Mental Stress Induces Transient Endothelial Dysfunction in Humans

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Background—Mental stress has been linked to increased morbidity and mortality in coronary artery disease and to atherosclerosis progression. Experimental studies have suggested that damage to the endothelium may be an important mechanism.

Methods and Results—Endothelial function was studied in 10 healthy men (aged 50.4±9.6 years) and in 8 non–insulin-dependent diabetic men (aged 52.0±7.2 years). Brachial artery flow-mediated dilation (FMD, endothelium dependent) and response to 50 μg of sublingual glyceryl trinitrate (GTN, endothelium independent) were measured noninvasively by use of high-resolution ultrasound before and after (30, 90, and 240 minutes) a standardized mental stress test. The same protocol without mental stress was repeated on a separate occasion in the healthy men. In healthy subjects, FMD (5.0±2.1%) was significantly (P<0.01) reduced at 30 and 90 minutes after mental stress (2.8±2.3% and 2.3±2.4%, respectively) and returned toward normal after 4 hours (4.1±2.0%). Mental stress had no effect on the response to GTN. In the repeated studies without mental stress, FMD did not change. The diabetic subjects had lower FMD than did the control subjects (3.0±1.5% versus 5.0±2.1%, respectively; P=0.02) but showed no changes in FMD (2.7±1.1% after 30 minutes, 2.8±1.9% after 90 minutes, and 3.1±2.3% after 240 minutes) or GTN responses after mental stress.

Conclusions—These findings suggest that brief episodes of mental stress, similar to those encountered in everyday life, may cause transient (up to 4 hours) endothelial dysfunction in healthy young individuals. This might represent a mechanistic link between mental stress and atherogenesis. (Circulation. 2000;102:2473-2478.)

Key Words: endothelium ■ atherosclerosis ■ mental stress ■ diabetes mellitus

In patients with coronary artery disease, mental stress is known to provoke myocardial ischemia1 and is linked to increased morbidity and mortality.2,3 In addition, chronic stress is associated with accelerated progression of atherosclerosis itself from an early stage, but the mechanisms involved remain unclear.4

Endothelial function plays a key role in determining the clinical manifestations of established atherosclerotic lesions and in the initiation of early atherosclerosis.5 Healthy endothelium maintains vascular tone and inhibits smooth muscle cell growth, the adhesion of white blood cells, and platelet aggregation by the production of NO.5,6 In humans, several risk factors for cardiovascular disease7–10 have been shown to induce endothelial dysfunction from an early stage, even from the first decade of life.11 In animal studies, chronic social stress impairs endothelial function by increasing the rate of endothelial cell damage12 and reduces NO availability in atherosclerotic arteries.13 We hypothesized that mental stress might initiate and/or promote atherogenesis by impairing endothelium-dependent vascular homeostasis in preclinical subjects. Therefore, the impact of an acute mental stress, of the type likely to be encountered frequently during normal daily activities, on endothelial function was examined in subjects with and without risk factors for coronary artery disease.

Our findings indicate that prolonged impairment of endothelium-dependent relaxation may result even after a brief episode of mental stress; this may represent an important link between repeated or chronic stress and acceleration of the atherogenic process.

Methods

Subjects
Healthy male subjects (aged 50.4±9.6 years, range 40 to 62 years) were recruited from a population-based cohort of civil servants if they had no known cardiovascular risk factors or clinical evidence of vascular disease (they were nonsmokers, had no family history of cardiovascular disease, and had normal blood pressure, cholesterol, and glucose levels). In addition, 8 subjects (aged 52.0±7.3 years, range 44 to 62 years) with non–insulin-dependent diabetes mellitus (NIDDM) but no clinical history of cardiovascular disease were...
recruited from our outpatient clinic. All the diabetic subjects were maintained on oral hypoglycemic agents (metformin and glibenclamide), but none were receiving antioxidants vitamins or known vasoactive drugs (e.g., calcium antagonists, ACE inhibitors, or statins) that could influence endothelial function.\textsuperscript{11,14–16}

**Experimental Protocol**

Subjects were given a broad outline of the nature of the investigation before the study but were not given precise details of the protocol for the mental stress test. None had previously taken part in any mental stress studies, and all subjects gave informed written consent. The present study was approved by the local ethics committee.

All subjects attended the laboratory in the morning in a fasting state. A small cannula was introduced into an antecubital vein for biochemical analyses. After 1 hour of rest, a baseline vascular study was performed immediately before performance of the mental stress test. Subjects were required to listen to a recorded message, which gave them instructions on how to perform the mental stress task. They were asked to imagine a situation in which they had been falsely accused of shoplifting. They were then required to prepare a statement in their defense for 2 minutes and to present it in front of an audience and video camera for 3 minutes.\textsuperscript{7} Blood pressure and heart rate were recorded at 2-minute intervals from the beginning of mental stress for 10 minutes with the use of an automatic oscillographic device (Dinamap, Critikon Inc). None of the investigators had any communication with the subjects during this time.

Before beginning the speech task, subjective stress levels were evaluated by use of a visual analogue scale (ranging from 1, signifying very little stress, to 7, signifying extreme stress). This was repeated at the end of the speech task, and subjects were asked to evaluate their maximum levels of stress.

Endothelium-dependent and -independent dilatation were assessed noninvasively by use of high-resolution ultrasound immediately before the mental stress test and 30, 90, and 240 minutes after its completion. Briefly, with subjects supine, at rest, and in a quiet air-conditioned room (22°C to 25°C), a B-mode scan of the right brachial artery was obtained in longitudinal section between 5 and 10 cm above the elbow by use of a 7.0-MHz linear array transducer and a standard Acuson XP10 system (Acuson, USA). The transducer was held at the same point throughout the scan by a stereotactic clamp, and fine adjustments were made by means of micrometer screws attached to the mount. To ensure consistency of the image with serial scans, the transducer position was marked on the skin, and a hard-copy print of the B-mode image was taken. ECG-gated end-diastolic frames were acquired at 3-second intervals throughout the study and stored on a personal computer by use of a video frame grabber. Blood flow was manipulated in the brachial artery by a pneumatic cuff placed around the forearm distal to the segment of artery being imaged. After 1 minute of baseline flow, the cuff was inflated to a suprasystolic pressure (300 mm Hg) for 5 minutes and released, resulting in a brief episode of reactive hyperemia. Brachial artery diameter changes in response to this increased blood flow were assessed for a further 5 minutes. Blood flow velocity was continuously monitored by pulsed-wave Doppler, with an angle of insonation of 70° to the vessel and with the range gate in the center of the artery. After 10 minutes of rest, brachial artery response to glyceryl trinitrate (GTN, 50 μg sublingual) was assessed in the same fashion. This dose was significantly lower than that used in previous studies\textsuperscript{17} and was selected to achieve a dilatation more closely related to that obtained for flow-mediated dilation (FMD).\textsuperscript{18}

To examine the effect of diurnal variations on endothelial function, healthy control subjects were asked to return on a second occasion, during which an identical measurement protocol excluding the mental stress intervention (time-control study) was performed. Salivary samples were collected in Salivettes (Sarstedt) before and at 10-minute intervals for 1 hour after mental stress to measure cortisol levels. Cortisol was measured in saliva to avoid the effect of repeated venous samples on the subjects’ stress levels and was closely correlated with plasma cortisol levels.\textsuperscript{19}

Inflammatory cytokines (interleukin [IL]-1, IL-6, and tumor necrosis factor [TNF]-α) were measured at baseline and 1 hour after mental stress by ELISA with use of capture and detection antibodies (R&D Systems) and developed with 3,3′,5,5′-tetramethylenediamine dihydrochloride (Sigma Chemical Co). The optical densities were measured on a Dynatec ELISA plate reader.\textsuperscript{20}

**Analysis of Data**

Brachial artery diameter was measured offline by an automatic edge-detection system (Information Integrity). All analyses were performed by the same experienced operator (A.E.D.), who was blinded to the patient, the stimulus, and the stage of the study. All scans from each patient were included in the analysis.

Baseline vessel size was taken as the mean of the measures obtained during the first minute. FMD was calculated as the percent increase in vessel size from baseline, obtained 60 seconds after the release of the cuff. GTN response was calculated as the maximum percent increase in vessel size from baseline. Volume blood flow was calculated by multiplying the Doppler flow velocity (corrected for the angle) by the heart rate and the vessel cross-sectional area (πr²). Blood flow velocity was measured offline from video-recorded tapes at baseline and at 15-second intervals after cuff release. Peak reactive hyperemia was calculated as the maximum percent increase in flow after cuff release, and the area under the curve of reactive hyperemia over the first 60 seconds after cuff release was calculated by triangulation.

Data were analyzed by use of an NCSS statistical package for Windows ‘95. Descriptive data are expressed as mean±SD. The diabetic and control groups were compared by the use of a 2-sample Student t test. ANOVA for repeated measures was used to test the effect of mental stress on vascular, humoral, and hemodynamic parameters. A value of $P<0.05$ was considered statistically significant.

**Results**

**Demographics of Subjects**

Clinical characteristics of the healthy control subjects and diabetic subjects are shown in the Table. Compared with control subjects, diabetic subjects had higher plasma glucose levels but worse lipid profiles and higher heart rates.

**Response to Mental Stress**

In the healthy control subjects, the speech task caused a significant increase in the subjective assessment of mental stress (from 2.6±1.2 to a peak of 4.9±1.2 and to 4.6±1.0 after mental stress, $P<0.01$). This was associated with a significant rise in blood pressure, heart rate, and blood flow.
(P<0.01), whereas brachial artery diameter was reduced (P<0.05) (Figure 1). All changes returned to baseline levels within 2 minutes after the mental stress test. After mental stress, there was a significant increase in salivary cortisol levels from 10.6±8.7 to a maximum of 14.1±10.7 nmol/L at 20 minutes after the mental stress tests, declining to 8.5±5.6 nmol/L at 60 minutes (P<0.05). Mental stress had no effect on resting vessel size or the intensity of reactive hyperemic blood flow at 30, 90, or 240 minutes (data not shown). In contrast, in 8 of the 10 healthy subjects, there was a reduction in FMD at 30 and 90 minutes after mental stress (Figure 2), such that mean FMD was significantly reduced at these time points, returning toward baseline levels by 240 minutes (baseline FMD 5.0±2.1%, 30-minute FMD 2.8±2.3% [P<0.05], 90-minute FMD 2.3±2.4% [P<0.01], and 240-minute FMD 4.1±2.0%; Figure 3). The vascular response to GTN was not influenced by mental stress (10.5±4.3% after 30 minutes, 11.1±4.8% after 90 minutes, and 10.9±2.7% after 240 minutes; Figure 3).

During the time-control study in the healthy subjects, FMD (5.0±2.9% at baseline, 4.8±2.3% after 30 minutes,

Figure 1. Changes in blood flow and diameter of brachial artery and in blood pressure (BP) and heart rate induced by mental stress in healthy subjects (●) and in NIDDM subjects (○). Values are mean±SEM. NIDDM subjects had lower flow response to mental stress than did healthy subjects, whereas diameter was reduced significantly only in healthy subjects (§P<0.05 vs baseline). BP increased significantly in healthy subjects (*P<0.01 vs baseline) and NIDDM subjects (§P<0.05 vs baseline), whereas heart rate increased significantly only in the healthy subjects (*P<0.01 vs baseline).

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Figure 2. Individual changes in FMD after mental stress test in healthy subjects (top) and NIDDM subjects (bottom).

5.6±3.0% after 90 minutes, and 5.5±3.4% after 240 minutes) and GTN response (9.3±3.3% at baseline, 10.3±4.9% after 30 minutes, 10.0±4.0% after 90 minutes, and 9.4±3.7% after 240 minutes) did not change (Figure 3).

Before mental stress, diabetic subjects had significantly lower FMD than did healthy control subjects (3.0±1.5%
versus 5.0±2.1%, respectively; *P*=0.02). This was not explained by any differences in baseline vessel size, flow, or the intensity of the reactive hyperemic stimulus. However, there was no significant difference in response to the direct smooth muscle dilator GTN (10.7±3.2% versus 12.1±3.6% for diabetic versus healthy subjects, respectively; *P*=NS).

Diabetic subjects’ subjective assessments of the mental stress were reduced compared with those of the healthy control subjects (from 2.3±0.8 to a peak of 4.7±1.6 and to 3.7±0.9 after mental stress), although this did not reach statistical significance. Moreover, in the diabetic subjects, although mental stress increased blood pressure (*P*=0.05), heart rate, brachial artery blood flow, and diameter did not change significantly (Figure 1). These differences were also reflected in a reduced salivary cortisol response (from 9.4±5.8 to 8.7±7.6 nmol/L at 20 minutes and to 7.4±5.7 nmol/L at 60 minutes after the mental stress test, *P*=NS). In the diabetic group, FMD did not change significantly after the mental stress test (3.0±1.5% at baseline, 2.7±1.1% after 30 minutes, 2.8±1.9% after 90 minutes, and 3.1±2.3% after 240 minutes; Figures 2 and 3). GTN response was also unchanged (12.1±3.6% at baseline, 12.8±3.8% after 30 minutes, 12.2±3.2% after 90 minutes, and 13.0±3.4% after 240 minutes; Figure 3). Reduction in FMD was significantly associated with the increase in heart rate during mental stress (*P*=0.05) but not with changes in blood pressure or cortisol levels.

There was no significant change in cytokine levels before and 60 minutes after acute mental stress in either the healthy control subjects (at baseline, TNF-α 20.7±9.6 pg/mL, IL-1 13.0±7.3 pg/mL, and IL-6 18.8±11.8 pg/mL; after mental stress, TNF-α 21.1±10.2 pg/mL, IL-1 13.5±6.7 pg/mL, and IL-6 20.9±12.9 pg/mL) or the diabetic subjects (at baseline, TNF-α 22.7±18.8 pg/mL, IL-1 17.0±9.5 pg/mL, and IL-6 29.7±26.7 pg/mL; after mental stress, TNF-α 22.7±18.5 pg/mL, IL-1 17.7±10.7 pg/mL, and IL-6 27.2±19.9 pg/mL). Cytokine levels were not related to FMD or changes in FMD.

**Discussion**

We have shown, in healthy subjects without clinical vascular disease or risk factors, that a very brief period of mental stress results in prolonged vascular endothelial dysfunction for up to 4 hours. Endothelium-dependent FMD was reduced to levels comparable to those associated with chronic endothelial dysfunction in diabetic subjects. This dynamic noxious response may represent the mechanism by which repetitive mental stress is linked to initiation or acceleration of the atherogenic process in preclinical subjects.

Epidemiological studies have shown that psychosocial stress is associated with increased cardiovascular morbidity and mortality. This may be due to exacerbation of unhealthy lifestyles and/or to direct pathophysiological effects. In patients with coronary artery disease, mental stress results in vascular constriction due to sympathetic activation, and in the presence of impaired NO production, this might contribute to the genesis of myocardial ischemia. However, these effects of mental stress in established disease do not explain the observed increase in the rate of development of atherosclerosis seen in animals with a chronic increase in stress levels or the causal association between coronary artery disease development and stress in humans.

The endothelium plays a key role not only in the pathophysiology of established cardiovascular disease but also in the initiation of atherosclerosis from a much earlier stage. NO production from healthy endothelial cells has an antiatherogenic effect by inhibiting cellular adhesion, migration, and proliferation responses involved in early lesion formation.

Transient reductions in endothelial function have previously been reported after a high fat meal, in drug-induced hyperhomocysteinemia, and in the inflammatory response to vaccination and might contribute to the pathogenesis of atherosclerosis. Studies in the cynomolgus monkey have shown that chronic social conflict increases the rate of endothelial cell damage and reduces NO bioavailability in the coronary arteries. Mental stress is known to result in rapid changes in systemic hemodynamics mediated by sympathetic activation. We purposefully elected to measure endothelial function at 30, 60, and 90 minutes after the mental stress test to determine whether more prolonged adverse effects on endothelial function might occur after complete resolution of hemodynamic changes.

A public-speaking task was chosen to induce mental stress because it has been shown to produce reliable hemodynamic and sympathetic nervous system responses. Furthermore, the level of mental stress was considered to be relevant to everyday life and likely to be encountered frequently. Mental strain was confirmed by a subjective visual analogue scale, and increased sympathetic drive was confirmed by the brisk increase in heart rate, blood pressure, and forearm blood flow and constriction of the radial artery seen during the test. Neuroendocrine activation was verified in the healthy subjects by increased salivary cortisol levels, as previously reported. The change in endothelial function after mental stress was not homogeneous in the control subjects. However, there was no difference in clinical characteristics, acute hemodynamic responses, or the subjects’ assessments of mental stress, which might explain this different behavior.

The noninvasive high-resolution ultrasound technique used to assess endothelial function in the present study has been developed in our laboratory and has been shown to be accurate and reproducible and to reflect NO bioavailability in conduit arteries of the systemic circulation. The results of our control studies in healthy subjects in which no mental stress test was performed confirm the reproducibility of both the endothelium-dependent and -independent vascular responses with repeated measures over 4 hours. We used a lower dose of GTN than previously reported, which resulted in submaximal dilatation. This will more accurately reflect smooth muscle sensitivity to nitroso vasodilators and permits multiple doses to be given to subjects over a relatively short period.

The response to mental stress in the NIDDM subjects was different from that in the healthy control subjects. FMD was significantly impaired in the NIDDM subjects at baseline, and no further reduction was noted in response to the mental stress test. Possible explanations for these findings include a different level of stress stimulus or a different vascular response. Diabetic subjects reported a lower level of mental stress, which may explain this different behavior.
stress after the speech task. This was associated with a lower heart rate and blood pressure response and no significant increase in cortisol levels after the same mental stress task. This might reflect the regular contact of the diabetic subjects with a hospital environment or might be due to subclinical autonomic dysfunction or an abnormality of the hypothalamus-pituitary-adrenocortical axis, with prolonged activation leading to downregulation of the coronary response to acute changes.\textsuperscript{33} This pattern has been noted in some patients with abnormal obesity and insulin resistance.\textsuperscript{34} An alternative explanation is that the vascular endothelial function could not be impaired further than the abnormality already present at baseline in the diabetic subjects.

A number of mechanisms might be involved in the pathogenesis of endothelial dysfunction after mental stress. Although the time course of blood pressure and heart rate changes was considerably shorter than the FMD response, the relationship between changes in heart rate and in FMD suggests a role for the sympathetic nervous system, as suggested in animal models.\textsuperscript{35} Consistent with a role for sympathetic activation is the observation that in the diabetic subjects, who had a significantly elevated resting heart rate but a reduced hemodynamic and cortisol response to the mental stress test, no change in endothelial function was seen. Chronic mental stress might also alter immunologic responsiveness, as evidenced by differences in T-cell subsets in subjects with low and high levels of stress.\textsuperscript{36} We have recently reported transient endothelial dysfunction after the acute inflammatory response to vaccination.\textsuperscript{27} However, in the present study, we did not demonstrate a significant change in plasma levels of the inflammatory cytokines TNF-\textalpha, IL-1, or IL-6 over 1 hour after the mental stress test. Endothelial dysfunction after mental stress might be mediated via increased oxidative stress or through the release of potent vasoconstrictors, such as endothelin\textsuperscript{29} and angiotensin II.\textsuperscript{37} However, the absence of a change in vessel size or basal blood flow over the 4 hours after mental stress would argue against an important role for vasoconstrictors as the mechanism of impaired FMD. Further studies using specific antagonists of potential pathways will be required to define precisely the mechanism(s) involved.

Our findings suggest that in healthy subjects without overt vascular disease, a brief episode of mental stress can induce a rapid impairment of endothelium-dependent relaxation in human conduit arteries that is apparent for up to 4 hours after resolution of the acute hemodynamic response. These findings suggest a potentially important mechanism linking short-lived episodes of mental stress, as encountered frequently during normal daily life, to a vascular abnormality relevant to early atherosclerosis. Atherosclerosis is known to have a long preclinical period, during which a range of risk factors may act on the vessel wall and to which the endothelium appears to be the key “transducer” determining physiological and pathological responses. The finding that a common behavioral stimulus can adversely affect this important homeostatic layer of cells both improves our understanding of atherogenesis and suggests potential therapeutic approaches to modify the disease process.


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