)
HR (bpm)	57.0 (4.9)	56.9 (6.3)	57.5 (5.8)	54.5 (8.0)	56.9 (6.4)	54.0 (5.9)	60.5 (9.2)	60.1 (9.0)	75.7 (13.0)	59.2 (7.3)	61.7 (6.4)	66.4 (6.2)
Lipid												
Total C (mg/dl)	156.3 (30.0)	156.8 (26.3)	157.9 (28.2)	138.8 (31.2)	138.6 (28.4)	138.5 (30.3)	142.9 (25.5)	142.9 (26.8)	146.1 (27.9)	142.9 (24.3)	142.1 (21.9)	143.7 (21.2)
HDL-C (mg/dl)	47.1 (11.2)	47.9 (10.5)	47.2 (10.1)	45.8 (7.3)	45.0 (24.2)	45.2 (7.2)	45.9 (7.6)	45.8 (7.8)	46.9 (8.1)	42.6 (8.3)	42.4 (8.1)	43.6 (8.8)
LDL-C (mg/dl)	91.4 (21.1)	91.3 (20.4)	94.0 (21.7)	78.0 (27.4)	78.2 (24.6)	77.8 (27.2)	85.0 (21.6)	84.9 (23.1)	86.3 (23.6)	85.8 (23.9)	85.1 (21.8)	86.2 (20.3)
Triglycerides (mg/dl)	89.8 (46.5)	88.4 (37.1)	83.3 (30.9)	75.5 (22.9)	78.0 (24.2)	77.2 (24.2)	60.2 (24.8)	62.2 (23.4)	64.5 (20.0)	73.3 (29.2)	73.2 (21.0)	70.0 (19.1)
FFA (mg/dl)	0.40 (0.19)	0.41 (0.19)	0.42 (0.19)	0.43 (0.15)	0.51 (0.16)	0.54 (0.15)	0.54 (0.12)	0.50 (0.13)	0.77 (0.29)	0.45 (0.18)	0.56 (0.23)	0.61 (0.25)
Hematologic												
Hgb (g/dl)	13.9 (1.9)	14.1 (1.4)	41.2 (1.4)	14.2 (0.8)	14.2 (0.6)	14.2 (0.7)	13.8 (0.9)	13.6 (1.1)	14.2 (1.0)	14.6 (0.5)	14.5 (0.7)	14.9 (0.5)
Hct (%)	40.0 (5.1)	40.6 (4.0)	40.5 (3.7)	41.0 (2.0)	40.9 (1.6)	40.8 (1.8)	39.8 (2.3)	39.3 (3.0)	41.0 (3.1)	42.5 (1.4)	41.9 (2.0)	43.0 (1.7)

[a] See text for statistical results.

differences between groups, with the exception of a slightly higher hematocrit in subjects who received labetalol ($F(1, 47) = 4.90$, $p < .05$). Analyses assessing changes following the infusion of drug or saline indicated that labetalol lowered resting systolic and diastolic BP by a mean of 6.7 and 6.6 mm Hg, respectively, and increased heart rate slightly, by a mean of 2.4 bpm (SBP: $F(1, 46) = 15.27$; DBP: $F(1, 46) = 28.02$; HR: $F(1, 46) = 7.98$, p values $< .01$). Free fatty acid concentrations increased somewhat from preinfusion baseline among labetalol-treated subjects ($F(1, 46) = 10.39$, $p < .01$), but no other changes were observed for lipid-related measurements or indices of hemoconcentration. As expected, no changes were observed among subjects receiving saline from the preinfusion to infusion baseline in any of the cardiovascular or blood measures.

No changes in blood pressure or any blood measure occurred in the two resting groups (saline-rest and labetalol-rest) from the infusion baseline to task periods. By the end of the task period, the initial slight heart rate elevation caused by labetalol returned to preinfusion levels in the labelalol-rest condition, $F(1, 23) = 5.97$, $p < .05$.

Task Period Changes in Stressed Subjects

We next examined changes between the infusion baseline and task periods in the two groups exposed to the stress condition (saline-stress and labetalol-stress). Here, it was predicted that labetalol would attenuate heart rate but not BP responses to the experimental task. Results of the analyses confirmed these predictions. Although heart rate increased significantly in both stressed groups relative to baseline (p values $< .05$), the increase in heart rate was greater in subjects receiving saline compared with those receiving labetalol, as reflected by a significant drug $\times$ period interaction, $F(1, 24) = 16.18$, $p < .01$ (heart rate rose by 26% and 8% in the saline-stress and labetalol-stress groups, respectively). Systolic and diastolic blood pressure rose significantly between the infusion baseline and task periods, and the magnitude of response did not differ between the saline and labetalol conditions (period main effects for SBP and DBP: $F(1, 24) = 146.18$ and $F(1, 24) = 103.16$, respectively, p values $< .001$). For the saline-stress group, SBP and DBP rose by 13% and 17%, respectively, with corresponding rises of 13% and 20% among stressed subjects administered labetalol.

Analyses involving hematocrit and hemoglobin indicated that hemoconcentration had occurred during stress (period main effects for hematocrit and hemoglobin: $F(1, 24) = 60.6$; $F(1, 24) = 110.38$, respectively,

p values <.001). Specifically, hematocrit and hemoglobin each rose by 3.5% in subjects exposed to the stressor. These effects were not altered by labetalol (drug × period interactions for hematocrit and hemoglobin: $F(1, 24)$'s = 2.42 and 3.37, respectively, p values > .05). Total, HDL, and LDL cholesterol rose by 2 to 3% in the two stressed groups ($F(1, 24)$'s = 9.81, 18.48, and 4.18, respectively, all p values < .05), and these lipid responses did not differ between the labetalol and saline conditions (drug × period interactions were all $F(1, 24)$'s < 1.09, NS). On the other hand, labetalol inhibited stress-related increases in free fatty acids and triglycerides, as reflected by significant drug × period interactions for each of these variables ($F(1, 24)$'s = 9.45 and 5.66, respectively, p values < .03). In the saline-stress group, free fatty acids rose ($p < .05$) by 50% but did not change significantly in stressed subjects pretreated with labetalol. Mean serum triglycerides in the saline-stress group rose by 6%, but neither this change nor a smaller magnitude decline in the labetalol-stress subjects was statistically significant.

Drug and Task Effects on Total, HDL, and LDL Cholesterol Levels, Corrected for Hemoconcentration

The previous analyses indicated that stress elicited both hemoconcentration and elevations in total, HDL, and LDL cholesterol concentration and that neither effect was significantly altered by labetalol pretreatment. Analyses that arithmetically correct for hemoconcentration were conducted to further determine whether the observed changes in total cholesterol and its HDL and LDL fractions were dependent on, or independent of, concomitant hemoconcentration. Consistent with our previous findings (9, 12), arithmetic correction of serum lipid concentrations for reductions in plasma volume ameliorated the rises in each of these lipid measures. Indeed, after correcting for hemoconcentration, results showed that total and LDL cholesterol actually decreased slightly from infusion to task in both stressed groups (p values < .05); however, decreases in HDL cholesterol reached significance only in the saline-stress condition (mean decrease of 1.7 mg/dl) (period main effects for total, HDL, and LDL cholesterol: $F(1, 24) = 34.25$; $F(1, 24) = 14.39$; $F(1, 24) = 21.21$, respectively, all p values < .01). Mean decreases in corrected total and LDL cholesterol were −5.2 mg/dl and −3.4 mg/dl for the two stressed groups combined.

DISCUSSION

It is now established that laboratory-administered psychological stressors of only 5- to 30-minute duration, such as common mental arithmetic and speech tasks, reliably elicit small increases in serum cholesterol and triglycerides and larger elevations in free fatty acids (4). Consistent with many earlier reports, the present study found that a series of three challenging tasks involving mental arithmetic, the Stroop test, and a speech task increased levels of total, HDL, and LDL cholesterol and free fatty acids (9–14, 30–37). Changes in triglyceride concentrations were of similar magnitude but failed to reach statistical significance. In comparison, free fatty acid levels rose by fully 50% over baseline values. Circulating free fatty acids are an energy substrate, and levels increase with sympathetic activation, as occurs during stress. However, free fatty acids may be part of a chain of metabolic events that leads to hypertriglyceridemia and elevated VLDL (6–8), may have deleterious effects during myocardial ischemia (38), and may mediate insulin resistance and the development of type 2 diabetes (39).

The primary purpose of this study was to examine two potential mechanisms mediating the relationship between acute stress and elevated serum lipid concentrations. By blocking the effects of sympathetic activation but not hemoconcentration, we were able to observe how each of these pathways contributes to changes in lipid profiles during brief exposures to mental stress. Partial pharmacologic blockade of adrenergic receptors by labetalol was verified by the two-thirds reduction in heart rate response to stress. Results of this study suggest that various lipid fractions are affected by different processes during periods of acute stress. Adrenergic blockade with labetalol did not inhibit increases in total, HDL, and LDL cholesterol concentrations, suggesting that these alterations were due to hemoconcentration rather than metabolic sequelae of beta-adrenoceptor activation. Indeed, subsequent arithmetic adjustment for hemoconcentration eliminated the increases in these lipid fractions. In contrast, labetalol clearly diminished the stress-induced increase in free fatty acid concentrations. Although concomitant triglyceride elevations were modest, the pattern of results suggests a similar inhibitory effect on triglycerides by labetalol. Hepatic release of triglyceride-rich very low-density lipoprotein particles is unlikely to be responsive to brief psychological stress, but the rise in serum triglyceride level could be due to reduced catabolism via adrenergically mediated inhibition of lipoprotein lipase (5, 40). Previously, McCann et al. (11) suggested that increases in cholesterol and lipoprotein concentrations during acute

mental stress are attributable to hemoconcentration, whereas stress-induced lipolysis rapidly releases free fatty acids from adipocytes. In this study, we directly support these hypotheses using intravenous pharmacologic blockade of adrenergic receptors. Thus, while the number of critical tests used in this study may invite cautious interpretation, the pattern of findings is consistent with predicted effects, particularly differential effects of labetalol on cholesterol and its subfractions vs. free fatty acids and triglycerides.

Future research should also investigate mechanisms of stress-induced lipid changes in larger and more diverse samples. Participants in this study were young, healthy men, and thus, the generalizability of our findings to other groups is unclear. For example, to the extent that women often manifest greater beta-adrenergic activation than men during acute stress exposure (41), they may experience greater acute elevations in free fatty acids and triglycerides. On the other hand, to the extent that men typically exhibit relatively larger net blood pressure responses than women (41–43), men may have greater hemoconcentration responses to acute stress. Based on our results, beta-adrenergic blockade would be expected to reduce the sympathetically mediated serum lipid changes (ie, rises in free fatty acids and triglycerides) but not those affected by hemoconcentration.

In order for our study to test the two mechanisms in question, it was important that labetalol block the effects of sympathetic activation while leaving hemoconcentration responses to stress intact in the group receiving labetalol and stress. We believe that hemoconcentration occurred in this group due to the much weaker alpha- than beta-blocking properties of labetalol. As previously seen, beta-adrenergic blockade does not generally prevent blood pressure responses to stress (44), and these responses, in turn, are likely to influence hemoconcentration. In the future, it would be interesting to conduct a similar experiment using an alpha-adrenergic antagonist. We would predict that such an intervention would effectively block blood pressure responses to stress and therefore inhibit changes in hemoconcentration and cholesterol as well.

While this study addressed serum lipid responses to acute laboratory stress, elevated lipid levels have also been observed during subjects' exposure to stressors of a more severe and chronic nature, such as examinations (45–48), low job security (49, 50), and earthquakes (51); for a review of the associations between naturalistic stress and lipids, see Niaura et al. (4). Hemoconcentration may remain an important mechanism in these contexts as well. For example, increases in hematocrit are associated with higher plasma cholesterol concentrations during periods of unemployment (50). In addition, it is likely that other mechanisms play a role in increasing blood lipid levels. Hormones, secreted during periods of chronic stress, may enhance sympathetically induced lipid metabolic processes and exert independent effects of their own. For example, cortisol is known to facilitate triglyceride synthesis and stimulate VLDL secretion by the liver (5, 52, 53). In addition, chronic stressors are often accompanied by health behavior changes such as an increased consumption of fatty foods, smoking, and alcohol use that, in turn, alter lipid profiles (4, 54–56).

Previously, it has been suggested that the nature or potency of a laboratory stressor influences the magnitude of lipid reactivity and that, for stressors eliciting greater hemodynamic responses, such as the speech task, hemoconcentration does not entirely explain concomitant increases in total cholesterol and its fractions (14, 15). The series of stressors used here included a speech task, which, like previous studies using this task, evoked substantial sympathetic activation. Thus, our pattern of findings is not limited to acute laboratory stressors evoking relatively weaker stress responses but is relevant to those stressors evoking more robust sympathetic activation.

The clinical importance of brief and small perturbations in serum lipid concentrations following exposure to short-term psychological stressors remains unclear. However, preliminary studies suggest that such lipid alterations may persist beyond termination of the stressor. For example, results of one study indicate that rises in total cholesterol persisted at least 30 minutes after exposure to a 20-minute mental arithmetic and Stroop task (9). Recent studies of animals exposed to repeated stressors suggest that cholesterol may remain elevated even longer. Thus, rats exposed to one or three daily sessions of uncontrollable tailshock exhibited elevated plasma cholesterol levels up to 22 hours after the final shock (57).

In summary, as in prior literature, we observed that acute psychological stress caused hemoconcentration and immediate rises in serum total cholesterol, cholesterol subfractions, free fatty acid levels, heart rate, and blood pressure. The stress-associated elevations in total, LDL, and HDL cholesterol were attributable to concomitant hemoconcentration and not to sympathetically mediated alterations in lipid metabolism. However, increased free fatty acid and triglyceride concentrations following stress were due to sympathetic activation, as evidenced by their amelioration under adrenoceptor blockade.

This research was supported, in part, by Grant IRG-58-31 from the American Cancer Society (E.B.) and by Grant HL 40962 (S.B.M.).

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