Social Status, Environment, and Atherosclerosis in Cynomolgus Monkeys

Jay R. Kaplan, Stephen B. Manuck, Thomas B. Clarkson, Frances M. Lusso, and David M. Taub

The purpose of this experiment was to examine the effects of social environment and social status on coronary artery and aortic atherosclerosis in adult male cynomolgus monkeys (Macaca fascicularis). Thirty experimental animals were assigned to six groups of five members each, and all animals were fed a moderately atherogenic diet (43% of calories as fat, 0.34 mg cholesterol/Cal) for 22 months. Group memberships were changed periodically among 15 monkeys (unstable social condition) and remained fixed throughout the experiment in the remaining animals (stable social condition). Within each condition, individual monkeys were classified as either dominant or subordinate animals, based on dyadic patterns of aggression and submission. At necropsy, the coronary arteries were subjected to pressure fixation and five sections each were taken from the left anterior descending, left circumflex, and right coronary arteries. The mean intimal area measurement, based on all arterial sections, served as a coronary index for each animal. Results indicated that dominant animals in the unstable condition had significantly greater coronary artery atherosclerosis than dominant monkeys housed in stable social groups. Coronary artery atherosclerosis in the unstable dominants was also greater than among similarly housed (i.e., unstable) subordinates. A similar pattern was observed in the abdominal aorta, but was not statistically significant. No significant differences or similar patterns were seen in the thoracic aorta. Additional analyses revealed that the coronary artery effects were not due to concomitant differences in total serum cholesterol or high density lipoprotein cholesterol concentrations, blood pressures, ponderosity, or fasting glucose concentrations among the experimental animals. Behaviorally, manipulation of group memberships intensified agonistic encounters and disrupted patterns of affiliative interaction between dominant and subordinate monkeys. Overall, these results suggest that social dominance (an individual behavioral characteristic) is associated with increased coronary artery atherosclerosis, but only under social conditions that provide recurrent threats to the status of dominant animals (i.e., under behavioral challenge). (Arteriosclerosis 2:359-368, September/October 1982)

Numerous investigators have suggested that psychosocial factors contribute in human beings both to the progression of atherosclerosis and to clinical manifestations of coronary artery disease.1-6 In addition, in studies using animal models, behavioral stressors such as cage restraint, crowding, social perturbation, and shock avoidance have been found to produce a variety of arterial lesions.7-12 It is noted frequently, however, that the influence of psychosocial variables may be mediated, in part, by behavioral characteristics of the individual organism. For instance, the coronary-prone behavior pattern, which is prospectively associated with coronary heart disease in human beings, is defined as a constellation of coping behaviors exhibited by only some...
individuals (Type A's) when encountering an appropriately challenging environment. Thus, individual differences in behavior and demands of the social environment (e.g., stress) may interact in their effects on atherosclerosis.

In cynomolagus monkeys, coronary artery atherosclerosis has been found recently to be related to differences in "competitiveness" (unpublished observation) yet within this model it is uncertain to what extent effects associated with behavioral attributes of the individual animal may be conditioned by accompanying social or environmental characteristics. Accordingly, in the study described here, both an individual variable (social dominance) and a psychosocial manipulation (periodic reorganization of group memberships) were used in examining the interaction of individual characteristics and social environment in the production of atherosclerosis in male cynomolagus monkeys. A major finding of the study was that social dominance was associated with increased coronary artery atherosclerosis, but only in an environment marked by social instability.

### Methods

#### Animals

The animals used were 30 male cynomolgus monkeys, imported as adults from Malaysia and the Philippine Islands (average age = 7.5 years, estimated by dentition). They were part of a group of 60 monkeys selected from a population of over 150 adult male *Macaca fascicularis* which had been fed a test diet containing approximately 0.34 mg cholesterol/Cal for 2 months. Blood samples for determination of total serum cholesterol (TSC) and high density lipoprotein cholesterol (HDLC) concentrations were taken from all animals at Weeks 6 and 8 during this period. The 60 animals selected represented the middle range of dietary cholesterol responses for this population (TSC responses between 200 and 350 mg/dl). When selected, these animals were brought to Bowman Gray School of Medicine, where 30 of the monkeys were used in the present study and the remaining 30 were assigned to another experiment.

#### Diet

An atherogenic diet, designed to mimic closely the nutritional composition of diets consumed by average North American human beings, was used in this study (table 1). This diet contained 43% of calories from fat and 0.34 mg cholesterol/Cal (156 mg cholesterol/100 g dry weight constituents). The diet was mixed with 20 ml H₂O/100 g dry weight constituents, and 100 g was fed to each animal twice a day. Animals fed this diet showed a mean total serum cholesterol of 471 ± 83 mg/dl (so) and a mean HDLC of 41 ± 10 mg/dl across 22 months.

### Table 1. Diet Composition

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, USP</td>
<td>8.00</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>8.00</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>35.00</td>
</tr>
<tr>
<td>Dextrin</td>
<td>6.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.00</td>
</tr>
<tr>
<td>Applesauce</td>
<td>4.50</td>
</tr>
<tr>
<td>Lard</td>
<td>12.00</td>
</tr>
<tr>
<td>Butter</td>
<td>3.00</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>3.00</td>
</tr>
<tr>
<td>Dried egg yolk</td>
<td>6.00</td>
</tr>
<tr>
<td>Complete vitamin mixture</td>
<td>2.50</td>
</tr>
<tr>
<td>Alphacel</td>
<td>0.90</td>
</tr>
<tr>
<td>Hegsted salts mixture</td>
<td>4.00</td>
</tr>
<tr>
<td>NaCl (table salt)</td>
<td>2.10</td>
</tr>
</tbody>
</table>

#### Design

This experiment consisted of a 2-by-2 factorial design involving two levels of a psychosocial manipulation (stable and unstable social housing) and two levels of a classificatory variable (dominant and subordinate social status).

#### Psychosocial Manipulation

Fifteen monkeys were assigned to each of two experimental conditions, which were equated for responsiveness to dietary cholesterol. Animals within each condition were divided randomly into three five-member groups, and all groups were housed in identical pens measuring 2 × 3.2 × 2.5 m (with outdoor exposure).

1. **Unstable Social Condition.** In one condition, designated the unstable social condition, group memberships were altered periodically during the experiment by redistribution of monkeys among the three affected groups. The unstable groups were reorganized using a procedure which allowed each animal to be placed with either three or four new monkeys on every reorganization; they were reorganized once every 12 weeks during the initial 12 months of the study, and once every 4 weeks in the following 10 months. Additionally, an estrogen implanted, ovariectomized female was placed in each of the unstable groups for the last 2 weeks of each 4-week period during the final 10 months of the study. All reorganizations were accomplished while monkeys were restrained with Ketamine hydrochloride (15 mg/kg of body weight).

2. **Stable Social Condition.** Group memberships in the second, or stable, social condition remained constant throughout the period of study.

At the end of the 22 months, monkeys housed in both the stable and unstable social conditions were necropsied.
**Social Status**

It is well established that macaque social groups are characterized by dominance or status hierarchies in which animals are ranked by virtue of their abilities to defeat group members in fights. Because the social structure and behavior of macaques are also highly dependent on status relationships, social dominance was employed as a primary behavioral descriptor of the experimental animals in this investigation. Therefore, monkeys housed in each of the two social conditions were characterized as being either more dominant or more subordinate, based on patterns of aggressive interaction observed over the course of the study.

**Psychosocial Observations**

The behavioral repertoire of macaques is well known. The majority of behaviors observed in a social context may be categorized as aggressive, submissive, or affiliative, and can be described by a limited number of relatively stereotyped motor patterns. In this study, 21 behaviors were assessed (see table 2). Technicians recorded the occurrence of these behaviors using a focal sampling technique, in conjunction with an electronic data collection device (Electro General Corporation, Minneapolis, Minnesota). Data were transmitted from the collection instruments to a PDP 11/44 computer, where they were corrected for errors in logic and transmission, and then tabulated by behavior and by animal into matrices of communication (e.g., grooming, aggression, submission, body contact). Periodic checks revealed a mean interobserver reliability of 94% in the recording of social behaviors. A total of 210 focal samples (each 15 minutes in length) was made on each experimental animal, and all observations were made between 0900 and 1100 hours. Rates of performance of all behaviors were calculated from focal samples on a per monkey per hour basis.

For purposes of later analysis, rates of performance related to the discrete behaviors representing aggressive and submissive motivations (table 2) were summed to yield an overall rate of aggression and rate of submission for each animal. Additionally, a ratio of the rate of aggressive to submissive behaviors was calculated for each monkey; this index allowed the overall interaction pattern of the animal to be categorized as either more aggressive or more submissive, depending upon which type of behavior was more prevalent.

Grooming is an indicator of affiliation and its quantification reflects both the direction and strength of social bonds. In particular, the balance between grooming others and being groomed is thought to be dependent, in part, on social status. This relationship may also be affected by the degree of stress in a social environment, since grooming provides a means of relieving or reducing tension among animals. Therefore, to describe the overall affiliative status of the experimental animals, a ratio of the rate at which an animal groomed others to the rate at which it was itself groomed was calculated for each monkey. Passive body contact, another important indicator of affiliation, was measured in terms of the percent of total time spent in this activity. Sitting alone was measured in the same way.

**Determination of Social Status**

Social rankings were determined on the basis of fight outcomes among individuals, and not on the rates of performance of these behaviors or on their severity. Winners of fights were judged dominant to losers. The aggression and submission matrices constructed for each of the social reorganization periods were used to determine the direction of fight outcomes for all pairings of animals in each social group. The one animal in each group that defeated all others, as evidenced by elicitation of flight gestures, was designated the first ranking monkey. The monkey that defeated all but the first ranking animal was labeled second ranking, and so forth. It may be noted that determination of dominance rankings is simplified in macaque groups by the tendency for dominance relationships to be transitive. That is, if Monkey A is dominant to Monkey B, and if B is dominant to Monkey C, then A is usually dominant to C also.

Rankings were established in each group for each social reorganization period. The number of animals over which a given monkey was dominant was recorded as a dominance "score" for that monkey during the particular experimental period in which a set of observations was made. Thus, the first ranking monkey in each group of five was assigned a score.

### Table 2. Behaviors of Cynomolgus Macaques

<table>
<thead>
<tr>
<th>Category</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggression Contact</td>
<td>Siap/grab/push</td>
</tr>
<tr>
<td></td>
<td>Grapple</td>
</tr>
<tr>
<td></td>
<td>Bite</td>
</tr>
<tr>
<td>Noncontact</td>
<td>Open-mouth threat</td>
</tr>
<tr>
<td></td>
<td>Stare threat</td>
</tr>
<tr>
<td></td>
<td>Chase</td>
</tr>
<tr>
<td></td>
<td>Supplant</td>
</tr>
<tr>
<td>Submission</td>
<td>Lip smack</td>
</tr>
<tr>
<td></td>
<td>Be supplanted</td>
</tr>
<tr>
<td></td>
<td>Cower</td>
</tr>
<tr>
<td></td>
<td>Flee</td>
</tr>
<tr>
<td></td>
<td>Jump/turn away</td>
</tr>
<tr>
<td></td>
<td>Grimace</td>
</tr>
<tr>
<td></td>
<td>Scream</td>
</tr>
<tr>
<td></td>
<td>Present</td>
</tr>
<tr>
<td>Affiliation</td>
<td>Groom</td>
</tr>
<tr>
<td></td>
<td>Passive body contact</td>
</tr>
<tr>
<td></td>
<td>Sitting alone (nonsocial)</td>
</tr>
</tbody>
</table>
of 4, the second ranking monkey a score of 3, and so forth. These scores were next averaged for each monkey over all experimental periods. Those monkeys having an average score greater than 2.0 were then designated dominant animals in the experiment, and those having an average score of 2.0 or less were labeled subordinates. This method of describing dominance is similar to that reported by others.26 Finally, Kendall coefficients of concordance27 revealed that in both stable and unstable conditions, social rankings were consistent over the course of the experiment (p < 0.001).

Clinical Pathologic Observations

Serum Lipid Concentrations

Blood samples for determination of TSC and HDLC concentrations were taken 22 times during the course of the study (approximately once a month). TSC determinations (in mg/dl) were carried out using the AutoAnalyzer II procedure.28 HDLC concentrations (in mg/dl) were assessed by the heparin manganese precipitation procedure, as described in the Lipid Research Clinics Manual.29 All serum lipids were evaluated in our Lipid Analytic Laboratory, which is in complete compliance with the Cooperative Lipid Standardization Program of the U.S. Department of Health and Human Services. Animals were fasted 24 hours prior to sampling, and during the sampling procedures were restrained with Ketamine hydrochloride (15 mg/kg).

Blood Pressure

Systolic and diastolic blood pressures were recorded on monkeys sedated with Ketamine hydrochloride using a Doppler ultrasound apparatus (Arteriosonde 1010, Roche, Cranbury, New Jersey). Blood pressures were determined 11 times during the course of the study (approximately once every other month). Previous work in our laboratory has shown that indirect measures of blood pressure recorded under Ketamine anesthesia correlate well with blood pressure measurements obtained from the same animals under fully conscious conditions.30

Ponderosity

To evaluate relationships between obesity and atherosclerosis, estimates of ponderosity were made on all animals. Ponderosity was measured as the body weight of the animal divided by the distance between the suprasternal notch and pubic symphysis (g/cm).31 These determinations were made four times during the study.

Carbohydrate Metabolism

Assessments of plasma glucose concentrations were made following an 18-hour fast on six occasions during the experiment. Glucose concentrations (mg/dl) were determined using a Beckman Glucose Analyzer.

Heart Rate Responsivity to Behavioral Stimuli

It is frequently reported that individuals who are behaviorally predisposed to coronary heart disease (Type A's) show enhanced cardiovascular responses to psychosocial challenges.32-34 As a result, it has recently been proposed that a cardiovascular hyperresponsivity under stress may promote atherosclerosis.1,35 In this regard, heart rate responses to a standard laboratory challenge involving threat of capture and physical handling were recorded on a single occasion near the end of this investigation, using an EKG telemetry system. Changes in heart rate observed under these stimulus conditions, adjusted for differences in resting heart rate across animals, were taken as an index of behaviorally-induced heart rate reactivity. Since preliminary analyses revealed no significant differences in the heart rate reactivity of dominant and subordinate monkeys of either experimental condition, and no overall differences between animals housed in stable and unstable social groups, these data are not described further in this report. A more detailed description of the measurement of individual differences in heart rate reactivity and of their retrospective association with atherosclerosis is presented elsewhere.36

Measurement of Coronary Artery Atherosclerosis

At the time of necropsy, the heart and aorta were removed and the coronary arteries were perfused with 10% neutral buffered formalin under a pressure of 100 mm Hg. Following pressure fixation, 15 tissue blocks (each 3 mm in length) were cut perpendicularly to the long axis of the coronary arteries. Five of these were serial blocks from the left circumflex, five from the left anterior descending, and five from the right coronary artery. Two sections were cut from each block and stained with either hematoxylin and eosin or Verhoeff van Gieson stains.

Each of the Verhoeff van Gieson stained sections was projected and the area occupied by intima and/or intimal lesion (i.e., the area between the internal elastic lamina and the lumen) was measured using a Zeiss MOP III Image Analyzer. The extent of coronary artery atherosclerosis for each animal was expressed as the mean intimal area (in mm²) of 15 sections of coronary artery.37 On 14 of the experimental animals, arterial sections were also projected and digitized by a second investigator; a comparison of intimal area values based on the two sets of measurements yielded a reliability coefficient of 0.96.

Measurement of Aortic Atherosclerosis

Two segments of the aorta were taken from each animal: 1) a 10 cm segment of the abdominal aorta, starting just distal to the branch of the celiac artery; and 2) a 10 cm segment of the thoracic aorta, beginning at a point just distal to the aortic arch. The adventitia was removed and the artery segments were...
open longitudinally, flat-mounted on cardboard and placed in 10% neutral buffered formalin for immersion fixation. After fixation, the aortic segments were stained with Sudan IV in isopropyl alcohol. Gross evaluations by four investigators provided an estimate of the percentage of total surface area of each artery segment that was affected with plaque; this value was converted to an area measurement, expressed in mm². Cross-sectional blocks (up to five) were then cut through plaques representative of the thoracic and abdominal segments, and the thickness of the arterial plaques (in mm) was calculated from microscopic slides using the Zeiss Mop III Image Analyzer. Plaque "volume," the index of aortic from microscopic slides using the Zeiss Mop III Image Analyzer. Plaque "volume," the index of aortic

Results

Coronary Artery Atherosclerosis

Two study animals died during the course of the experiment; therefore, results reported here are based on 28 monkeys. Among these animals, six were dominant and nine were subordinate animals in the unstable social condition, and seven were dominants and six subordinates in the stable social condition.

Mean intimal areas (in mm² ± SEM) among dominant and subordinate animals in the two experimental conditions are presented in table 3. To determine whether there were significant group differences in extent of coronary atherosclerosis, a 2-by-2 (Conditions-by-Status) ANOVA was performed.* Due to slightly skewed distributions of intimal area measurements, however, an initial data transformation of the form X' = √X + √X + 1 was carried out as recommended by Sokol and Rohlf38 and Freeman and Tukey.37 The ANOVA performed on these data showed no significant main effect for Conditions or Status, but did yield a significant Conditions-by-Status interaction term (p = 0.034). Contrasts among group means by Dunn's procedure38 (at p < 0.05) revealed that dominant monkeys in the unstable social condition had significantly greater atherosclerosis than dominant animals housed in stable social groups. Severity of coronary artery atherosclerosis in the unstable dominants was also significantly greater than among similarly housed (i.e., unstable) subordinates. Interestingly, dominant animals in the stable social condition had slightly less atherosclerosis than their subordinate counterparts, although this difference was not statistically significant.

While extent of intimal plaque varied among groups, pathologic characteristics of the lesions seen in coronary arteries from the four groups were qualitatively similar. Generally, the intimal lesions contained numerous foam cells, extracellular lipid, and increased amounts of collagen and elastin. Accumulations of lipid and, occasionally, mineralization were noted as medial changes in the coronary arteries of some of the animals. The medial changes seen were comparable to those observed in other macaques fed equivalent diets.

Figure 1 shows photomicrographs of a section of left circumflex coronary artery in four animals, one from each of the experimental groups. Though presented only for purposes of illustration, the extent of intimal plaque depicted in these photomicrographs approximates mean values for the respective groups from which these animals were selected.

Lumen Stenosis

Intimal area is preferred as a measure of atherosclerosis since it is not affected by changes in artery size.40 However, we have also examined the influence of psychosocial factors on lumen stenosis. This variable was calculated here as the ratio of intimal area to total area within the internal elastic lamina, averaged over all arterial sections. Lumen stenosis among animals housed in the stable social condition averaged 26% ± 6.7% (SEM) in dominant monkeys and 40% ± 5.6% in subordinates. Mean values in the unstable condition were 50% ± 5.5% among dominants and 27% ± 4.8% in subordinate monkeys.

A 2-by-2 (Conditions-by-Status) ANOVA performed on these measures revealed no significant main effect for Conditions or Status, but again showed the significant Conditions-by-Status interaction (p = 0.003). Subsequent group contrasts by Dunn's procedure (at p < 0.05) indicated that lumen stenosis in unstable dominants was significantly greater than among dominant animals in the stable social condition. Conversely, subordinates housed in stable social groups had significantly greater lumen stenosis than subordinate monkeys in the unstable condition. Lumen stenosis was also signifi-

<table>
<thead>
<tr>
<th>Social condition</th>
<th>Dominant (mm²)</th>
<th>Subordinate (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>0.32 ± 0.13</td>
<td>0.45 ± 0.12</td>
</tr>
<tr>
<td>Unstable</td>
<td>0.74 ± 0.12</td>
<td>0.38 ± 0.10</td>
</tr>
</tbody>
</table>

* All analyses of variance and covariance reported here were carried out on a Honeywell 6620 computer, using the BMDP statistical package. All findings are based on two-tailed tests of significance.
FIGURE 1. Photomicrographs of sections of left circumflex coronary arteries representing the mean intimal area measured in the socially stable dominant (A), stable subordinate (B), unstable dominant (C), and unstable subordinate (D) groups. All photomicrographs are of paraffin sections stained by the Verhoeff van Geison method, x 40.

Aortic Atherosclerosis

As with the coronary arteries, atherosclerosis extent measured in the abdominal aorta was first subjected to a square root transformation and then entered as a dependent measure in a 2-by-2 (Conditions-by-Status) ANOVA. Although this analysis revealed no significant effects, the Conditions-by-Status interaction term approached significance \( p = 0.067 \). In addition, mean plaque volume (± SEM) among the four experimental groups varied directionally in a manner similar to that observed based on intimal area measurements in the coronary arteries (Stable = 25.8 ± 13 mm\(^3\) dominant, 69.9 ± 18 mm\(^3\) subordinate; Unstable = 52.2 ± 22.8 mm\(^3\) dominant, 41.4 ± 14 mm\(^3\) subordinate). Unfortunately, transformation applied to measures of plaque volume in the thoracic segment of the aorta failed to normalize group distributions, thus precluding parametric analyses. Nonparametric comparisons (Mann-Whitney \( U \)), moreover, showed no significant differences between animals housed in stable and unstable social groups, or among dominant and subordinate monkeys of either social condition. Rank order correlations between measures of atherosclerosis in abdominal and thoracic segments of aorta were relatively low \( r_s = 0.42, p < 0.05 \), as were correlations of intimal area measurements in the coronary arteries with plaque volume in both the abdominal \( r_s = 0.32, p = 0.05 \) and thoracic \( r_s = 0.11, \text{NS} \) segments of the aorta. These low correlations between measures of aortic and coronary artery atherosclerosis parallel observations made previously by other investigators.

Serum Lipids, Blood Pressure, Ponderosity, and Glucose

Having observed interactive effects of social conditions and status on coronary atherosclerosis, we examined: 1) whether other physiologic variables recorded in this study related appreciably to the extent of atherosclerosis, and 2) where they did, whether these variables might account for the apparent atherogenic influences of the behavioral factors. For this purpose, mean values for TSC, HDLC, systolic and diastolic blood pressure, ponderosity, and fasting
glucose concentration were first calculated for each animal, based on all measurements obtained during the course of the study. To determine if these values adequately reflected the physiologic status of the monkeys over the entire experiment, Pearson correlations were then computed (across all animals) between the overall mean scores and recordings made at successive measurement periods. These coefficients averaged 0.84 for TSC, 0.87 for HDLC, and 0.96 for ponderosity; thus, the mean values calculated for these variables were representative of the several periodic measurements recorded over the experiment. Coefficients for systolic blood pressure averaged 0.66, for diastolic blood pressure, 0.68, and for fasting glucose, 0.66. While somewhat more variable than the lipid and ponderosity measurements, individual differences in blood pressure and fasting glucose concentration were still reasonably stable during the experiment, and therefore were represented by the animals’ overall mean scores.

Pearson correlations were computed next between mean scores on each of the foregoing physiologic variables and intimal area measurements. These coefficients are presented in Table 4. The correlations are listed separately for monkeys housed in the stable (n = 13) and unstable (n = 15) social conditions, and for all animals together (n = 28). Both TSC and HDLC correlated significantly, overall, with extent of coronary atherosclerosis. Corresponding coefficients within the two social conditions were similar as well, although due to reduced degrees of freedom, only TSC among monkeys living in stable social groups covaried significantly with intimal areas. Blood pressure, ponderosity and fasting glucose concentrations were unrelated to atherosclerosis in this study, both in the stable and unstable conditions, and therefore were represented by the animals’ overall mean scores.

Finally, since blood lipids were found to be associated with atherosclerosis, TSC and HDLC were entered as covariates in 2-by-2 (Conditions-by-Status) analyses of covariance, employing intimal area and lumen stenosis as dependent variables. As in the previously reported ANOVA’s, these analyses revealed no reliable main effects for either Conditions or Status. Moreover, the Conditions-by-Status interaction terms were again significant for both dependent measures (intimal area: p = 0.038; lumen stenosis: p = 0.004). These findings indicate, then, that the behavioral influences on atherosclerosis observed here were statistically independent of effects due to differences in serum lipid values among the experimental animals.

**Social Behaviors**

It was expected that patterns of agonistic and affiliative behavior would vary between the two social conditions, in both dominant and subordinate animals. Tests for behavioral differences among groups were therefore carried out based on overall rates of aggression and submission, directionality of grooming behaviors, and amount of time spent alone and in passive body contact during the course of the experiment. Because these measures of social behavior were not normally distributed in the present data, all analyses reported below involve nonparametric tests of significance (Mann-Whitney U).

Median values on each of the behavioral measures are presented in Table 5 for dominant and subordinate animals in the stable and unstable conditions. As expected, ratios of aggressive to submissive behavior were greater for dominant, relative to subordinate, monkeys in both stable (p < 0.002) and unstable (p < 0.002) social groups. Yet, more extreme forms of agonistic behavior tended to vary by social condition. Dominant animals in the unstable social condition, for example, showed higher rates of contact aggression than did dominant monkeys housed in stable groups (p = 0.074). In contrast, noncontact aggression did not differentiate the two sets of dominant animals (p > 0.10). Among subordinate monkeys, more pronounced gestures of submission (e.g., fleeing, cowering, grimacing) also tended to occur with greater frequency in the unstable groups (p = 0.075), while milder forms of submissive behavior (e.g., lip smacking, moving away) did not differ between the two social conditions (p > 0.10).

With respect to affiliative behaviors, subordinate monkeys in the unstable groups spent more time sitting alone (p < 0.02) and less time in passive body contact with other animals (p < 0.01) than did their dominant counterparts. Dominant and subordinate animals in the stable social condition did not differ significantly on either of these behavioral indices (p > 0.10). As noted previously, directionality of grooming was measured as a ratio of the rate at which an animal groomed others to the rate at which it was itself groomed. This index was significantly higher in subordinate, relative to dominant, animals in the unstable social condition (p < 0.02) and tended to be higher for dominant than subordinate monkeys in the stable condition. Concerning these results, the median ratios listed in Table 5 describe
Table 5. Median Values for Social Behaviors Observed Among Dominant and Subordinate Animals in Stable and Unstable Social Conditions

<table>
<thead>
<tr>
<th>Social behavior</th>
<th>Stable condition</th>
<th>Unstable condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dominant</td>
<td>Subordinate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dominant</td>
</tr>
<tr>
<td>Ratio: aggression*</td>
<td>2.55</td>
<td>0.20</td>
</tr>
<tr>
<td>Submission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of aggression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact aggression*</td>
<td>0.80</td>
<td>0.50</td>
</tr>
<tr>
<td>Noncontact aggression*</td>
<td>5.02</td>
<td>1.36</td>
</tr>
<tr>
<td>Rate of submission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extreme submission*</td>
<td>0.50</td>
<td>1.56</td>
</tr>
<tr>
<td>(e.g., flee)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild submission*</td>
<td>1.39</td>
<td>5.45</td>
</tr>
<tr>
<td>(e.g., be supplanted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio: grooming others*</td>
<td>1.18</td>
<td>0.68</td>
</tr>
<tr>
<td>Being groomed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive body contact†</td>
<td>26.7</td>
<td>31.5</td>
</tr>
<tr>
<td>Sitting alone†</td>
<td>60.6</td>
<td>48.6</td>
</tr>
</tbody>
</table>

*Incidents per hour.
†Percent of total time.

Asymmetric grooming relationships in which in unstable social groups, dominant monkeys were groomed at a higher rate than they groomed others and subordinates groomed others more than they were themselves groomed; a similar, but reversed asymmetry tended to characterize grooming relationships among dominant and subordinate animals in the stable social condition.

Discussion

Results of this investigation demonstrated that, in male cynomolgus monkeys, social status influences the development of coronary artery atherosclerosis, but only under certain environmental conditions. Specifically, dominant animals living in unstable (i.e., periodically reorganized) social groups exhibited, on necropsy, significantly greater coronary artery atherosclerosis than did subordinate monkeys housed under the same social conditions. Dominants in the unstable social groups also had more extensive arterial lesions than dominant monkeys housed in groups with stable, or fixed memberships. These findings are consistent, in turn, with current hypotheses concerning the role of individual behavioral characteristics in the development of coronary disease in humans. It is well established, for example, that certain individuals (Type A's) are predisposed to coronary disease due to their hard-driving competitiveness, impatience, and aggressiveness. However, the Type A behavior pattern is not defined as a trait of personality, but as a set of overt behaviors that are elicited in susceptible individuals only under sufficiently challenging environmental conditions. Persons who have a propensity to exhibit Type A behaviors, but who are exposed to little challenge or stress, would therefore be expected to develop less coronary artery disease than Type A-prone individuals living under more demanding circumstances.

It is interesting that epidemiologic investigations among human beings have not directly examined the Type A pattern in relation to concomitant environmental or stress-related variables. Hence, it has not been demonstrated previously that behavioral attributes of the individual and qualities of the psychosocial environment interact in their influences on coronary artery disease. We believe, however, that the present data provide initial support for this hypothesis, albeit in an animal model. Thus, a significant association was observed here between social dominance (an individual behavioral characteristic) and coronary artery atherosclerosis, although only under social conditions that provided recurrent threats to the status of dominant animals (i.e., under behavioral challenge). Dominant monkeys housed in stable social groups did not show an increased severity of coronary artery atherosclerosis.

Regarding atherosclerosis at other arterial sites, we found a similar relationship involving psychosocial variables in the abdominal segment of the aorta, although the effect was of borderline statistical significance. This result is perhaps not surprising, though, given the low correlation between measures of atherosclerosis in coronary and noncoronary arteries, both as observed in the current study and as reported previously by other investigators.

Behavioral effects associated with reorganization of group memberships (the psychosocial manipulation) were, like atherosclerosis, dependent on the
social status of the animals. Characteristic forms of agonistic interaction, for instance, varied between the two social conditions, but in a different manner for dominant and subordinate monkeys. Thus, in the unstable social condition, dominant animals tended to engage in greater contact aggression, and subordinates in more extreme forms of submission (e.g., cowering, fleeing), relative to dominant and subordinate monkeys in the stable social groups. Among affiliative behaviors, the most striking effect of social conditions involved differences in the overall directionality of grooming behaviors. In direct contrast to the stable social conditions, dominant monkeys in the unstable groups were groomed at a greater rate than they groomed others, and subordinates groomed other animals more frequently than they were themselves groomed. In this regard, other investigators have reported that grooming may represent both an affiliative act and a gesture of appeasement. With respect to the latter, we suspect that frequent grooming by subordinate monkeys in the unstable social condition may have served two functions: for the alleviation of tension, and as a strategy for maintaining proximity to the dominant animals, thereby preserving access to necessary resources such as food, water, and perch space. Yet, it should be noted that even manipulation of group memberships intensified agonistic encounters and disrupted other patterns of behavioral interaction among animals, the social positions of individual monkeys tended to remain stable over the course of the study. Social status per se, then, was relatively constant over time in both experimental conditions, whereas behavioral demands associated with dominant and subordinate status varied between the stable and unstable social groups.

Finally, the relationship of behavioral factors to coronary artery atherosclerosis in this experiment was independent of the influences of other physiologic variables, most notably TSC and HDLC concentrations. Observations among human subjects similarly reveal that the epidemiologically established Type A-coronary disease association is not accounted for by elevated serum lipid concentrations, or by several other "traditional" risk factors (hypertension, smoking and age). Hence, it remains unclear at present by what mechanism(s) behavioral variables contribute to the development of atherosclerotic disease. Several investigators have suggested, however, that centrally mediated autonomic and neuroendocrine responses to stress may play a role in atherogenesis, if experienced frequently enough or over intervals of sufficient duration. In relation to behavioral models in particular, Henry and Stephens propose that recurrent activation of the sympathetic-adrenomedullary system is induced by repeated challenges to social status, especially among high ranking or dominant animals. Accompanying increases in blood pressure might promote arterial "injury" via hemodynamic forces such as turbulence and sheer stress, while elevated levels of circulating catecholamines may foster arterial lesions directly and through influences on other pathogenic factors (e.g., platelet aggregation). Since we do not have data reflecting the neurohumoral status of our animals over the period of the study or during significant behavioral interactions, these comments are necessarily only speculative. Still, the failure of other physiologic variables (e.g., lipids, blood pressure, ponderosity) to "explain" effects of behavioral variables in this experiment, as well as in studies of the Type A behavior pattern in human beings, at least warrants inclusion of autonomic indices in subsequent investigations regarding psychosocial influences on atherosclerosis.

Acknowledgments

The authors thank Debra Campbell, Anne Marie Laughery, Eric Miller, Elizabeth Nicks, Linda Pate, and Evan Zucker for their technical assistance. We thank James Rose for comments on carbohydrate metabolism and Richard St. Clair for his comments on the manuscript. Finally, we thank Irwin Bernstein for his ideas concerning socially induced stress.

References

1. Williams RB. Psychophysical differences between Type A and B individuals that may lead to coronary heart disease. In: Dembroski TM, ed. Proceedings of the forum on coronary prone behavior. DHEW publication no. (NIH) 78-1451, 1977:165-191
10. St Clair RW, MacNinch JE, Middleton CC, Clarkson TB, Loffband HB. Changes in serum cholesterol levels of squirrel monkeys during importation and acclimation. Lab Invest 1957;16:828-832


40. Clarkson TB, Bond MG, Bullock BC, Marzetta CA. A study of atherosclerosis regression in Macaca mulatta. IV. Changes in coronary arteries from animals with atherosclerosis induced for 19 months and then regressed for 14 or 48 months at plasma cholesterol concentrations of 300 or 200 mg/dl. Exp Mol Pathol 1981;34:345–358


49. Haft JL. Cardiovascular injury induced by sympathetic catecholamines. Prog Cardiovasc Dis 1974;17:73–86

Index Terms: atherosclerosis • social behavior • stress • monkeys • social status
Social status, environment, and atherosclerosis in cynomolgus monkeys.
J R Kaplan, S B Manuck, T B Clarkson, F M Lusso and D M Taub

doi: 10.1161/01.ATV.2.5.359
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/2/5/359

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/