

Changes in plasma lipids with psychosocial stress are related to hypertension status and the norepinephrine stress response

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Received 28 November 2007; accepted 19 August 2008

Abstract

Hypertension is a known risk factor for cardiovascular disease. Hypertensive individuals show exaggerated norepinephrine (NE) reactivity to stress. Norepinephrine is a known lipolytic factor. It is unclear if, in hypertensive individuals, stress-induced increases in NE are linked with the elevations in stress-induced circulating lipid levels. Such a mechanism could have implications for atherosclerotic plaque formation. In a cross-sectional, quasi-experimentally controlled study, 22 hypertensive and 23 normotensive men (mean \pm SEM, 45 ± 3 years) underwent an acute standardized psychosocial stress task combining public speaking and mental arithmetic in front of an audience. We measured plasma NE and the plasma lipid profile (total cholesterol [TC], low-density-lipoprotein cholesterol [LDL-C], high-density-lipoprotein cholesterol, and triglycerides) immediately before and after stress and at 20 and 60 minutes of recovery. All lipid levels were corrected for stress hemoconcentration. Compared with normotensives, hypertensives had greater TC ($P = .030$) and LDL-C ($P = .037$) stress responses. Independent of each other, mean arterial pressure (MAP) upon screening and immediate increase in NE predicted immediate stress change in TC (MAP: $\beta = .41$, $P = .003$; NE: $\beta = .35$, $P = .010$) and LDL-C (MAP: $\beta = .32$, $P = .024$; NE: $\beta = .38$, $P = .008$). Mean arterial pressure alone predicted triglycerides stress change ($\beta = .32$, $P = .043$) independent of NE stress change, age, and BMI. The MAP-by-NE interaction independently predicted immediate stress change of high-density-lipoprotein cholesterol ($\beta = -.58$, $P < .001$) and of LDL-C ($\beta = -.25$, $P < .08$). We conclude that MAP and NE stress reactivity may elicit proatherogenic changes of plasma lipids in response to acute psychosocial stress, providing one mechanism by which stress might increase cardiovascular risk in hypertension.

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1. Introduction

Systemic hypertension ranks among the leading risk factors for adverse cardiovascular outcomes [1]. However, the mechanisms that link hypertension with increased incidence of cardiovascular events are not fully understood. Sympathetic predominance is a risk factor both for developing sustained hypertension [2] and for developing cardiovascular disease (CVD) in those with sustained hypertension [3,4]. Markers of sympathetic activity such as norepinephrine (NE) levels are generally higher in hypertensives than normotensives, both at baseline and when confronted with a psychosocial stressor [5–9]. Furthermore,

hypertensives tend to have greater total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) levels, and lower levels of high-density lipoprotein cholesterol (HDL-C) [10]. The prevalence of hyperlipidemia in hypertension is as high as 40% [11]. It is suggested that acute lipid stress responsivity may reflect processes that contribute to the development of raised blood cholesterol levels. Greater acute stress lipid responses predicted higher LDL-C, HDL-C, and TC/HDL-C ratios several years later [12]. However, little is known about interactions between sympathetic activity and plasma lipid levels during stress responses in hypertensive patients.

Like NE, a raise in TC and its fractions can be elicited in response to acute psychosocial stressors in a laboratory setting [13,14]. One explanation for stress-related changes in blood lipids is through multifactorially caused changes in hemoconcentration [15], whereby acute loss of plasma

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volume within the intravascular space concentrates non-diffusible blood constituents and, thereby, increases blood lipid concentration [16,17]. However, findings are inconsistent, with some studies suggesting elevations in blood lipids even after adjustment for changes in hemoconcentration [18,19].

A second explanation for stress-related changes in lipid concentrations is the metabolic effect of catecholamine spillover in response to acute psychosocial stress. Increases in circulating NE induce lipolysis and release free fatty acids into the circulation [20], which, in turn, serve as a substrate for the resynthesis of TG and hepatic production of very low-density lipoprotein cholesterol [21]. Increases in NE are associated with elevated plasma levels of TC, LDL-C, and HDL-C [22]. Furthermore, blood levels of TC, LDL-C, and TG are lower and HDL-C levels are higher with α -adrenergic than with β -adrenergic blockade in patients with systemic hypertension [23–25]. However, effects of an acute psychosocial stressor shown to elicit large increases in NE on blood lipid levels have not been compared between hypertensive and normotensive individuals, particularly not while controlling for hemoconcentration. Research linking NE to blood lipid increases in response to stress may be important for developing target interventions to reduce CVD risk in hypertension.

The purpose of this study was 3-fold: First, we investigated blood lipid changes to the widely used Trier Social Stress Test (TSST), which combines a public-speaking task and a mental arithmetic task performed in front of an audience [26], in a sample of otherwise healthy and unmedicated hypertensive and normotensive men. We hypothesized that hypertensives exhibit exaggerated changes in blood lipids to acute stress. Second, to investigate underlying mechanisms of such differences, we tested whether blood lipid levels are associated with NE levels and mean arterial blood pressure (MAP), both at baseline and in response to stress. Because the TSST is known to evoke large increases in plasma NE [27,28], we hypothesized that NE levels would directly relate to stress-induced changes in plasma lipids corrected for hemoconcentration. Third, we tested whether interactions between MAP and NE stress change affected stress-induced changes in lipids.

2. Methods

2.1. Study population

The study was part of a project assessing stress reactivity in systemic hypertension [9] and was formally approved by the Ethics Committee of the State of Zurich, Switzerland. We analyzed plasma lipids from 45 subjects representing the final sample for this part of the study. All participants provided written informed consent. With the aid of the Swiss Red Cross of Zurich and through advertisements, we recruited nonsmoking hypertensive and normotensive men who, apart from hypertension,

were otherwise in excellent physical and mental health, as confirmed by an extensive health questionnaire and telephone interview. Specific exclusion criteria, obtained by subjects' self-report, were as follows: regular heavy exercise, alcohol and illicit drug abuse, any heart disease, varicosis or thrombotic diseases, elevated blood glucose level and diabetes, elevated cholesterol level, liver and renal diseases, chronic obstructive pulmonary disease, allergies and atopic diathesis, rheumatic diseases, and current infectious diseases. In addition, participants were included only if they reported taking no medication, either regularly or occasionally. If the personal or medication history was not conclusive, the subjects' primary care physician was contacted for clarification.

2.2. Assessment of hypertension

After a 15-minute rest, 3 seated screening blood pressure (BP) measurements were obtained on 3 separate days by a fully automated sphygmomanometry device (Omron 773; Omron Healthcare Europe, Hoofddorp, the Netherlands); and the average BP was computed. Subjects were categorized into hypertensive and normotensive individuals following the World Health Organization/International Society of Hypertension definition (systolic BP ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg) [29]. For the purpose of data reduction, the average MAP was calculated across all individuals according to the formula two-thirds diastolic BP + one-third systolic BP and was used in analyses. The screening procedure yielded 22 hypertensive and 23 age-matched normotensive men (mean \pm SEM age, 45.0 ± 2 years; range, 22–65 years) with a complete plasma lipid profile.

2.3. Psychosocial stress procedure

All experimental sessions commenced between 2:00 PM and 4:00 PM and lasted for approximately 2 hours. Participants abstained from food and drinks (other than water) for 2 hours before the experiment, and from physical exercise, alcohol, and caffeinated beverages from the evening before the test day. To inflict acute psychosocial stress, we used the standardized and well-established TSST, which elicits social evaluative threat and unpredictability of the stressor [26,30]. The task comprises 5 minutes of preparation, a mock job interview (5 minutes), and a mental arithmetic task (serial subtraction, 5 minutes) in front of an unknown panel of 1 man and 1 woman [26]. The TSST enables a naturalistic exposure to a psychosocially stressful situation and has repeatedly been found to induce profound endocrine and cardiovascular responses [26,30]. During the 45 minutes before introduction to the TSST and another 60 minutes after task completion, subjects remained seated in a quiet room.

Via an indwelling catheter, blood samples were obtained under resting conditions (ie, baseline levels) 1 minute before subjects were introduced to the TSST and immediately after completion of the TSST. Additional blood

samples were drawn 20 and 60 minutes after completion of the TSST. At the end of blood sampling, participants were debriefed; and participation was financially remunerated with 80 Swiss francs.

2.4. Biochemical measures

For NE assessment, venous blood was drawn into EDTA-coated Monovette tubes (Sarstedt, Numbrecht, Germany) and immediately centrifuged for 10 minutes at 2000g; obtained plasma was stored at -80°C until analysis. Plasma NE was determined by high-pressure liquid chromatography (detection limit, 0.25 pg/mL; inter- and intraassay coefficients of variation, $< 5\%$; Laboratory for Stress Monitoring, Göttingen, Germany). All samples from one subject were analyzed in the same run.

Blood lipids (TC, HDL-C, and TG) were measured by standard laboratory procedures in plasma (Synlab, Augsburg, Germany) using a calorimetric system (AU, Olympus, Hamburg, Germany) and are expressed in milligrams per deciliter. Low-density lipoprotein cholesterol was calculated using the Friedewald formula: $\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/2.19)$. Hemoglobin (grams per deciliter) and hematocrit (percentage) were obtained by processing whole blood collected in 2.7-mL EDTA tubes (Sarstedt, Rommelsdorf, Germany) on an automated hematology system (Advia 120, Bayer Diagnostics, Fernwald, Germany).

2.5. Hemodynamic measures

Heart rate data were obtained continuously via a portable heart rate monitor (Polar system, S810; Polar, Kempele, Finland) [31]. Following previous methods, BP was measured continuously from 5 minutes before the introduction to the TSST to 5 minutes after completion of the TSST (ie, average of speech and arithmetic BP) by the Vasotrac APM205A device (Medwave, St Paul, MN) [9].

2.6. Statistical analyses

All calculations were performed using SPSS (11.0.1) software packages (SPSS, Chicago, IL). Data are presented as mean \pm SEM. All tests were 2-tailed with the level of significance set at $P < .05$ and the level of borderline significance set at $P \leq .10$. In the case of missing data, cases were excluded listwise. Data were normally distributed and homogeneity of variance was given as indicated by Kolmogorov-Smirnov and Levene tests, respectively. These tests were performed before statistical procedures were applied.

We corrected all plasma lipid levels for stress hemoconcentration following previous methods by computing stress-induced changes in plasma volume (ie, stress hemoconcentration) from hemoglobin and hematocrit measures according to the formula by Dill and Costill [32,33].

Univariate analyses of variance (ANOVAs) were calculated to test for differences in hypertensive vs normotensive subjects in terms of demographic characteristics and baseline levels of NE and plasma lipids (Table 1).

Table 1
Characteristics of the 45 subjects studied

	Hypertensives (n = 22)	Normotensives (n = 23)	P
Age (y)	46.3 \pm 3.0	44.6 \pm 2.4	.659
Body mass index (kg/m ²)	27.1 \pm 0.65	25.1 \pm 0.49	.016
Systolic BP (mm Hg)	150 \pm 1.9	121 \pm 1.5	<.001
Diastolic BP (mm Hg)	95 \pm 1.8	78 \pm 1.4	<.001
MAP (mm Hg)	113.3 \pm 1.7	92.4 \pm 1.4	<.001
Cholesterol (mg/dL)	217.8 \pm 8.9	210.4 \pm 7.7	.534
TG (mg/dL)	218.6 \pm 34.7	216.6 \pm 25.5	.962
LDL-C (mg/dL)	134.6 \pm 5.8	129.8 \pm 5.6	.554
HDL-C (mg/dL)	49.5 \pm 3.0	49.8 \pm 2.8	.938
NE (pg/mL)	426.6 \pm 24.7	327.8 \pm 24.0	.006

Values are given as mean \pm SEM. Plasma lipid and stress hormone measures reflect plasma levels at baseline (ie, immediately before the stressor).

To test whether hypertensives exhibit exaggerated changes of blood lipids to acute stress as compared with normotensives, we calculated repeated-measures ANOVAs with 2 groups (hypertensive, normotensive) as an independent variable and the 4 periods in which lipids were measured (baseline, 1 minute, 20 minutes, and 60 minutes poststress) as repeated dependent variables. Repeated-measures ANOVAs were also calculated for group differences in repeated NE, BP, hematocrit, and hemoglobin levels. We applied the Huynh-Feldt correction for the degrees of freedom.

To investigate underlying mechanisms of elevated BP effects on blood lipids, we used MAP as a continuous variable instead of categorizing hypertensives and normotensives into a dichotomous variable. The use of a continuous variable increases the effect size and the statistical power in regression analyses [34]. This is particularly desirable in the present analyses because we are including several control variables, which can affect statistical power in a sample of this size. To test whether blood lipid levels at baseline are associated with NE levels at baseline and MAP, we calculated hierarchical linear regression equations entering all independent variables in 1 block. As the dependent variable, we entered baseline levels of lipid measures. As independent continuous variables, we entered MAP and NE baseline levels. To test whether stress-induced blood lipid changes are associated with stress changes in NE and MAP, we again calculated hierarchical linear regression equations regressing MAP and stress change in NE in 1 block on lipid stress changes due to stress (lipid level immediately after stress minus baseline level). As independent variables, we entered MAP and the stress change of NE. To test whether an interaction between MAP and NE stress change affected stress changes in lipids, we additionally included interaction terms into the previous regression equations. Interaction terms were formed by multiplying MAP with the NE stress change score.

We controlled for age and BMI in all regression analyses. However, to prevent model overfitting, none of the regression models considered more than 5 independent variables [35]. The optimal total sample size of $n = 42$ to

detect an expected effect size of 0.35 in regression analyses with a power between 0.80 (maximum of 5 predictors) and 0.95 (minimum of 1 predictor) was calculated a priori with the statistical software G-Power [36]. All regression parameters were Z-transformed before regression analyses to allow computation of interaction terms. Change scores

for plasma lipids were computed on original data and transformed subsequently. We did not perform statistical adjustment for multiple tests because, in the case of our specific preestablished hypotheses, statistical adjustment might deem truly and clinically important associations insignificant [37].

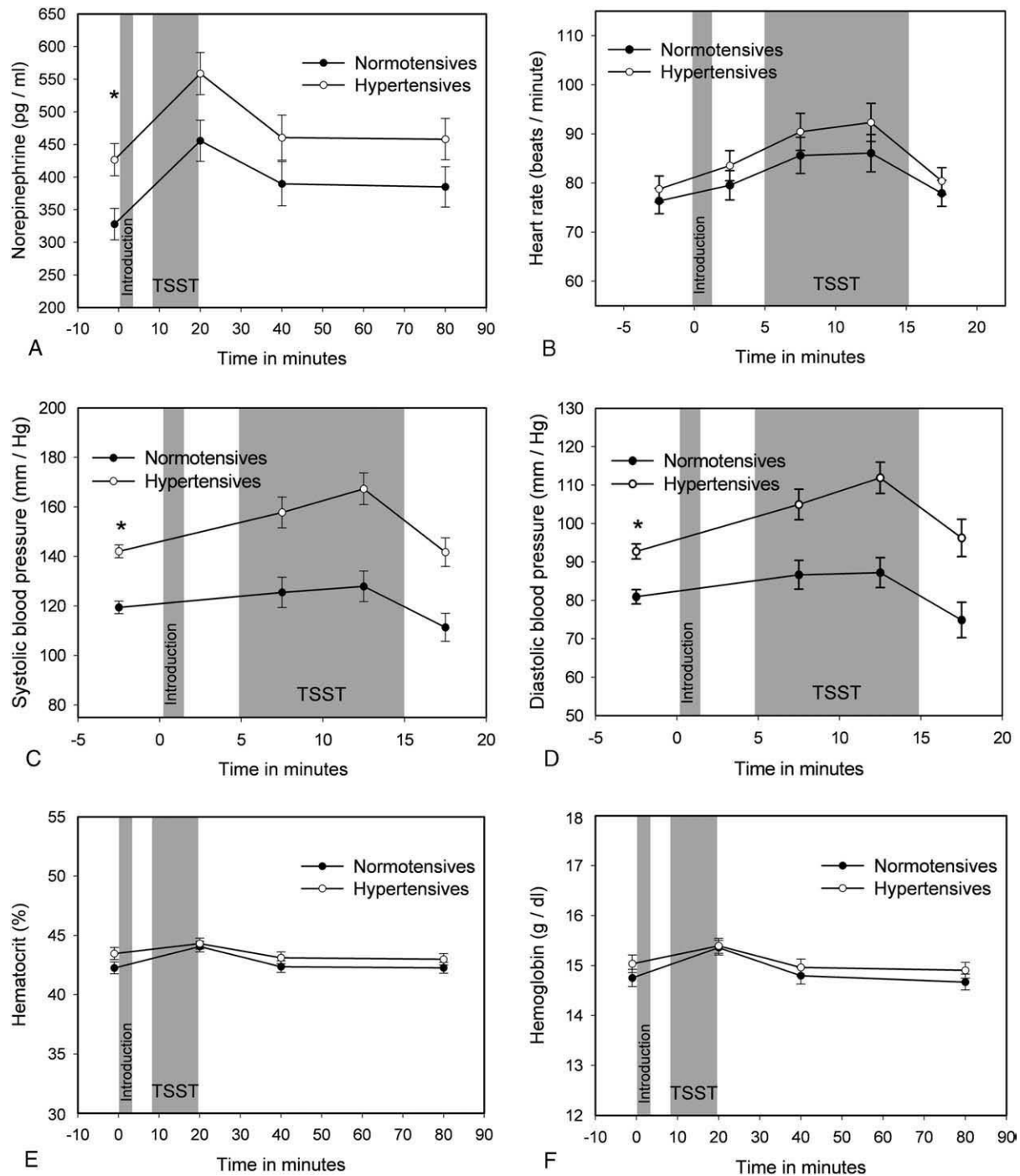


Fig. 1. A to F, Changes of NE, heart rate, BP, hematocrit, and hemoglobin to psychosocial stress in hypertensive and normotensive men. Values are means \pm SEM. Across all subjects, the stressor elicited significant responses in NE, HR, BP, hematocrit, and hemoglobin (P s < .001). Hypertensive men showed relatively higher NE (P = .033, A) as well as higher systolic and diastolic BP levels (P s < .001) before and after stress (C and D). Hypertensives and normotensives did not significantly differ in their hematocrit (E) and hemoglobin (F) levels before and after stress.

3. Results

3.1. Subjects' characteristics

Table 1 provides the characteristics of the 45 subjects studied according to their hypertension status. As expected, hypertensive subjects had higher systolic and diastolic BP than normotensive subjects. In addition, hypertensives had higher BMI and also higher plasma levels of NE at rest (ie, immediately before stress) than normotensives.

3.2. Hypertension status and stress changes of lipids and other physiologic parameters

The TSST caused significant increases in NE, HR, BP, hematocrit, and hemoglobin (Fig. 1) as well as in TG (all P s < .05), with a trend toward statistical significance seen for TC (P < .07) (Fig. 2). In addition, HDL-C showed an immediate decrease to stress exposure in all subjects (P < .05).

3.2.1. Lipids

Between rest and 60 minutes poststress (Fig. 2), repeated-measures ANOVA revealed a greater response to stress in TC (interaction group-by-stress: $F[2.5/107.8] = 3.31$, $P = .030$) and LDL-C (interaction group-by-stress: $F[3.0/127.6] = 2.92$,

$P = .037$) in hypertensives than normotensives. In contrast, there was no difference in the stress response for HDL-C ($P = .52$) and TG ($P = .13$) levels between the 2 groups.

3.2.2. NE, HR, BP, hematocrit, and hemoglobin

Fig. 1 shows that hypertensives had higher NE levels both at baseline and after psychosocial stress relative to normotensives (group effect: $F[1/43] = 4.83$, $P = .033$). As expected, systolic BP and diastolic BP were higher in hypertensives than in normotensives (P s < .001). In contrast, hypertensives did not significantly differ from normotensives before and after stress in terms of HR, hematocrit, and hemoglobin (P s > .26).

3.3. Associations between lipid levels and NE

3.3.1. At rest

Norepinephrine baseline level significantly predicted TC ($\beta = .32$, $P = .030$, $\Delta R^2 = .105$). This effect lost significance ($P = .22$) after controlling for age ($P = .09$), BMI ($P = .93$), and MAP ($P = .66$). Similarly, NE baseline level significantly predicted LDL-C ($\beta = .32$, $P = .032$, $\Delta R^2 = .102$). This effect became nonsignificant ($P = .22$) after controlling for age ($P = .20$), BMI ($P = .85$), and MAP ($P = .76$). There

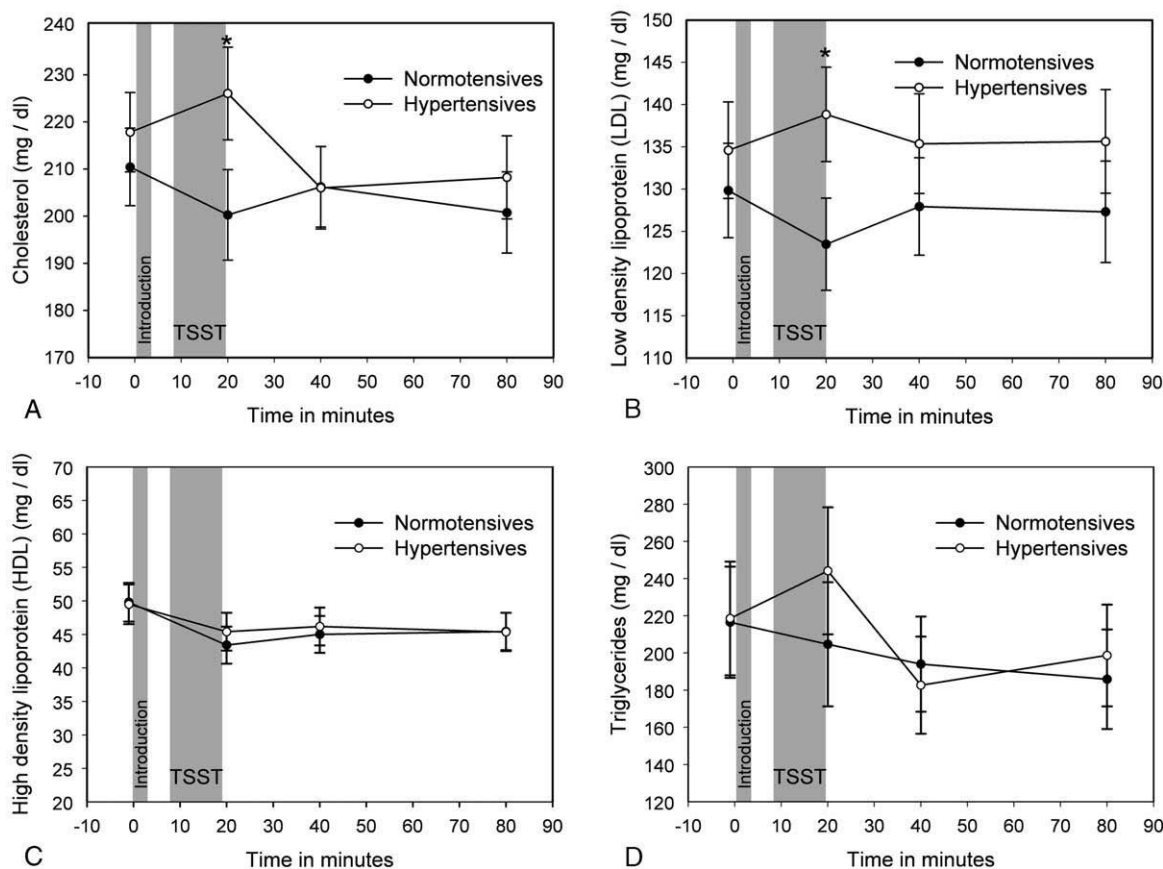


Fig. 2. A to D, Plasma lipid changes to psychosocial stress in hypertensive and normotensive men (A–D). Values are means \pm SEM. Stress elicited significant changes in TG and HDL-C (P s < .05), with a similar trend seen for TC (P < .07) (A–D). Hypertensives showed greater TC (A) and LDL-C (B) changes relative to normotensives (P s < .037). The groups were not significantly different in HDL-C (C) and TG (D) stress responses.

were no associations between NE baseline levels and TG ($P = .77$) and HDL-C ($P = .23$).

3.3.2. Stress changes from baseline to immediately poststress

Mean arterial pressure and stress change in NE predicted TC and LDL-C stress changes independently of each other; MAP and NE stress change together explained 28% of the total variance in stress change of TC (MAP: $\beta = .41$, $P = .003$, $\Delta R^2 = .166$; NE: $\beta = .35$, $P = .010$, $\Delta R^2 = .125$) and 23% of the total variance in stress change of LDL-C (MAP: $\beta = .32$, $P = .024$, $\Delta R^2 = .101$; NE: $\beta = .38$, $P = .008$, $\Delta R^2 = .143$). Additional controlling for age and BMI did not significantly change results of TC but did change results of LDL-C (Table 2). Mean arterial pressure significantly predicted TG stress change (MAP: $\beta = .32$, $P = .040$, $\Delta R^2 = .092$) after controlling for age ($P = .11$) and BMI ($P = .10$). Additional controlling for NE stress change ($P = .68$) did not significantly affect this relationship. Neither MAP nor NE stress change significantly predicted HDL-C stress change.

3.4. Interactions of MAP with NE stress change and stress change in lipids

The MAP-by-NE stress change interaction significantly predicted stress change in LDL-C ($\beta = -.27$, $P = .045$, $\Delta R^2 = .072$) and in HDL-C ($\beta = -.61$, $P < .001$, $\Delta R^2 = .39$) when controlling for MAP and NE stress change. Additional controlling for age and BMI marginally affected the relationship with stress change in LDL-C ($\beta = -.25$, $P = .073$) but not with stress change in HDL-C ($\beta = -.58$, $P < .001$). The interaction between MAP and NE stress change was not significantly associated with stress changes in TC and in TG.

Table 2
Hierarchical regression analyses for associations between stress changes in NE and lipids

Variables entered	Standardized β -coefficient	t	P	R^2 change
Cholesterol stress change				
Age	.08	.53	.43	.00
Body mass index	.15	.80	.43	.01
MAP	.35	2.51	.016	.11
NE stress change	.35	2.48	.017	.11
LDL-C stress change				
Age	.08	.55	.59	.01
Body mass index	.21	1.38	.18	.03
MAP	.24	1.65	.11	.05
NE stress change	.38	2.70	.01	.13
TG stress change				
Age	.26	1.65	.11	.06
Body mass index	-.28	-1.72	.09	.06
MAP	.32	2.09	.043	.09
NE stress change	-.06	-.37	.71	.00
HDL-C stress change				
Age	-.04	-.26	.80	.00
Body mass index	.34	2.06	.046	.09
MAP	.02	.14	.89	.00
NE stress change	.10	.62	.54	.01

Stress change indicates the difference between immediate poststress level and baseline.

4. Discussion

We examined whether hypertensives exhibit exaggerated changes of blood lipids to acute stress and whether blood lipid levels are associated with NE plasma levels and MAP (as continuous assessment of hypertension status), both at baseline and in immediate response to stress. Moreover, we tested whether interactions between MAP and NE stress change affected stress-induced changes in lipids. Our findings extend prior research on acute stress reactivity in hypertension and associated risk for CVD, elucidating potential mechanisms involving associations between MAP, sympathetic activity, and plasma lipid levels during acute stress responses in hypertensive patients.

We found that hypertensives showed greater TC and LDL-C stress changes that were sustained up to 60 minutes poststress than normotensives. Screening MAP, independent of NE, BMI, and age, was associated with immediate stress changes in TC, TG, and LDL-C such that the higher the MAP was, the greater were the stress-induced changes in plasma levels of these proatherogenic blood lipids. Moreover, independent of age, BMI, and MAP, greater stress-induced NE release was independently associated with increased immediate stress-associated changes in TC and LDL-C levels. These findings suggest that both NE and MAP are important factors associated with the observed differences between hypertensives and normotensives in the lipid response to stress. In other words, our data suggest that there is a second path linking BP or hypertension status with short-term stress changes of proatherogenic lipids that is not mediated via NE, as would be predicted [17,23,24]. In addition, greater baseline NE levels were associated with higher baseline TC and LDL-C levels in hypertensives and normotensives, although not independent of age and BMI. This is consistent with previous studies showing that blood lipid levels are partially regulated by steady-state sympathetic activity [38].

Our findings extend previous observations of acute lipolytic properties of NE [20] for the first time to hypertensive patients and suggest a potentially important clinical mechanism contributing to the association between stress and CVD [39–41], particularly in hypertension.

How could hypertension status and NE influence blood lipid changes? Lipolysis is thought to occur through catecholamine stimulation of adrenergic receptors expressed on the cell surface membrane of fat cells [20,42]. Notably, some recent findings also suggest additional mechanisms for NE-induced lipolysis not related to adrenoceptors [42]. Our data further suggest that during acute stress hypertension might be associated with hitherto unknown lipid metabolic processes that are unrelated to NE change and stress hemoconcentration [17]. However, we feel that it is premature to speculate on such mechanisms because stress-triggered NE release from sympathetic nerve endings and the adrenal medulla only partially reflects sympathetic nervous system activity during stress. For instance, we did not assess

adrenergic receptor functioning and polymorphisms and their role in stress-induced lipid changes in relation to hypertension [43].

Although it is known that elevated baseline levels of TC and LDL-C are associated with increased risk of atherogenesis [10], it is unknown whether acute changes in lipids, as observed in our study, are of clinical relevance. To what extent do acutely increased lipids deposit at sites of endothelial lesions, thereby contributing to the initiation of the fatty streak, atherosclerosis progression, and ultimately coronary occlusion [44]? Do such processes increase the CVD risk particularly in hypertension? It is suggested that the frequency of stress-induced bouts of NE release are of clinical importance [45], and the same may be true in terms of changes in lipid levels. Over a lifespan, an acutely exaggerated lipid response could contribute to clinically manifest atherosclerosis in hypertensives. Such reasoning is corroborated by prior research suggesting that, among hypertensives, exaggerated behaviorally evoked cardiovascular reactivity is associated with greater carotid intima-media thickness, a subclinical marker of atherosclerosis [46].

The interaction between MAP and NE stress change was negatively associated with the immediate stress-associated change of HDL-C ($\beta = -.64$) and LDL-C ($\beta = -.31$). In other words, the higher the MAP and the NE stress response were, the stronger was the decrease of HDL-C and LDL-C. A negative association may be anticipated with the potentially antiatherogenic lipid HDL-C, which corresponds with the idea that a decrease in “good lipids” in hypertensives during stress increases CVD risk [1,39]. However, MAP and NE stress change were also negatively associated with the potentially proatherogenic lipid LDL-C, which seems counterintuitive; however, the association was not independent of age and BMI and should therefore not be overinterpreted. Thirty-nine percent of the total variance in HDL-C was explained by the interaction of MAP with NE. This suggests a mechanism potentially mediating cardiovascular risk of hypertension in response to mental stress. Furthermore, HDL-C levels were reduced immediately after stress in all subjects, suggesting that acute stress effects on HDL-C may lead to potential atherosclerotic harm in healthy individuals independent of NE.

Our study possesses several strengths, including recruitment of apparently healthy and unmedicated subjects with reasonable health habits. This is important because blood lipid metabolism is affected by numerous drugs (including antihypertensives) and lifestyle factors [47]. Potential confounding factors were reduced in the analyses by controlling for age and BMI. In addition, plasma lipid levels were corrected for stress-induced shifts in hemoconcentration before all analyses. The study also has its limitations. The significant associations between MAP, NE levels, and their interactions in determining lipid levels are correlational and do not prove a causal link. For instance, some investigators suggest that having high normal BP and concomitant elevated serum cholesterol may lead to

exaggerated cardiovascular responses to stress due to endothelial dysfunction resulting from abnormalities of lipid metabolism [48]. Because of the sample size, conclusions pertaining to relationships observed between MAP and NE and specific lipids must be drawn with caution. In addition, our findings were obtained in a sample of apparently healthy men with BP in the normotensive and mildly hypertensive range and may not be generalizable to individuals with more severe hypertension and to women. Furthermore, we did not include a nonstressed control group to validate whether changes in lipid levels were unequivocally caused by the TSST. However, because decrease in TC takes several hours during the morning [49] and LDL-C and HDL-C both remain relatively constant over 24 hours [50], it is likely that diurnal effects did not evidently affect the time course of changes in plasma lipids.

In sum, we found evidence for our hypothesis that screening MAP and NE stress change are associated with acute stress changes of several blood lipids that are associated with CVD. By eliciting a proatherogenic lipid profile, stress may accelerate the atherogenic process early on in otherwise healthy hypertensive men or may even contribute to artery occlusion in coronary patients. Our observations require replication in larger samples and other populations, and the underlying mechanisms remain to be elucidated.

Acknowledgment

The study was funded by research grant 2003 from the University of Zurich, Switzerland (to PHW). We thank the Swiss Red Cross of Zurich, particularly BM Frey, MD, and H Jehle, for their invaluable help in study enrollment.

References

- [1] Kaplan NM. Importance of coronary heart disease risk factors in the management of hypertension. An overview. *Am J Med* 1989;86:1-4.
- [2] Palatini P, Longo D, Zaetta V, Perkovic D, Garbelotto R, Pessina AC. Evolution of blood pressure and cholesterol in stage 1 hypertension: role of autonomic nervous system activity. *J Hypertens* 2006;24:1375-81.
- [3] Julius S, Nesbitt S. Sympathetic overactivity in hypertension. A moving target. *Am J Hypertens* 1996;9:113S-20S.
- [4] Everson SA, Lynch JW, Kaplan GA, Lakka TA, Sivenius J, Salonen JT. Stress-induced blood pressure reactivity and incident stroke in middle-aged men. *Stroke* 2001;32:1263-70.
- [5] Goldstein DS. Plasma catecholamines and essential hypertension. An analytical review. *Hypertension* 1983;5:86-99.
- [6] Rahn KH, Barenbrock M, Hausberg M. The sympathetic nervous system in the pathogenesis of hypertension. *J Hypertens Suppl* 1999;17:S11-4.
- [7] Scheuch K, Hanefeld M, Grässler J, Seibt R, Naumann HJ. Hypertriglyceridaemia in mild hypertension: impact on cardiovascular and hormonal reactivity under different stress tests. *J Hum Hypertens* 1999;13:533-9.
- [8] Flaa A, Mundal HH, Eide I, Kjeldsen S, Rostrup M. Sympathetic activity and cardiovascular risk factors in young men in the low, normal, and high blood pressure ranges. *Hypertension* 2006;47:396-402.

- [9] Wirtz PH, von Känel R, Mohiyeddini C, Emini L, Ruedisueli K, Groessbauer S, et al. Low social support and poor emotional regulation are associated with increased stress hormone reactivity to mental stress in systemic hypertension. *J Clin Endocrinol Metab* 2006;91:3857–65.
- [10] Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA* 1986;256:2835–8.
- [11] Bona KH, Thelle DS. Association between blood pressure and serum lipids in a population. The Tromsø Study. *Circulation* 1991;83:1305–14.
- [12] Steptoe A, Brydon L. Associations between acute lipid stress responses and fasting lipid levels 3 years later. *Health Psychol* 2005;24:601–7.
- [13] Niaura R, Stoney CM, Herbert PN. Lipids in psychological research: the last decade. *Biol Psychol* 1992;34:1–43.
- [14] Muldoon MF, Herbert TB, Patterson SM, Kameneva M, Raible R, Manuck SB. Effects of acute psychological stress on serum lipid levels, hemoconcentration, and blood viscosity. *Arch Intern Med* 1995;155:615–20.
- [15] Allen MT, Patterson SM. Hemoconcentration and stress: a review of physiological mechanisms and relevance for cardiovascular disease risk. *Biol Psychol* 1995;41:1–27.
- [16] Patterson SM, Gottdiener JS, Hecht G, Vargot S, Krantz DS. Effects of acute mental stress on serum lipids: mediating effects of plasma volume. *Psychosom Med* 1993;55:525–32.
- [17] Bachen EA, Muldoon MF, Matthews KA, Manuck SB. Effects of hemoconcentration and sympathetic activation on serum lipid responses to brief mental stress. *Psychosom Med* 2002;64:587–94.
- [18] Stoney CM, Matthews KA, McDonald RH, Johnson CA. Sex differences in lipid, lipoprotein, cardiovascular, and neuroendocrine responses to acute stress. *Psychophysiology* 1988;25:645–56.
- [19] Stoney CM, Bausserman L, Niaura R, Marcus B, Flynn M. Lipid reactivity to stress. II. Biological and behavioral influences. *Health Psychol* 1999;18:251–61.
- [20] Amer P. Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab* 2005;19:471–82.
- [21] Hjelm Dahl P, Linde P. Adrenergic control of blood flow and lipolysis in human adipose tissue. In: Refsum HM, editor. *Alpha-adrenoreceptor blockers in cardiovascular disease*. London: Churchill Livingstone; 1985. p. 151–64.
- [22] McCann BS, Magee MS, Broyles FC, Vaughan M, Albers JJ, Knopp RH. Acute psychological stress and epinephrine infusion in normolipidemic and hyperlipidemic men: effects on plasma lipid and apoprotein concentrations. *Psychosom Med* 1995;57:165–76.
- [23] Ligueros M, Unwin R, Wilkins MR, Humphreys J, Coles SJ, Cleland J. A comparison of the effects of the selective peripheral alpha 1-blocker terazosin with the selective beta 1-blocker atenolol on blood pressure, exercise performance and the lipid profile in mild-to moderate essential hypertension. *Clin Auton Res* 1992;2:373–81.
- [24] Andersen P, Seljeflot I, Herzog A, Amesen H, Hjermann I, Holme I. Effects of doxazosin and atenolol on atherothrombotic risk profile in hypertensive middle-aged men. *J Cardiovasc Pharmacol* 1998;31:677–83.
- [25] Hobbs FR, Khan T, Collins B. Doxazosin versus bendroflumazide: a comparison of the metabolic effects in British South Asians with hypertension. *Br J Gen Pract* 2005;55:437–43.
- [26] Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 1993;28:76–81.
- [27] Buske-Kirschbaum A, Ebrecht M, Kern S, Hellhammer DH. Endocrine stress responses in TH1-mediated chronic inflammatory skin disease (psoriasis vulgaris)—do they parallel stress-induced endocrine changes in TH2-mediated inflammatory dermatoses (atopic dermatitis)? *Psychoneuroendocrinology* 2006;31:439–46.
- [28] Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, et al. Stress induced changes in human salivary alpha-amylase activity—associations with adrenergic activity. *Psychoneuroendocrinology* 2006;31:49–58.
- [29] Kjeldsen SE, Erdine S, Farsang C, Sleight P, Mancia G. 1999 WHO/ISH hypertension guidelines—highlights & ESH update. *J Hypertens* 2002;20:153–5.
- [30] Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 2004;130:355–91.
- [31] Nater UM, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol* 2005;55:333–42.
- [32] Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974;37:247–8.
- [33] Bacon SL, Ring C, Lip GY, Carroll D. Increases in lipids and immune cells in response to exercise and mental stress in patients with suspected coronary artery disease: effects of adjustment for shifts in plasma volume. *Biol Psychol* 2004;65:237–50.
- [34] MacCallum RC, Zhang S, Preacher KJ, Rucker D. On the practice of dichotomization of quantitative variables. *Psychol Methods* 2002;7:19–40.
- [35] Babyak MA. What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models. *Psychosom Med* 2004;66:411–21.
- [36] Buchner A, Faul F, Erdfelder E. G-Power: a priori, post-hoc, and compromise power analyses for the Macintosh. 2.1.2 ed. Trier: University of Trier; 1997.
- [37] Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998;316:1236–8.
- [38] Berlan M, Galitzky J, Riviere D, Foureau M, Tran MA, Flores R, et al. Plasma catecholamine levels and lipid mobilization induced by yohimbine in obese and nonobese women. *Int J Obes* 1991;15:305–15.
- [39] Wahrborg P. Mental stress and ischaemic heart disease: an underestimated connection. *Eur Heart J* 1998;19(Suppl O):O20–3.
- [40] Esch T, Stefano GB, Frichione GL, Benson H. Stress in cardiovascular diseases. *Med Sci Monit* 2002;8:RA93–RA101.
- [41] Strike PC, Steptoe A. Behavioral and emotional triggers of acute coronary syndromes: a systematic review and critique. *Psychosom Med* 2005;67:179–86.
- [42] Tavernier G, Jimenez M, Giacobino JP, Hulo N, Lafontan M, Muzzin P, et al. Norepinephrine induces lipolysis in beta1/beta2/beta3-adrenoreceptor knockout mice. *Mol Pharmacol* 2005;68:793–9.
- [43] Biaggioni I. Sympathetic control of the circulation in hypertension: lessons from autonomic disorders. *Curr Opin Nephrol Hypertens* 2003;12:175–80.
- [44] Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115–26.
- [45] McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med* 1998;338:171–9.
- [46] Gianaros PJ, Bleil ME, Muldoon MF, Jennings JR, Sutton-Tyrrell K, McCaffery JM, et al. Is cardiovascular reactivity associated with atherosclerosis among hypertensives? *Hypertension* 2002;40:742–7.
- [47] Ansell B. Future directions in lipid therapies. *Adv Ther* 2002;19:61–72.
- [48] Borghi C, Veronesi M, Bacchelli S, Esposti DD, Cosentino E, Ambrosioni E. Serum cholesterol levels, blood pressure response to stress and incidence of stable hypertension in young subjects with high normal blood pressure. *J Hypertens* 2004;22:265–72.
- [49] Jones PJ, Schoeller DA. Evidence for diurnal periodicity in human cholesterol synthesis. *J Lipid Res* 1990;31:667–73.
- [50] Miettinen TA. Diurnal variation of cholesterol precursors squalene and methyl sterols in human plasma lipoproteins. *J Lipid Res* 1982;23:466–73.