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Serum lipids and cardiovascular reactivity to stress

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Abstract

Several studies have reported an association between serum lipid levels and cardiovascular reactivity to laboratory stressors. Their findings, however, are equivocal. The inconsistencies may be due to shortcomings such as the small number of subjects, the inclusion of patient groups, no control for medication, and no control for age effects. Two studies are presented investigating the relationship in large groups of adolescent and middle-aged males and females. Cholesterol, triglycerides and HDL were measured. Subjects were exposed to mental stressors, and in one study also to a cold pressor test. In addition to heart rate and blood pressure, in one study impedance cardiography was used to measure pre-ejection period, stroke volume and total peripheral resistance. Canonical correlation analysis suggested an association between triglycerides and decreased cardiac reactivity to mental stressors in middle-aged females. Trends in the same direction were found in both middle-aged males and females with respect to reactivity to the cold pressor. These associations, however, were not confirmed when the extreme deciles of the triglyceride distributions were compared with respect to stress reactivity. The fact that associations were completely absent in youngsters but sometimes showed up in older persons suggested an age dependency of the association. In post hoc analyses, indeed, some evidence was found for stronger cardiac responsivity being associated with cholesterol specifically in relatively older males. In females, in contrast to this, the association between triglycerides and cardiac responsivity was stronger in the younger group. More detailed measurement techniques, of specifically vascular processes, may be needed to explore further the effects of sex and age on the association between lipids and stress reactivity.

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1. Introduction

The association between serum lipid levels and the risk of coronary heart disease (CHD) is well established. Lipids, including serum cholesterol and triglycerides, are transported in the circulation bound to (apo)proteins, the cluster together forming the lipoproteins. The different lipoproteins can be separated on the basis of their densities (lipid/protein ratios). The major classes of lipoproteins are very low-density lipoprotein (VLDL), mainly consisting of triglycerides, low-density lipoprotein (LDL), the main transporter of cholesterol, and high-density lipoprotein (HDL). HDL exhibits a reverse relationship with CHD risk. Serum lipid and lipoprotein levels have a substantial genetic/constitutional component (Heller et al., 1993; Snieder et al., 1997) and are also influenced by behavioral factors like diet (Hopkins, 1992), exercise (Tran et al., 1983) and smoking (Freeman et al., 1993). Periods of episodic or chronic stress have shown to be associated with elevated cholesterol levels. This has, for example, been demonstrated for undergoing an earthquake (Trevisan et al., 1992), job loss (Kasl et al., 1968), threat of unemployment (Mattiasson et al., 1990), perceived job insecurity (Siegrist et al., 1988), perceived workload (McCann et al., 1990) or examinations (van Doornen and van Blokland, 1987). A rise in cholesterol level during stress is, however, not a universal finding (Niaura et al., 1991).

The mechanism underlying a stress-induced cholesterol elevation is supposed to be the sympatho-adrenergic system, because adrenaline is a potent lipolytic agent. Exaggerated cardiovascular reactivity to stress has been proposed as a risk factor for future CHD (Matthews et al., 1986; van Doornen, 1991). The propensity of individuals to be sympathetically reactive to stress is generally assessed by exposing subjects to short-term laboratory stressors. Large individual differences in cardiovascular reactivity are generally observed, which seem to reflect differences in adrenergic mobilisation (Eisenhofer et al., 1985). Because the sympatho-adrenergic system is both involved in lipolysis and in stress reactions several studies have explored the association between cardiovascular reactivity to stress and serum lipids. According to this idea lipid levels and stress reactivity are associated because of a common mechanism: sympatho-adrenal activity.

There is yet another mechanism that might be responsible for a possible association. Hypercholesteremia has been shown to be associated with an augmented response to vasoconstrictors (Heistad et al., 1984) and impaired endothelial-dependent vascular relaxation (Casino et al., 1993). This points to a more peripheral vascular explanation for the association between stress reactivity and lipids.

The idea of a common sympathetic mechanism would show up as a relationship between cardiac responsivity and lipids, the more peripheral explanation would predict lipids to be associated with a stronger rise in peripheral resistance in response to stress.

To date, seven studies have investigated the relationship between lipid and lipoprotein levels and cardiovascular reactivity to laboratory stressors. The studies have been empirical in their approach in the sense that relationships between reactivity and lipids were studied without paying much attention to the physiological basis to expect such a relationship. Some refer briefly to the possible role of sympathetic activity. This might apply to an association of reactivity with cholesterol or triglyceride levels, but for HDL there is no clear physiological basis to expect an association with reactivity.

We will first summarize the empirical evidence for the existence of a relationship, and in case associations are observed, whether they support a common sympathetic basis or a peripheral explanation. A schematic overview of the studies is presented in Table 1.

McKinney et al. (1987) measured cholesterol, HDL and triglycerides in 50 physicians and exposed them to two mental stressors and the cold pressor test. Cholesterol and triglycerides correlated positively with the diastolic blood pressure (DBP) and total peripheral resistance (TPR) response to the cold pressor and to one of the mental tasks. For HDL, the reverse pattern was observed. Lipid parameters were unrelated to the heart rate (HR) and systolic blood pressure (SBP) reactions to the stressors. The correlations with DBP and TPR suggest an association between vascular responsiveness and lipids. Jorgensen et al. (1988) showed in a group of 59 mild hypertensives that those above the median with respect to the average HR response to two mental stressors had significantly higher total cholesterol and triglyceride levels than those below the median of HR reactivity. Results for a median split on the basis of SBP or DBP reactivity were not mentioned and so probably were not significant. Fredrikson and Blumenthal (1988) found no association between total cholesterol/HDL ratio and HR and BP responses to a mental arithmetic task in a group of 42 infarction patients. Fredrikson et al. (1991) exposed 60 healthy subjects in their 40s to six stressors. Compared to low-cholesterol subjects, high-cholesterol subjects showed a stronger SBP response, but the same DBP response across the tasks. HDL was unrelated to reactivity. Suarez et al. (1991) observed a negative correlation (-0.40) between the DBP response to mental arithmetic and cholesterol level in 24 healthy males. The SBP and HR responses were unrelated to cholesterol. HDL, LDL, VLDL and triglycerides levels were unrelated to reactivity. No relations were observed with the reactivity to another task. Owens et al. (1993) observed positive correlations between cholesterol level and both systolic and diastolic reactivity to the delivery of a short speech in a group of 48 subjects. No correlations were observed for two other tasks. In the most recent study by Burker et al. (1994), those above the median of blood pressure reactivity to a series of four stressors had higher levels of cholesterol and LDL and lower levels of HDL.

These results suggest some connection between lipid parameters and cardiovascular reactivity but the pattern of results is confusing, not consistent, and often contradictory between studies. A peculiar aspect is that within studies only for certain tasks an association was observed, whereas for other tasks the results were negative. The results do not allow a conclusion concerning the question whether

Table 1
Overview of studies to stress reactivity and lipid levels

Study	(1) Subjects (2) Age (3) Medication	Tasks	(1) Cardiovascular variables (2) Lipids	(1) Analysis (2) Age correction	Results	
McKinney et al. (1987)	(1) 49 m 1 f; hypertension + gastrointest. patients	Video game; mental arithmetic; cold pressor	(1) SBP, DBP, MAP, HR, TPR, SV	(1) Correlative	Video game:	No associations
	(2) 46.5 ($\pm$10.4)		(2) Cholesterol, HDL, triglycerides	(2) No	Mental arithmetic:	Trigl $\times$ DBP $r = +0.32$ Trigl $\times$ MAP $r = +0.35$ HDL $\times$ DBP $r = -0.46$ HDL $\times$ TPR $r = -0.35$
	(3) 48%				Cold pressor:	Chol $\times$ DBP r = +0.33 HDL $\times$ TPR $r = -0.44$
Jorgensen et al. (1988)	(1) 59 m; mild hypertension	Video game, Stroop	(1) HR, SBP, DBP	(1) Median split HR; response aggregated across tasks	High HR reactors Higher cholesterol and triglycerides	
	(2) 28–69, median 48 (3) No		(2) Cholesterol, triglycerides	(2) Median split age	No age interaction	
Fredrikson and Blumenthal (1988)	(1) 42 m MI patients	Mental arithmetic	(1) HR, SBP, DBP	(1) Median split cholesterol/HDL	No effects	
	(2) 28–66, median 52 (3) $\pm$70%, β-block; $\pm$50% calcium antag.		(2) Cholesterol, HDL, triglycerides	Task levels (2) Yes		

Table 1 (continued)

Study	(1) Subjects (2) Age (3) Medication	Tasks	(1) Cardiovascular variables (2) Lipids	(1) Analysis (2) Age correction	Results	
Fredrikson et al. (1991)	(1) 30 m 29 f (2) 30–55, m = 41.2 (3) No	Mirror drawing; mental arithmetic; stroop; cold pressor; handgrip; type A-interv.	(1) HR, SBP, DBP (2) Cholesterol, LDL, HDL	(1) Median split lipids Responses aggregated across tasks	High chol > SBP resp. High LDL > HR resp.	
Suarez et al. (1991)	(1) 24 m (2) 35–50 (3) No	Mental arithmetic; word identification	(1) HR, SBP, DBP, FBF (2) Cholesterol, HDL, LDL, VLDL, triglycerides	(1) Correlative (2) No	Mental arithmetic:	Chol × DBP, $r = -0.40$
Owens et al. (1993)	(1) 15 m 34 f (2) 40–55 (3) No	Handgrip; mirror drawing; speech task	(1) HR, SBP, DBP (2) Cholesterol, HDL, LDL, triglycerides	(1) Correlative (2) Yes	Speech task:	Chol × SBP, $r = 0.28$ Chol × DBP, $r = 0.28$
Burker et al. (1994)	(1) 62 m 37 f (2) 45–60 (m = 44.5 (m); m = 47 (f)) hypertensives (3) Discontinued	Mental arithmetic; public speaking; cold pressor; video game	(1) SBP, DBP, HR (2) Cholesterol, LDL, HDL, VLDL, apo-AI, Apo-AII, Apo-B, Lp(a)	(1) Median split SBP DBP, HR Responses aggregated across tasks (2) No	Total group: Women:	High SBP reactors, higher Chol, LDL, apo-B High DBP reactors, higher LDL, apo-B, lower HDL High HR reactors, higher Apo-AI/A-II ratio

lipids are associated with cardiac or with vascular responding. Only McKinney et al. (1987) measured the response of the total peripheral resistance and indeed observed a stronger response to be associated with elevated lipids. Other studies, however, showed HR and SBP reactions to be related to lipid levels, supporting an association of lipids with cardiac responding.

Limitations of these studies are the small number of subjects and the use of clinical groups in some of them, sometimes without control for medication. In McKinney's study half of the subjects were on antihypertensive medication, which probably comprised of β-blockers. In the study of Fredrikson and Blumenthal (1988) study more than two thirds of the subjects were using β-blockers. β-Blockers are known to influence both lipid profile and HR and DBP reactivity to stress. Other points of concern are the influence of age, sex, and female contraceptive use. In several studies the age range of the subjects was large. This may influence the size and the direction of the correlations, because serum cholesterol is known to rise with age, whereas HR responsivity declines with age (Snieder et al., 1995). Some studies corrected for age effects, whereas others did not. Because there are sex differences in lipoprotein levels and in cardiovascular reactivity it is indicated to analyse males and females separately. Contraceptive use is known to influence lipid profile thus contraceptive users should be excluded.

In the present study the relationship between cardiovascular reactivity and plasma lipid and lipoprotein levels was explored in four groups of middle-aged subjects, and in two subject groups of pubertal age. Males and females, and the older and younger groups will be analysed separately. To find out to what extent age is of influence on the association between lipids and reactivity, in case age is significantly associated with lipid level within a group, relations will be presented with and without age correction. Subjects on medication and female contraceptive users were excluded. In addition to heart rate and blood pressure the cardiac pre-ejection period (PEP) was measured as a specific sympathetic index and total peripheral resistance was measured in part of the groups. These parameters will give some clue to decide between the two explanatory options for an association between lipids and reactivity: either a common sympathetic mechanism or a more peripheral explanation.

2. Methods

2.1. Subjects

2.1.1. Study 1

This study is part of a larger project in which risk factors for CHD and stress reactivity were studied in 160 adolescent twin pairs and their parents (Boomsma et al., 1990, 1996). For the present analyses subjects were excluded when currently taking anti-hypertensives, lipid lowering drugs or contraceptives. The final groups consisted of 146 males, 141 females, 160 boys, and 135 girls. All subjects were paid Hfl. 25 (15 US$) for their participation.

2.1.2. Study 2

This study is part of a larger project studying the genetics of risk factors for CHD in a group of 206 middle-aged male and 220 middle-aged female twin members (Snieder et al., 1995). Exclusion criteria were the same as in study 1. The final groups consisted of 178 males and 169 females.

2.2. Procedure: study 1

Testing took place in a sound-attenuated, electrically shielded cabin, where subjects were seated in a comfortable chair. The sequence of events in fixed order was: practising for a couple of minutes the task that would be presented, a pause (10 min), a resting period (10 min), a choice reaction time task (8.5 min), a pause (10 min), a resting period (10 min), and a mental arithmetic task (8.5 min).

2.2.1. Tasks

As a mental arithmetic (MA) task, three digits were displayed consecutively on a screen during a 5-s interval. To induce time pressure, in the left margin of the screen an illuminated bar was displayed which gradually shortened and indicated the 5-s interval during each trial. The subject was instructed to add the three numbers and to compare their answer to another number displayed on the screen. The number presented could at random be the right or the wrong answer. Within a period of 2 s the subjects had to compare the answer with the digit displayed, and had to press a 'Yes' or a 'No' button dependent on their judgment of correctness. Feedback was furnished 2 s later concerning the correctness of their choice. After a further 2 s the next sum was presented. The task had 10 levels of difficulty: ranging from three one-digit numbers to three two-digit numbers. The level reached by the subjects after 36 practice trials determined the starting level of the task. An algorithm adapted the task difficulty according to performance. This procedure was developed to attain about equal stress levels for all subjects and a more or less constant stress level during the task.

In the choice reaction time (RT) task, each trial started with the simultaneous onset of an auditory warning stimulus and the appearance of a vertical bar on a monitor. After 5 s, a high or a low pitch tone was presented. Subjects had to react to high tones by pressing the 'Yes' key and to a low tone by pressing the 'No' key. Two seconds later feedback was presented. After a further 2 s, the next trial was presented.

2.3. Procedure: study 2

After a resting period of 3 min, three mental stress tasks were presented in fixed order: the RT task, the MA task (identical to those in study 1) and a tone-avoidance task (TA). Each task lasted 8.5 min with pauses of 3 min between them. After a post-stress relaxing period of 8.5 min, the session was concluded with a cold pressor test of 2 min. In the tone-avoidance task, subjects had to attend to the occurrence of an 'X' (500 ms) in one of the corners of a TV screen. A response had

to be made by pressing one of four buttons on a response panel opposite to the position where the stimulus was presented. Incorrect or too slow responses were punished with an 80-dB noise of 2 s duration presented by headphones. An algorithm continuously adapted the response criterion to the performance of the subject to attain about equal stress levels for the subjects and a more or less constant load during the task. The cold pressor test consisted of immersion of a hand into a bucket of ice water for 2 min.

2.3.1. Physiological recordings

Blood pressure was measured with the Dinamap (Critikon model 845XT) using an oscillometric technique. Blood pressure was measured three times during each condition. Responses were defined as the absolute differences between the average level during the tasks minus the last measurement during the preceding rest period. The electrocardiogram (ECG) and the impedance cardiogram (ICG) were obtained from disposable pregelled Ag/AgCl electrodes (AMI type 1650-005 Medtronic). Measuring electrodes for the ECG were placed on the sternum over the first rib and between the eighth/ninth ribs, just above the apex of the heart on the left lateral margin of the chest. An ECG ground electrode was placed on the abdomen above the right iliac crest. ECG was recorded with an amplifier with a time constant of 0.1 s and 1 MΩ input impedance.

The impedance cardiogram (ICG) was recorded with Nihon-Kohden Impedance Plethysmograph (AI-601G) with a tetrapolar spot electrode system as described by Boomsma et al. (1989). Current electrodes were placed on the back of the subject at the height of the cervical vertebra C4 and thoracic vertebra T9, respectively, imposing a current of 350 μA, with a frequency of 50 kHz and output impedance of >40 Ω. Measuring electrodes were placed directly below the laryngeal cartilage projection (Adam's apple) and over the tip of the sternum. The basal thorax impedance (Z_o) was continuously displayed and recorded by the experimenter three times per task. Thorax impedance change (ΔZ) was recorded with the Nihon-Kohden Differentiator (ED-601G) using a time constant of 5 ms and a high-frequency cut off of 75 Hz. This yielded the first derivative of the thorax impedance, dZ/dt. To get rid of artefacts both the ECG and ICG signal were filtered with 30.0-Hz high-frequency cut-off (6-dB roll off). ECG and ICG complexes were ensemble averaged in reference to the ECG R-wave, for periods of 101 s. For the cold pressor the averaging was done for two 1-min periods. The averaged complexes were used to compute the pre-ejection-period (PEP), left ventricular ejection time (LVET) and the maximal rate of change of impedance (dZ/dt). PEP was defined as the time in ms from Q-onset in the ECG till the B-point (upstroke) in the ICG. PEP is an index of cardiac contractility, and changes in PEP during stress are considered to reflect predominantly a β-adrenergic effect on the heart (Cacioppo et al., 1994). LVET was defined as the time in ms between the B-point in the ICG and the X-point in the ICG (representing the end of ejection: the closure of the aortic valves). dZ/dt-max and LVET can be used to calculate stroke volume (SV) using the formula proposed by Kubicek et al. (1974). Cardiac output was obtained by multiplying SV and HR. Combining the blood pressure measurements with the CO

value of the corresponding minute, total peripheral resistance (TPR) was estimated by the formula: $TPR = (MAP/CO) \times 80$ (in dyne-s/cm^5). Because the interindividual validity of impedance cardiography-derived measures is a matter of debate (Sherwood et al., 1990), percent changes from resting level were used to define responses for these variables.

In study 1, the amplitude of the dZ/dt registrations could not be scored in a considerable part of the subjects because of an undetected mistake in the adjustment of the range of the A–D converter. For this reason signal amplitudes were truncated and thus no assessment of SV was possible. For this reason in study 1 the ICG could only be used to measure the PEP.

2.4. Lipid measurements

In both studies EDTA blood was obtained after arrival in the lab by venipuncture after overnight fasting. Plasma was separated from cells after centrifugation for 10 min at 3000 rpm. Part of the plasma was kept at $+4$°C for lipid determinations within the next 5 days. Cholesterol and triglyceride levels were determined using enzymatic methods (Boehringer, Mannheim, Germany; CHOD-PAP kit no. 236691 and GPO-PAP kit no. 701904). HDL cholesterol was measured after precipitation with phosphotungstate Ng^{2+} of VLDL, IDL, and LDL according to Lopes-Virella et al. (1977).

2.5. Statistical analysis

The inconsistency of the results between tasks in previous studies may be due to the limited reliability of responses to single tasks. Taking the aggregated response across tasks will furnish a more reliable characterization of an individuals' typical responsivity (Kamarck et al., 1992). This will increase the chance of revealing an association with another quite stable characteristic, such as a serum lipid level. To maintain the comparability of the results of our two datasets, the cold pressor responses from study 2 were not included in the averaging. Thus, for both studies the responses to the mental stressors will be averaged. Another reason to treat the cold pressor separately is that it induces a type of response that differs from the response to mental stressors, namely a strong rise in peripheral resistance. This allows a more direct test of the possible association between lipids and vascular reactivity.

A response was defined as task level minus baselevel. For PEP and IBI this leads to negative values. To avoid confusion in the interpretation of the direction of the correlations with lipids, the sign of the correlations was reversed for PEP and IBI. Thus, for example, a positive correlation of cholesterol with PEP points to a stronger PEP response associated with higher cholesterol values.

First, zero-order correlations were calculated per group between the reactivity measures and lipid/lipoprotein values. To check on the influence of age, partial correlations adjusting for age will be presented as well.

For three reasons multivariate analyses were done also: (1) the large number of tests on the correlations elevates the chance of type I errors; (2) because both the lipids and lipoproteins are correlated, as are cardiovascular reactivity measures, the correlations are not independent results; (3) it is possible that some combination of lipid measures rather than a single measure is related to reactivity. Per group canonical correlation analysis was performed (Stevens, 1992). This analysis describes the number and nature of independent relationships between two sets of variables. The multivariate effects were tested by using Wilks' Lambdas. The *F*-transformed values are presented. The SPSS statistical package was used.

3. Results

3.1. Study 1

Lipid levels and the average cardiovascular reactions to the stressors of the four groups of subjects are presented in Table 2.

Means and standard deviations of cholesterol and HDL of these middle-aged males and females closely resemble the values from a large-scale Dutch epidemiological study including data from 5622 males and 6062 females in the age range from 40 to 49 years (Verschuren et al., 1994). The tendency for males of this age to have somewhat higher cholesterol and triglyceride levels, but lower HDL levels than females, is in line with this Dutch study and with findings abroad (Brunner et al., 1993; Schaefer et al., 1994). The values for the boys and girls, and the tendency of boys to have somewhat lower cholesterol and HDL values at the age of 16, correspond to reports from other studies on this age group in The Netherlands and abroad (Lauer et al., 1988; Srinivasan et al., 1993; Twisk et al., 1995).

In middle-aged males age was unrelated to lipid values and reactivity to the tasks. HDL was associated with a somewhat stronger HR reaction to the tasks ($r = 0.18$,

Table 2
Lipid levels and the average cardiovascular responses to two mental stressors (study 1)

	Middle aged males ($n = 146$)	Middle aged females ($n = 141$)	Boys ($n = 160$)	Girls ($n = 135$)
Age	47.9 (6.4) (35–65)	46.0 (5.9) (35–59)	16.8 (1.8) (14–21)	16.2 (1.8) (13–21)
Cholesterol (mmol/l)	5.8 (1.0)	5.6 (1.1)	4.1 (0.6)	4.3 (0.7)
HDL (mmol/l)	1.1 (0.3)	1.4 (0.3)	1.2 (0.2)	1.4 (0.3)
Triglycerides (mmol/l)	1.4 (0.7)	0.9 (0.4)	0.7 (0.3)	0.7 (0.2)
IBI (ms)	−62.9 (51.6)	−72.7 (51.5)	−82.2 (52.8)	−82.8 (49.6)
SBP (mmHg)	9.1 (6.1)	7.8 (7.1)	8.3 (5.4)	7.9 (6.1)
DBP (mmHg)	5.0 (3.2)	3.5 (3.7)	6.3 (3.6)	5.6 (3.5)
PEP (ms)	−6.4 (11.2)	−7.0 (10.4)	−4.8 (9.7)	−5.9 (9.4)

Table 3
Canonical variate–variable loadings of lipids and cardiovascular responses to the mental stressors in middle-age females (study 1)

	Canonical variate
Cholesterol	0.87
Triglycerides	0.83
HDL	−0.14
IBI	−0.45
PEP	−0.51
SBP	0.36
DBP	−0.10

$p < 0.05$). In middle-aged females, increasing age was associated with higher cholesterol ($r = 0.37$, $p < 0.01$), triglycerides ($r = 0.21$, $p < 0.05$) and HDL ($r = 0.17$, $p < 0.05$). A higher cholesterol level was associated with a smaller cardiac sympathetic reaction as indicated by the PEP, but this only after age correction ($r = -0.17$, $p < 0.05$). Higher triglyceride levels were associated with a stronger SBP response ($r = 0.18$, $p < 0.05$), though this correlation became non-significant after age correction. In the two young groups, no significant associations emerged between lipid values and reactivity. Thus the evidence for an association between lipids and reactivity in these rather large groups is meager. The scarce significant correlations are low and may well be chance findings.

Yet in a multivariate approach we found some evidence for an association. In the group of middle-aged females, canonical analysis (on the age-corrected values) revealed a significant multivariate effect ($F(12,331) = 1.83$, $p = 0.04$). The loadings of the variables on the one significant canonical variate are shown in Table 3.

This canonical variate is mainly defined by cholesterol and triglycerides, their loadings being 0.87 and 0.83, respectively. The canonical correlation was 0.33. This indicates that the set of lipid variables and the set of reactivity variables share 10% common variance. The same analysis on the non-age-corrected correlations showed a canonical correlation of 0.36. The negative loadings of IBI and PEP on this canonical variate suggest smaller cardiac responsivity to be associated with higher cholesterol and triglycerides in these middle-aged females.

Our second study, comprising middle-aged males and females, allows a check of the replicability of this finding.

3.2. Study 2

Lipid levels and the cardiovascular reactions to the stressors are presented in Table 4.

The response pattern to the mental stressors contrasted in the expected way with the one to the cold pressor. Mental stress led to a predominantly cardiac response and a slight decrease in peripheral resistance, whereas the cold pressor mainly triggered a vascular response. In Table 5 the correlations between lipid values and the cardiovascular responses are presented.

In both males and females, age was associated with both the lipid values and the reactivity measures. Because of this, we will focus on the age-corrected correlations further. In males, cholesterol showed a positive correlation with the PEP response to the mental tasks. HDL was associated with smaller DBP reactivity to both the mental tasks and the cold pressor. Triglyceride level was related to a slightly stronger rise in TPR in response to the cold pressor.

In females, HDL was associated with stronger SV and CO reactions to the mental tasks. Cholesterol level was associated with smaller responses of PEP and CO to the cold pressor, and triglycerides with a smaller heart rate response.

For comparability with study 1, canonical analyses were run for the mental tasks and the cold pressor separately. As in study 1, canonical analysis only evidenced an association between the sets of lipids and reactivity to the mental tasks for the females. The association, however, was weaker and statistically only a trend ($F(21,442) = 1.42,\ p = 0.10$). The canonical correlation was 0.30. Age correction does not seem to have been of any influence, the canonical correlation on the non-age-corrected data being 0.31. Table 6 (left column) shows the loadings of the variables on the canonical dimension.

HDL and the DBP, CO and SV reactivity showed the highest loadings in the same direction, suggesting a stronger cardiac reaction being associated with high HDL. Triglycerides load negatively on this dimension, suggesting an association

Table 4
Lipid levels and the average cardiovascular responses to three mental stressors and to the cold pressor (study 2)

	Males ($n = 178$)	Females ($n = 169$)
Age	43.1 (6.2)	45.1 (6.7)
	(35–63)	(65–61)
Cholesterol (mmol/l)	5.4 (1.0)	5.5 (1.1)
HDL (mmol/l)	1.1 (0.3)	1.4 (0.3)
Triglycerides (mmol/l)	1.3 (0.8)	1.0 (0.4)
Mental stressors		
IBI (ms)	−118.5 (59.7)	−103.3 (64.0)
SBP (mmHg)	12.0 (7.9)	10.4 (8.8)
DBP (mmHg)	6.7 (5.7)	6.1 (5.4)
PEP (ms)	−9.0 (11.6)	−10.3 (9.0)
SV (%)	5.5 (25.9)	11.7 (25.8)
CO (%)	20.8 (31.6)	27.2 (33.9)
TPR (%)	−1.2 (26.7)	−6.7 (26.0)
Cold pressor		
IBI (ms)	−49.1 (76.0)	−66.2 (62.3)
SBP (mmHg)	13.2 (7.8)	14.9 (9.7)
DBP (mmHg)	12.1 (7.6)	11.5 (7.2)
PEP (ms)	−1.5 (10.1)	−1.2 (7.0)
SV (%)	−8.8 (16.8)	−7.1 (15.4)
CO (%)	−3.8 (17.3)	0.7 (18.9)
TPR (%)	23.8 (41.3)	17.2 (23.7)

Table 5
Correlations between lipid values and the average cardiovascular responses to three mental stressors, and to the cold pressor (study 2) (age-corrected values between brackets)

	Age	Chol.	HDL	Trigl.
Males ($n = 178$)				
Mental stressors				
Age		0.19**		
IBI	−0.16*			
SBP				
DBP			−0.13 (−0.18*)	
PEP		0.15* (0.15*)		
SV%	0.19**			
CO%				
TPR%	−0.17*			
Cold pressor				
IBI				
SBP				
DBP			−0.17* (−0.20*)	
PEP	0.19**	0.16* (0.13)		
SV%	0.16*			
CO%	0.17*			
TPR%				0.17* (0.17*)
Females ($n = 165$)				
Mental stressors				
Age		0.48**	0.19*	0.19*
IBI				
SBP				
DBP				
PEP				
SV%	0.20**	0.17* (0.08)	0.20** (0.17*)	
CO%	0.20**		0.25** (0.22**)	
TPR%	−0.19**	−0.15* (−0.14)		
Cold pressor				
IBI				−0.19* (−0.19*)
SBP	0.20**			
DBP				
PEP		−0.08 (−0.15*)		
SV%				
CO%		−0.09 (−0.15*)		
TPR%				

*$p < 0.05$; **$p < 0.01$ (two-sided).

between high triglycerides and lower cardiac reactivity. In study 1 the canonical analysis also suggested such a relationship. One should, however, be careful in comparing the results of the analyses because the pattern of loadings of the lipid parameters on the canonical dimensions differ between the two studies.

A more direct test of the association between vascular responsiveness and lipids can be furnished by canonical analyses on the cold pressor results. For both males and females the canonical analyses showed a trend. ($F(21,482) = 1.44$, $p = 0.09$, and

$F(21,442) = 1.48$, $p = 0.08$, respectively)). Both canonical correlations were 0.28. Again the canonical correlations on the non-age-corrected data were about the same: 0.29 and 0.30 for males and females, respectively. In Table 6 (middle and right column) the loadings of the variables on the canonical variates are presented. In males high triglycerides seem to be associated with a stronger rise in TPR, a smaller rise (or stronger decrement) in CO, and smaller heart rate and SBP reactivity. The pattern of loadings in females is different. Here, also, triglycerides have the highest loading (even 1.00) on the canonical dimension. As in males, high triglycerides seem to be associated with a smaller heart rate reaction. The sign of TPR is, however, negative, which suggests an opposite relation between triglycerides and vascular reactivity in males and females. We should interpret the results of these canonical analyses with caution because the multivariate associations were only trends.

4. Discussion

The study of the relationship between cardiovascular reactivity to lab stressors and serum lipids has shown inconsistent and even contradictory findings. The purpose of the present study was to investigate this association in much larger groups of subjects than in previous studies, thereby controlling for confounding factors that may have been responsible for the inconsistencies between the previous studies: the use of medication, the inclusion of patient groups, and the influence of age and gender. Despite these improvements, no clear and convincing picture emerged. Although some significant correlations were observed, they were low and not consistent across the two studies. The canonical analyses on lipids and the reactivity to the mental stressors showed some association in the females. If anything, there was some slight evidence in both studies for an association in middle-aged females between triglycerides and smaller cardiac responding to mental

Table 6
Canonical variate–variable loadings of lipids and cardiovascular responses to the mental stressors in females and to the cold pressor in males and females (study 2)

	Females, mental stress	Males, cold pressor	Females, cold pressor
Cholesterol	0.33	0.01	0.46
Triglycerides	−0.60	0.74	1.00
HDL	0.73	0.04	−0.41
IBI	0.36	−0.49	−0.68
SBP	0.33	−0.54	−0.16
DBP	0.70	−0.13	−0.40
PEP	0.42	−0.05	−0.16
SV%	0.53	−0.37	0.28
CO%	0.65	−0.58	−0.03
TPR%	−0.33	0.59	−0.40

stress. Also the HR response to the cold pressor was negatively related to triglycerides in females. We should note, however, that the direct correlations between triglycerides and the reactions to the mental stressors were not significant. As a decisive test to find out if 'there is something there', we compared the highest and lowest deciles of the triglyceride distributions of the females in both studies with respect to reactivity to the mental stressors, and in study 2 also with respect to the cold pressor responses. These groups, differing strongly in triglyceride levels, did not differ on any of the reactivity measures. The analyses for the extreme cholesterol groups were also negative.

We expected that part of the positive results of previous studies, and the lack of correspondence between their results, might have been due to the influence of confounders. Control for these confounders in our two studies did not lead to a complete vanishing of a relationship between lipids and stress reactivity. Age correction sometimes led to a small rise and sometimes to a small decline in the size of correlation. The canonical correlations on age-corrected or non-age-corrected data differed only slightly. This implies that the positive findings of the previous studies probably cannot be completely attributed to the influence of age. What about the influence of another confounder mentioned: the use of medication? Including the subjects on medication in the canonical analyses even led to decrements in the canonical correlations. Thus the inclusion of subjects on medication in the previous studies cannot explain their more positive results.

Which other factors may play a role in the inconsistencies between our two studies and between the previous ones? Are certain tasks more successful than others? Because of the heterogeneity in tasks used in the previous studies, and the aggregation of task responses across very different types of tasks, this is difficult to answer. Tasks can differ in two respects: the size of the response they induce and the response pattern. Concerning the first aspect: the strength of a stressor can influence the chance to find an association with lipids. The mean response to the mental tasks was stronger in study 2. Nevertheless, the canonical analysis was significant in middle-aged females in study 1, but only a trend in study 2. Moreover, we used types of laboratory tasks that are commonly used in other reactivity studies and they induced responses of comparable magnitude. Thus, a difference in response amplitude can hardly be an explanation for the differences in results between our two studies or for our less positive results than previous studies. Concerning the aspect of the response pattern: if we assume that cholesterol level is associated with stronger vascular responding, an association should be revealed more clearly by a task inducing a predominantly vascular response. Of all tasks, the cold pressor test clearly raised TPR most. Indeed in males triglycerides were associated with a slightly stronger TPR reaction to the cold pressor. The correlation, however, was low ($r = 0.17$) and was absent in females. From the previous studies no further information concerning this point can be derived. The authors never commented on their observations that the response to one task was associated with a lipid level, whereas the response to another was not (Suarez et al., 1991; Owens et al., 1993). Moreover, the only study that also measured the cardiovascular response in more detail (McKinney et al., 1987) does not give information about the difference in response pattern between their tasks.

The possibility exists that the association between lipid levels and reactivity is not a simple linear association in the normal range of lipid values. Maybe lipid levels affect reactivity only when they reach high levels. Fredrikson et al. (1991) compared a high- and a low-cholesterol group with mean values of 7.2 and 5.6 mmol/l, respectively, and observed higher SBP reactivity in the high-cholesterol group. We compared in the males and females of both studies the extreme deciles of the cholesterol distribution with respect to reactivity. All low-cholesterol groups had a mean cholesterol level < 4.0 and all high-cholesterol groups had mean levels > 7.5. Apart from some incidental trends the results were not more positive than in the correlational analyses we presented.

This leaves us to explain why our results are less positive than of previous studies. The number of subjects in our study, especially, gives credence to a higher reliability of our findings. Whereas in the other studies the number of subjects varied between 24 and 59, our groups varied in size between 135 and 178. Moreover, a closer inspection of the number of significances reported by the previous studies gives rise to the suspicion that part of them may be chance findings. For example, in the study by McKinney et al. (1987), the responses to three tasks of six cardiovascular variables were correlated with three lipid parameters. Out of these 54 correlations, six were significant. In the study by Suarez et al. (1991) the responses to two tasks of four cardiovascular variables were correlated with five lipid parameters. Out of 40 correlations only one was significant. In the study by Owens et al. (1993) two out of 36 correlations were significant. Moreover, these correlations were across males and pre- and postmenopausal females. These groups differed, according to their own results, both in stress reactivity and in lipid values. This leads to the conclusion that the positive results reported should be looked at with great caution.

Should we completely rule out the possibility of some connection between stress reactivity and lipid values? Interestingly, no associations at all were observed in the youngsters but some significances showed up in the older groups. This suggests that the association might emerge with increasing age. To investigate this possibility, we divided each middle-aged group by median split into an older and a younger group, and checked whether correlations between lipids and reactivity would be more manifest in the older groups. For the groups in study 1 this was not the case. For the groups in study 2, however, some evidence was found for this supposition. In older males, cholesterol correlated positively with the cardiac reaction to the mental stressors. The correlations with PEP, CO and SV were 0.22, 0.24 and 0.22, respectively (all p values < 0.05). In the younger group the correlations were 0.13, -0.06 and -0.06. This was also the case for the response to the cold pressor. Cholesterol correlated 0.24 with the PEP response and 0.26 with the HR response (-0.12 and -0.11 in the younger group). In the group of older males, HDL correlated negatively with the SBP and DBP response to the cold pressor: -0.35 and -0.24, respectively (0.09 and -0.09 in the younger group). Strange enough, we found an opposite pattern when older and younger women were compared. In the younger group cholesterol was positively related to the CO and SV response to mental stress, 0.25 and 0.28 ($p < 0.05$), whereas the correlations in the older group

were both −0.05. In response to the cold pressor, triglycerides correlated positively with the SV and CO response (0.23 and 0.32) and negatively with the TPR response (−0.29). In the older group these correlations were close to zero. Thus, some evidence was found for an age dependency of the association between lipids and reactivity, though this was only the case in study 2. Moreover, it is difficult to explain why in females the association seemed to decrease with age.

A last comment concerns the tenability of the assumptions underlying the expectation of finding an association between acute stress reactivity and lipid levels. The argument generally found in the introduction of papers on this topic is that a common denominator of lipid levels and cardiovascular reactivity may be 'sympathoadrenal activity'. It is obvious that catecholamines are involved in lipolysis, and that cardiovascular reactivity is, at least partly, sympathetically driven. However, acute cardiovascular reactivity to stress is not necessarily an indicator of a generally elevated sympathetic tone. Even if this was the case, a further necessary assumption is that basal lipid levels are related to sympathetic tone. Ward et al. (1994), however, found no association between 24-h urine adrenaline excretion and cholesterol level in 615 males. A small positive correlation was found with HDL ($r = 0.16$) and an inverse one with triglycerides (−0.14). Noradrenaline was not related to lipid levels. How attractive and plausible the idea of a common sympathetic basis may sound, it rests on fragile assumptions. Another reason to expect a relation between stress reactivity and lipids has a stronger basis. In several animal and human studies, lipid elevations have been shown to be causally related to an augmented response to vasoconstrictors or to impaired vascular dilation (Heistad et al., 1984; Howes and Krum, 1989; Tesfamariam et al., 1989; Casino et al., 1993). A reason we did not find an association between lipid values and the response of the TPR to the stressors may be the lack of sensitivity and indirectness of measuring TPR with impedance cardiography. More direct estimation of local vasoactivity in specific areas may be required to demonstrate this effect in humans in response to stress.

References

Boomsma, D.I., de Vries, J., Orlebeke, J.F., 1989. Comparison of spot and band impedance cardiogram electrodes across different tasks. Psychophysiology 26, 695–699.

Boomsma, D.I., van Baal, G.C.M., Orlebeke, J.F., 1990. Genetic influences on respiratory sinus arrhythmia across different task conditions. Acta Genet. Med. Gemellol. 39, 181–191.

Boomsma, D.I., Kempen, H.J.M., Gevers Leuven, J.A., Havekes, L., de Knijff, P., Frants, R.R., 1996. Genetic analysis of sex and generation differences in plasma lipid, lipoprotein and apolipoprotein levels in adolescent twins and their parents. Genet. Epidemiol. 13, 49–60.

Brunner, E.J., Marmot, M.G., White, I.R., O'Brien, J.R., Etherington, M.D., Slavin, B.M., Kearny, E.M., Davey-Smith, G., 1993. Gender and employment grade differences in blood cholesterol, apolipoproteins and haemostatic factors in the Whitehall II study. Atherosclerosis 102, 195–207.

Burker, E.J., Fredrikson, M., Rifai, N., Siegel, W., Blumenthal, J.A., 1994. Serum lipids, neuroendocrine, and cardiovascular responses to stress in men and women with mild hypertension. Behav. Med. 19, 155–161.

Cacioppo, J.T., Berntson, G.G., Binkley, P.F., Quigley, K.S., Uchino, B.N., Fieldstone, A., 1994. Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockade. Psychophysiology 31, 586–598.

Casino, P.R., Crescence, M., Kilcoyne, M., Quyyumi, A.A., Hoeg, J.M., Panza, J.A., 1993. The role of nitric oxide in endothelium-dependent vasodilation of hypercholesteremic patients. Circulation 88, 2541–2547.

Eisenhofer, G., Lambie, D.G., Johnson, R.H., 1985. Beta-adrenoceptor responsiveness and plasma catecholamines as determinants of cardiovascular reactivity to mental stress. Clin. Sci. 69, 483–492.

Fredrikson, M., Blumenthal, J.A., 1988. Lipids, catecholamines, and cardiovascular responses to stress in patients recovering from myocardial infarction. J. Cardiopulm. Rehabil. 12, 513–517.

Fredrikson, M., Lundberg, U., Tuomisto, M., 1991. Serum lipid levels and cardiovascular reactivity. J. Psychophysiol. 5, 89–95.

Freeman, D.J., Griffin, B.A., Murray, E., Lindsay, G.M., Gaffney, D., Packard, C.J., Shepherd, J., 1993. Smoking and plasma lipoproteins in man: effects on low density lipoprotein cholesterol levels and high density lipoprotein subfraction distribution. Eur. J. Clin. Invest. 23, 630–640.

Heistad, D.D., Armstrong, M.L., Marcus, M.L., Piegors, D.J., Mark, A.L., 1984. Augmented responses to vasoconstrictor stimuli in hypercholesteremic and atherosclerotic monkeys. Circ. Res. 54, 711–718.

Heller, D.A., de Faire, U., Pedersen, N.L., Dahlen, G., McClearn, G.E., 1993. Genetic and environmental influences on serum lipid levels in twins. New Engl. J. Med. 328, 1150–1156.

Hopkins, P.N., 1992. Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. Am. J. Clin. Nutr. 55, 1060–1070.

Howes, L.G., Krum, H., 1989. Plasma lipoproteins, cardiovascular reactivity and the sympathetic nervous system. J. Autonom. Pharmacol. 9, 293–301.

Jorgensen, R.S., Nash, J.K., Lasser, N.L., Hymowitz, M., Langer, A.W., 1988. Heart rate acceleration and its relationship to total cholesterol, triglycerides, and blood pressure reactivity in men with mild hypertension. Psychophysiology 25, 39–44.

Kamarck, T.W., Jennings, J.R., Debski, T.T., Glickman-Weiss, E., Eddy, M.J., Manuck, S.B., 1992. Reliable measures of behaviorally evoked cardiovascular reactivity from a PC-based test battery: results from student and community samples. Psychophysiology 29, 17–28.

Kasl, S.V., Cobb, S., Brooks, G.W., 1968. Changes in serum uric acid and cholesterol level in men undergoing job loss. J. Am. Med. Assoc. 206, 1500–1507.

Kubicek, W.G., Kottke, F.J., Ramos, M.V., Patterson, R.P., Witsoe, D.A., Labree, J.W., Remole, W., Layman, T.E., Schoenig, H., Garamela, J.T., 1974. The Minnesota Impedance cardiograph. Theory and applications. Biomed. Eng. 9, 410–417.

Lauer, R.M., Lee, J., Clarke, W.R., 1988. Factors affecting the relationship between childhood and adult cholesterol levels: The Muscatine Study. Pediatrics 20, 671–684.

Lopes-Virella, M.F., Stone, P., Ellis, S., Colwell, J.A., 1977. Cholesterol determination in high density lipoproteins separated by three different methods. Clin. Chem. 23, 882–884.

Mattiasson, I., Lindgarde, F., Nilsson, J.A., Theorell, T., 1990. Threat of unemployment and cardiovascular risk factors: longitudinal study of quality of sleep and serum cholesterol concentrations in men threatened with redundancy. Br. Med. J. 301, 461–466.

Matthews, K.A., Weiss, S.M., Detre, T., Dembroski, T.M., Falkner, B., Manuck, S.B., Williams, R.B., 1986. Handbook of Stress, Reactivity and Cardiovascular Disease. John Wiley, New York.

McCann, B.S., Warnick, G.R., Knopp, R.H., 1990. Changes in plasma lipids and dietary intake accompanying shifts in perceived workload and stress. Psychosom. Med. 52, 97–108.

McKinney, M.E., McIlvain, H.E., Hofschire, P.J., Collins, R.E., Somers, J.A., Ruddel, H., Buell, J.C., Eliot, R.S., 1987. Cardiovascular changes during mental stress: correlations with a presence of coronary risk factors and cardiovascular disease in physicians and dentists. J. Hum. Hypertens. 1, 137–145.

Niaura, R., Herbert, P.N., Saritelli, A., Goldstein, M., Flynn, M., Follick, M., Gorkin, L., Ahern, D., 1991. Lipid and lipoprotein response to episodic occupational and academic stress. Arch. Intern. Med. 151, 2175–2179.

Owens, J.F., Stoney, C.M., Matthews, K.A., 1993. Menopausal status influences ambulatory blood pressure changes during mental stress. Circulation 88, 2794–2802.

Schaefer, E.J., Lamon-Fava, S., Cohn, S.D., Schaefer, M.M., Ordovas, J.M., Castelli, W.P., Wilson, P.W.F., 1994. Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. J. Lipid Res. 35, 779–792.

Sherwood, A., Allen, M.T., Fahrenberg, J., Kelsey, R.M., Lovallo, W.R., van Doornen, L.J.P., 1990. Methodological guidelines for impedance cardiography. Psychophysiology 27, 1–23.

Siegrist, J., Matschinger, H., Cremer, P., Seidel, D., 1988. Atherogenic risk in men suffering from occupational stress. Atherosclerosis 69, 211–218.

Snieder, H., van Doornen, L.J.P., Boomsma, D.I., 1995. Developmental genetic trends in blood pressure levels and blood pressure reactivity to stress. In: Turner, J.R., Cardon, L.R., Hewitt, J.K. (Eds.), Behavior Genetic Approaches in Behavioral Medicine. Plenum, New York.

Snieder, H., van Doornen, L.J.P., Boomsma, D.I., 1997. The age- dependency of gene expression for plasma lipids, lipoproteins and apolipoproteins. Am. J. Hum. Genet. 60, 638–650.

Srinivasan, S.R., Bao, W., Berenson, G.S., 1993. Coexistence of increased levels of adiposity, insulin, and blood pressure in a young adult cohort with elevated VLDL cholesterol: The Bogalusa Heart Study. Metabolism 42, 170–176.

Stevens, J., 1992. Applied Multivariate Statistics for the Social Sciences. Lawrence Erlbaum, Hillsdale, New Yersey.

Suarez, E.C., Williams, R.B., Kuhn, C.M., Zimmerman, E.H., Schanberg, S.M., 1991. Biobehavioral basis of coronary prone behavior in middle-aged men. Part II: Serum cholesterol, the type A behavior pattern, and hostility as interactive modulators of physiological reactivity. Psychosom. Med. 53, 528–537.

Tesfamariam, B., Weisbrod, R.M., Cohen, R.A., 1989. Augmented adrenergic contractions of carotid arteries from cholesterol-fed rabbits due to endothelial cell dysfunction. J. Cardiovasc. Pharmacol. 13, 820–825.

Tran, Z., Weltman, A., Glass, G.V., Mood, D.P., 1983. The effects of exercise on blood lipids and lipoproteins: a meta-analysis of studies. Med. Sci. Sports Exercise 15, 393–402.

Trevisan, M., Jossa, F., Farinario, E., Krogh, V., Panico, S., Giumetti, D., Mancini, M., 1992. Earthquake and coronary heart disease risk factors: A longitudinal study. Am. J. Epidemiol. 135, 632–637.

Twisk, J.W.R., Kemper, H.C.G., Mellenbergh, G.J., 1995. Longitudinal development of lipoprotein levels in males and females aged 12-28 years: The Amsterdam Growth and Health Study. Int. J. Epidemiol. 24, 69–77.

van Doornen, L.J.P., 1991. Stress and the dynamics behind hypertension, cholesterol and atherosclerosis. In: Appels, A. (Ed.), Behavioral Observations in Cardiovascular Research. Swets and Zietlinger, Amsterdam.

van Doornen, L.J.P., van Blokland, R.W., 1987. Serum cholesterol: sex-specific psychological correlates during rest and stress. J. Psychosom. Res. 31, 239–249.

Verschuren, W.M.M., Boerma, G.J.M., Kromhout, D., 1994. Total and HDL-cholesterol in The Netherlands: 1987–1992. Levels and changes over time in relation to age, gender and educational level. Int. J. Epidemiol. 23, 948–956.

Ward, K.D., Sparrow, D., Landsberg, L., Young, J.B., Vokonas, P.S., Weiss, S.T., 1994. The relationship of epinephrine excretion to serum lipid levels: The Normative Aging Study. Metabolism 43, 509–513.