Influence of mental stress and circadian cycle on postprandial lipemia1–3

Catherine Le Fur, Monique Romon, Pascal Lebel, Patrick Devos, Alain Lancry, Laurence Guédon-Moreau, Jean-Charles Fruchart, and Jean Dallongeville

ABSTRACT

Background: Mental stress produces alterations in serum lipids and lipoproteins.
Objective: The objective was to assess the effect of mental stress during the day and night on postprandial lipoproteins.
Design: Fourteen healthy subjects aged 26.6 ± 5.0 y were given randomly the same meal either at night (0100) or during the day (1300), with or without (control session) a mental stress challenge. The meal contained 40% of estimated daily energy needs. The mental task was performed on a computer and consisted of a task of choice reaction. Blood samples were drawn at baseline and hourly for 7 h after the meal.
Results: Urinary epinephrine concentrations were higher (P < 0.012) during the mental task than during the control sessions. Repeated-measures analysis of variance showed that mean postprandial triacylglycerol concentrations were significantly higher (P < 0.02) and total cholesterol (P < 0.0001) and HDL-cholesterol concentrations were significantly lower (P < 0.0001) at night than during the day. The mean postprandial VLDL-triacylglycerol concentration was significantly higher (P < 0.04) during the mental task than during the control sessions. Similarly, the VLDL-cholesterol response, calculated as the area under the postprandial curve, was significantly greater (P < 0.02) during the mental task than during the control sessions. There was no interaction between mental stress and nyctohemeral cycle on postprandial lipoprotein responses, suggesting that both indexes act independently on postprandial lipid metabolism.
Conclusions: Mental stress is associated with increased concentrations of postprandial triacylglycerol-rich lipoprotein fractions. Therefore, postprandial hyperlipidemia is one possible mechanism contributing to the higher risk of ischemic heart disease in stressed people. Am J Clin Nutr 1999;70:213–20.

KEY WORDS  Nutrition, postprandial metabolism, cholesterol, triacylglycerol, lipoproteins, circadian cycle, mental stress, epinephrine, humans, men

INTRODUCTION

Numerous studies have shown that mental stress influences lipid and lipoprotein concentrations (1–7). In these experiments, an increase in total cholesterol, triacylglycerol, LDL cholesterol, and HDL cholesterol was generally observed (1–7). These lipid and lipoprotein changes have been attributed to the effect of epinephrine on lipoprotein lipase (8, 9), hepatic lipase (10), and hormone-sensitive lipase (11) activities. The overall effect of epinephrine action on these enzymes is to increase fatty acid efflux from adipose tissue (11), providing the liver with substrate for triacylglycerol synthesis and VLDL production. Additional investigations showed that short-term stress caused by tests results in hemoconcentration, which contributes to increases in the concentrations of circulating lipids and lipoproteins (1, 2).

Postprandial lipemia is a dynamic process characterized by increased concentrations of triacylglycerol after fat ingestion. Many conditions, such as age, physical activity, body weight, dyslipidemia, and apolipoprotein E polymorphism, affect the intensity of postprandial lipemia (12–15). The circadian cycle also influences postprandial lipid metabolism, with higher triacylglycerol concentrations after a meal eaten at night than during the day (16).

Studies to date that analyzed the effect of stress on lipoprotein metabolism were performed in fasting subjects during the day. The aim of the present study was to test the hypothesis that mental stress alters postprandial lipid and lipoprotein responses. Furthermore, because the circadian cycle also influences postprandial triacylglycerol metabolism, we assessed the possible effect of this cycle on stress-related changes in postprandial lipids.

SUBJECTS AND METHODS

Subjects

Fourteen healthy men aged 26.6 ± 5.0 y were recruited for the study from the University of Lille II through advertisements

1 From CERESTE, Service de Nutrition, and Service de Pharmacologie Hospitalière, CHR et U de Lille, Lille, France; INSERM U-325, INSERM CJF 95–05, and Département d’Athérosclérose, Institut Pasteur de Lille, Lille, France; CERIM, Faculté de Médecine Lille II, Lille, France; and ECCHAT, Faculté de Philosophie, Sciences Humaines, Amiens, France.
2 Supported by grant 931501 from INSERM and by an unrestricted grant from GIP Cereste, Ministère de la Santé et Solidarité Nationale.
3 Address reprint requests to J Dallongeville, Institut Pasteur de Lille, 1 rue du Professeur Calmette, 59019 Lille, Cedex, France. E-mail: jean.dallongeville@pasteur-lille.fr.
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and posters. The mean age and body mass index (BMI; in kg/m²) were 25.6 ± 5.0 y and 22.4 ± 2.2, respectively. Exclusion criteria were as follows: obesity (BMI > 27), unstable weight (change in weight > 2 kg in the last 3 mo), high blood pressure, smoking, medication use that might affect the sympathetic or parasympathetic systems, night or shift work, and travel across time zones in the preceding 6 mo. All potential volunteers had a fasting blood sample drawn for screening of diabetes, hyperlipidemia, and impaired thyroid, hepatic, or renal function. Furthermore, they had to answer a questionnaire concerning their daily routines and sleep and meal schedules. Those with irregular habits were excluded. Each subject was informed about the nature and the purpose of the study and was enrolled after giving informed consent. The hospital ethics committee (CCPRB de Lille, CHR et U de Lille) approved the protocol according to the current regulations in France. During the 24 h preceding each session, the subjects were reminded to refrain from exercising and consuming alcohol.

Experimental design

The study was performed in the Centre d’Investigation Clinique of the CHR et U de Lille. Four experimental procedures took place in a randomized sequence: 2 sessions during the day (between 0730 and 2030), 1 with and 1 without a mental task, and 2 sessions at night (between 1930 and 0830), 1 with and 1 without a mental task. Between sessions, there was a minimum time span of 5 d. All subjects completed the 4 sessions within 5 wk. On arrival, participants consumed a light meal providing 20% of their theoretical energy needs (calculated as 1.5 × basal metabolic needs; 17) and stayed in the ward, reading or watching TV; they were allowed to drink water only. At 1300 and 0100, a blood sample was collected and the subjects then consumed a test meal. Heart rates were recorded with an electrocardiogram during each experiment with a Holter HELA device recorder (18).

Test meal

The test meal was a mixed meal composed of bread, butter, ham or cheese, apple marmalade, and cottage cheese. Each subject consumed exactly the same meal during the 4 sessions. The meal provided 4186 kJ and the percentage of energy derived from protein, fat, and carbohydrate was 16%, 34%, and 50%, respectively.

Mental task

The mental challenge was performed on a computer and consisted of a task of choice reaction. Briefly, the subjects were presented rapidly and simultaneously 3 geometric figures on the computer screen. They had to recognize as soon as possible the shape of the figure and indicate by pressing a key on the computer keyboard the one with bold lines. The task lasted 10 min and was presented every 30 min during the 5 h after the meal. During the control sessions, the subjects operated the computer keyboard according to the same schedule used in the experimental sessions. Between mental challenges, the subjects were allowed to watch light-entertainment movies, but were not allowed to sleep.

Blood samples

An indwelling venous canula was inserted into an antecubital vein 1 h before the test meal. The first sample was drawn before the meal was eaten and then hourly for the 7 h after the meal.

Lipid measurements

Blood was collected into dry tubes and allowed to clot for 1 h. Serum was separated by centrifugation at 3300 × g for 20 min at 4°C. Lipoproteins were separated according to standard procedures by a combination of ultracentrifugation (see below) [at a density (d) of 1.006 kg/L] and Mg²⁺-phosphotungstate precipitation. HDL cholesterol was measured after precipitation by using commercially available reagents (CHOD/PAP; Boehringer Mannheim, Mannheim, Germany). VLDLs were separated from 0.5 mL serum by ultracentrifugation with a Beckman TL100 (Palo Alto, CA) in a single spin (d = 1.006 kg/L) (19) with modifications. Briefly, 0.5 mL of 0.9% NaCl was added to 0.5 mL serum and spun in a polycarbonate tube (400000 × g at 20°C for 2.5 h) in a Beckman 100.2 Ti rotor for 3 h. The tube was cut in 2 parts and the remaining 0.5-mL infranate was analyzed for lipids. The triacylglycerol-rich-lipoprotein (TRL) (d < 1.006 kg/L) fraction was measured by subtracting infranatant values from serum concentrations and LDL cholesterol was quantified by subtracting the HDL-cholesterol concentration from the infranatant cholesterol concentration. Cholesterol and triacylglycerols were measured by using Boehringer Mannheim reagents (reference nos. 1.040.1839 and 1.058.550, respectively). The intra- and interseries CVs were 1% and 1.3% for cholesterol and 1.1% and 1.8% for triacylglycerol, respectively. Six-hour urine samples were collected during each session into containers containing hydrochloric acid. Urinary epinephrine concentrations were measured by HPLC with electrochemical detection as described previously (20).

Statistical analysis

Three-way analysis of variance (ANOVA) with repeated measures was performed to assess the effect of mental task (stress or no stress), mealtime (night or day), and postprandial interval (t₀h, t₁h, t₂h, t₃h, t₄h, t₅h, t₆h, or t₇h) on lipid and lipoprotein concentrations. The percentage change was calculated at each time point as follow:

\[ \frac{(t_x - t_{0h})}{t_{0h}} \]

where \( t_x \) varies from \( t_{0h} \) (baseline) to \( t_{7h} \). The lipid postprandial areas under the curves (AUC) were calculated as follow:

\[ \text{AUC} = \sum (t_x - t_{0h}) \]

where \( t_x \) varies from \( t_{0h} \) to \( t_7 \). Because of the small number of subjects in this study and to avoid the potential confounding effect of non-Gaussian distribution and outlier values, a non-parametric statistical procedure was preferred for three-way ANOVA with repeated measures (21). This procedure included a rank transformation of each variable followed by the standard ANOVA procedure (22). The level of statistical significance was set at \( P < 0.05 \).

RESULTS

Baseline lipid concentrations

Baseline serum cholesterol (range: 2.9–5.7 mmol/L) and triacylglycerol (0.55–1.73 mmol/L) concentrations were within the expected normal range for age and sex. Three-way ANOVA with repeated measures did not show any significant differences in baseline lipid and lipoprotein concentrations between the 4 sessions (Table 1).
Catecholamine concentrations

Three-way ANOVA with repeated measures showed significantly higher urinary epinephrine concentrations during the mental task than during the control sessions (Figure 1). In addition, urinary epinephrine excretion was significantly lower at night than during the day. There was no evidence of a significant interaction between mental task and mealtime on epinephrine excretion. Finally, to evaluate whether the order of testing influenced the magnitude of stress reaction, urinary epinephrine concentrations during the first mental task session were compared with those during the second session. Three-way ANOVA with repeated measures showed no evidence of a significant effect of meal order between experiments. There was no effect of mental task on the urinary excretion of norepinephrine.

Effect of a mixed meal on postprandial lipemia: main effect of postprandial interval

As expected, three-way ANOVA with repeated measures showed that ingestion of the mixed meal resulted in a significant increase in postprandial triacylglycerol and VLDL-triacylglycerol concentrations (Figure 2) and a significant decrease in postprandial total and HDL-cholesterol concentrations (Figure 3). There was no significant effect of the mixed meal on postprandial LDL- and VLDL-cholesterol concentrations.

Influence of mealtime on postprandial lipemia: mealtime × postprandial interval interaction

Three-way ANOVA with repeated measures indicated a significant interaction between mealtime (night or day) and postprandial interval for triacylglycerol, total cholesterol, and HDL cholesterol, and a nearly significant interaction for VLDL triacylglycerol (P for trend = 0.07) (Figures 2 and 3). Triacylglycerol and VLDL triacylglycerol were higher, whereas total cholesterol and HDL cholesterol were lower at night than during the day. Because baseline lipid concentrations influence postprandial lipid responses (23), the analyses were repeated after the values were expressed as percentage changes. After adjustment, the interaction persisted for triacylglycerol (P < 0.04), total cholesterol (P < 0.0006), HDL cholesterol (P < 0.0001), and VLDL triacylglycerol (P for trend = 0.08). The postprandial concentrations of VLDL- and LDL cholesterol were not significantly affected by mealtime.

Influence of mental stress on postprandial lipemia

Three-way ANOVA with repeated measures indicated a significant interaction between mental task (stress) and postprandial interval for VLDL triacylglycerol (P < 0.04). One to 2 h after the meal, mean VLDL-triacylglycerol concentrations were similar during the mental task and control sessions, but were higher (NS) during the

### TABLE 1

Baseline lipid concentrations of the men participating in the study

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>Day + stress</th>
<th>Night</th>
<th>Night + stress</th>
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<tbody>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>0.92 ± 0.23</td>
<td>0.97 ± 0.23</td>
<td>0.98 ± 0.25</td>
<td>1.0 ± 0.29</td>
</tr>
<tr>
<td>VLDL triacylglycerol</td>
<td>0.66 ± 0.23</td>
<td>0.64 ± 0.23</td>
<td>0.69 ± 0.23</td>
<td>0.75 ± 0.28</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.90 ± 0.41</td>
<td>0.80 ± 0.49</td>
<td>0.95 ± 0.39</td>
<td>0.70 ± 0.36</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.23 ± 0.64</td>
<td>4.18 ± 0.64</td>
<td>4.20 ± 0.67</td>
<td>4.23 ± 0.72</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.24 ± 0.62</td>
<td>2.27 ± 0.62</td>
<td>2.11 ± 0.85</td>
<td>2.37 ± 0.67</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.08 ± 0.18</td>
<td>1.08 ± 0.20</td>
<td>1.13 ± 0.28</td>
<td>1.16 ± 0.28</td>
</tr>
</tbody>
</table>

* x ± SD. There were no significant differences between sessions by repeated-measures ANOVA.

**FIGURE 1.** Mean (±SEM) urinary epinephrine excretion of the 14 subjects during the day and at night during the mental task (stress) and control (no stress) sessions. Two-way ANOVA with repeated measures indicated a significant main effect of mealtime (P < 0.0001) and stress (P < 0.012), but no significant interaction between the 2 variables.
mental task than during the control session 3 h postprandially and later. Postprandial concentrations of triacylglycerol, cholesterol, VLDL cholesterol, LDL cholesterol, and HDL cholesterol were not significantly affected by the mental task. There was, however, a nearly significant main effect of mental task for VLDL cholesterol (P < 0.075) and LDL cholesterol (P < 0.086). VLDL cholesterol and LDL cholesterol tended to be higher and lower (NS), respectively, during the mental task than during the control session.

Because baseline lipid concentrations influence postprandial lipid responses, the analyses were repeated after the values were expressed as percentage changes. Three-way ANOVA with repeated measures indicated a significant main effect of postprandial interval on TG (P < 0.0001) and VLDL-TG (P < 0.0001) concentrations as well as a significant interaction between mental task and postprandial interval for VLDL-TG (P < 0.02). The percentage increase in VLDL triacylglycerol was higher late postprandially during the mental task than during the control sessions.

Postprandial changes in triacylglycerol, cholesterol, and HDL cholesterol were not significantly affected by mental task after adjustment for baseline values. There was, however, a significant main effect of stress on the percentage change in VLDL cholesterol (P < 0.05) and a nearly significant effect of stress on the percentage change in LDL cholesterol (P < 0.092).

A two-way repeated-measures ANOVA with the AUCs of triacylglycerol and the lipoproteins as dependent variables showed no evidence of a significant interaction between mealtime (night or day) and mental task (stress or no stress) for any of these variables (Figure 4). There was, however, a significant main effect of mental task on the AUC for VLDL cholesterol, but not on the AUCs for total triacylglycerol, VLDL triacylglycerol, total cholesterol, LDL cholesterol, or HDL cholesterol. The AUC for VLDL cholesterol was greater during the mental task than during the control sessions. There was also a significant main effect of nyctohemeral cycle on the AUCs for total cholesterol and HDL cholesterol, but not on the AUCs for total triacylglycerol, VLDL triacylglycerol, VLDL cholesterol, and LDL cholesterol. The AUCs for total cholesterol and HDL cholesterol were lower at night than during the day.

**DISCUSSION**

The goal of the present study was to assess the effect of stress and of the circadian cycle on postprandial concentrations of lipids and lipoproteins. Postprandial TRL responses increased in response to mental stress. However, there was no evidence of an additional effect of the circadian cycle on this relation. These findings suggest that stress affects postprandial TRL concentrations, which could contribute to the risk of ischemic heart disease in patients exposed to stress.

The protocol used in the present study differed from the protocols used in other investigations. In previous studies, the
stressor included the Stroop interference test, mental arithmetic calculation, motor coordination challenge, or speaking before a camera. The duration of the stress test ranged from a few to 20 min and blood sampling was performed either immediately or shortly after the stress challenge. In the present study, the stress constraint lasted 10 min and was repeated every half-hour for 5 h after the meal. This strategy was used to simulate a condition with repeated stress, such as job stress. One limitation of this strategy, however, was that the subjects were progressively accustomed to the stressors after repeated stimulation. Therefore, the magnitude of the reaction to the stress, assessed by the transient increase in heart rates during the mental task, declined during the experiment (Figure 5). However, the increase in urinary epinephrine indicated that the mental task in the present study induced a significant amount of stress. Urinary epinephrine secretion was lower at night than during the day, an observation that is compatible with a circadian cycle of epinephrine secretion (24). These lower nocturnal epinephrine concentrations did not affect the reaction to stress, suggesting independent influences of circadian rhythm and mental stress.

Mental task was associated with increased postprandial TRL concentrations, independently of baseline triacylglycerol concentrations, suggesting a specific effect of mental stress on postprandial TRL concentrations. This effect was delayed after the stress challenge was initiated. The VLDLs measured in this study represent a mixture of TRLs that included particles of intestinal and hepatic origin and did not allow an independent assessment of the effect of stress on intestinal or hepatic lipoprotein metabolism. The mechanism by which lipoprotein concentrations change after a stress challenge has been attributed to epinephrine’s action on lipid and lipoprotein metabolism enzyme activities. In vitro, epinephrine inhibits lipoprotein lipase and hepatic lipase secretions at a posttranslational level (8, 10). If such inhibition occurs in vivo, it could affect TRL clearance with subsequent accumulation of TRLs in the bloodstream. Hormone-sensitive lipase, which regulates the release of fatty acids from adipose tissue, is activated by epinephrine (25, 26). In fasting human subjects, epinephrine infusion at physiologic concentrations increases the net efflux of nonesterified fatty acids from adipose tissue (11). This flux of fatty acids from peripheral tissue to the liver could increase the substrate availability for triacylglycerol synthesis and VLDL formation and secretion. In the postprandial state, such as in the present study, insulin secretion may inhibit the hormone-sensitive release of fatty acids (27). Therefore, the contribution of the fatty acid flux from adipose

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**FIGURE 3.** Mean (± SD) serum LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) concentrations at baseline ($t_0$) and every hour postprandially ($t_1$ to $t_7$) during the day and at night during the mental task (stress; closed symbols) and control (no stress; open symbols) sessions. Three-way ANOVA with repeated measures indicated a significant main effect of postprandial interval on total cholesterol ($P < 0.001$) and HDL-C ($P < 0.0001$) concentrations, but no significant interaction between mental task and postprandial interval for the 2 variables. $n = 14$. 

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tissue to the liver to VLDL secretion is difficult to evaluate. However, the finding that postprandial VLDL-triacylglycerol curves diverge late postprandially, at a time when insulin concentrations have returned to baseline concentrations, supports the concept that insulin may mask the stress-related lipid changes that occur shortly after a meal. Finally, hemoconcentration has been observed during exposure to short-term mental stress (1, 2), which, theoretically, could contribute to increased postprandial TRL concentrations.

The postprandial responses of serum total cholesterol, LDL cholesterol, and HDL cholesterol were not affected by stress. This finding contrasts with the findings of studies performed in fasting subjects in whom short-term stress challenges were usually associated with increased cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations (1–7). During postprandial metabolism, total cholesterol concentrations tend to decrease as the result of decreased LDL and HDL concentrations (28). This postprandial change could theoretically mask the potential increase in LDLs and HDLs resulting from stress. Overall, these findings highlight the differences in stress-related lipoprotein changes between fasting and fed conditions (29).

As in our previous study (16), we found that circadian cycle has a clear influence on postprandial lipemia. Postprandial serum triglyceride was higher (NS) and total cholesterol and HDL cholesterol were significantly lower at night than during the day, independent of baseline concentrations. Despite this clear influence, the intensity of the stress-related postprandial lipid response was not significantly affected by the circadian cycle. Moreover, the lower secretion of epinephrine at night than during the day was not associated with a decreased response of lipoproteins to stress, suggesting that circadian cycle and stress act independently on postprandial lipemia.

In conclusion, evidence has accumulated that postprandial dyslipidemia is a risk factor for ischemic heart disease (13–15).
Increased chylomicron-remnant concentrations and decreased HDL-cholesterol concentrations have been observed in patients with angiographically verified ischemic heart disease and survivors of myocardial infarction (30, 31). In the present study, we showed that stress alters postprandial triacylglycerol metabolism. This finding suggests that postprandial dyslipidemia is one possible mechanism contributing to the higher risk of ischemic heart disease in stressed people.

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REFERENCES

FIGURE 5. Heart rate of a representative subject during a mental task (stress) and control (no stress) session.


