Body Fat Distribution as a Risk Factor for Coronary Artery Atherosclerosis in Female Cynomolgus Monkeys

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Central fat deposition is associated with increased risk of coronary heart disease morbidity and mortality in women. Subcutaneous fat distribution was investigated as a potential factor that might exacerbate diet-induced coronary artery atherosclerosis (CAA) in female cynomolgus monkeys (Macaca fascicularis) which share with North American Caucasian women a gender-related protection against CAA. In a retrospective necropsy study (n = 36), the distribution of the antemortem ratio of subscapular/triceps skinfold thickness was divided at the mean and the two resulting groups were designated high and low for this variable. CAA was quantified as the mean cross-sectional intimal area based on nine coronary artery sections. The extent of CAA was significantly greater in the high skinfold ratio group as compared to the low skinfold ratio group. Ponderosity was closely associated with the skinfold ratio but was not a useful predictor of CAA. These findings suggest that female cynomolgus macaques may provide a primate model of the health consequences of regional fat distribution in women. (Arteriosclerosis 7:226-231, May/June 1987)

There is an emerging interest in body fat distribution, rather than quantity, as a risk factor for coronary artery atherosclerosis (CAA). It has been shown that weight gain after reaching adulthood is a more sensitive predictor of subsequent coronary heart disease than actual weight and that fat accumulated in adulthood tends to be distributed more centrally than peripherally. Several studies indicate that a central pattern of fat deposition is associated with disorders of carbohydrate metabolism, hypertension, lipid metabolism, and risk of coronary heart disease morbidity and mortality. The distribution of fat has a stronger relationship with coronary heart disease than obesity. The interaction between obesity and fat distribution as risk factors for coronary heart disease and death from all causes in women appears to be a paradoxical effect in need of further study.

In view of the potential insight to be gained from further investigation of fat distribution as a cardiovascular risk factor, it would be helpful to have an animal model in which to study this phenomenon. Female cynomolgus macaques (Macaca fascicularis) share with Caucasian North American women protection against CAA. However, some of these monkeys, like some women, are not protected. Specifically, it is known that female Macaca fascicularis that are socially subordinate and those that have poor ovarian function or low high density lipoprotein (HDL) cholesterol levels are at a higher risk for CAA.

The following study is a preliminary investigation of the role of body fat and its regional distribution as a risk factor for CAA in female M. fascicularis. It was found that whole body ponderosity increased, and peripheral fat deposition decreased in adulthood. Ponderosity was not a good indicator of CAA, whereas, females with a relatively high central/peripheral skinfold thickness had more extensive CAA than females with low ratios. These results indicate that female cynomolgus macaques may provide an animal model to study regional fat distribution in women.

Methods

Animals

The subjects in this study were 36 adult feral female cynomolgus macaques, imported from Malaysia (Primate Imports, Port Washington, New York), that lived in harem breeding colony groups until natural death. Females entered the breeding colony as 4- to 6-year-old adults (estimated by dentition) and were housed there for 4.00 ± 0.18 years (SEM) while consuming a moderately atherogenic diet that averaged 0.39 mg cholesterol/calorie. The causes of death were unrelated to cardiovascular disease and consisted mainly of postpartum complications, fight trauma, or gastrointestinal disorders. It is possible that gastrointestinal disorders of long duration prior to death may have caused a slight decrease in mean ponderosity values.

Experimental measurements were made after anesthetizing the animals with ketamine hydrochloride (15 mg/kg body weight) administered intramuscularly. All procedures involving animals were conducted in compliance with state and federal laws, standards of the Department of Health.
Ponderosity and Skinfold Thickness

The ponderosity index (PI) used in this study is a determinant of body mass adjusted for body length and is mathematically derived as the ratio of body weight (g) to trunk length (cm) measured from the suprasternal notch to the pubic symphysis. Skinfold thickness (mm) was measured immediately inferior to the inferior angle of the scapula (subscapular skinfold), and over the triceps muscle, midway along the posterior brachium (triceps skinfold), using springloaded calipers. Skinfold thickness was also measured just superior to the iliac crest (supra-iliac skinfold). Because this measurement was characterized by high intraindividual variability due to changing reproductive condition, it was excluded from the analysis. Skinfold thickness and ponderosity were measured biannually. Subject criteria for inclusion were: 1) a minimum of 2.5 years spent in the colony, and 2) four adiposity measures taken at (at least) 6-month intervals.

Measurement of Coronary Artery Atherosclerosis

At necropsy the hearts, along with other organs, were removed and were immersion-fixed in 10% neutral buffered formalin. Three consecutive tissue blocks were cut perpendicular to the long axis of the left anterior descending, left circumflex, and right coronary arteries. Histological sections prepared from each of the nine blocks and stained with Verhoeff van Gieson stain were projected, and the cross-sectional area of intimal lesion was measured using a Zeiss MOP III Image Analyzer. The extent of CAA for each animal was expressed as a mean intimal area (mm²) of the nine sections.

Statistical Analyses

Univariate linear regression, t test, Pearson’s r correlation, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to determine statistical significance. All reported significance levels are the result of two-tailed tests unless otherwise indicated. The data were transformed, when necessary, to meet the assumptions of the statistical tests. Untransformed means and standard errors are depicted in all figures. In order to meet the assumptions of linearity and homogeneity of regression for ANCOVA, one outlier was deleted from that analysis. This female did not tolerate the atherogenic diet well, and thus it was drawn, which ranged in ponderosity from 91 to 217 g/cm, with an overall mean of 128.3 (± 3.5 SEM) (Figure 1). The frequency distribution shown in Figure 1 is skewed and 16% of the animals fall above one standard deviation above the mean.

In order to characterize the stability of ponderosity, the PI was analyzed with respect to the time individuals spent in the colony (Figure 2A). Although the entire colony was sampled during a given week, this measure corresponded to different numbers of days spent in the colony for different individuals, as females entered the colony on different dates. For this reason it was possible to identify only 15 females (of the sample of 36) who had adiposity measures taken four times at consecutive yearly intervals during the time spent in the colony. A repeated measures (1 x 4) ANOVA revealed that PI increased with time spent in the

Results

Ponderosity

Initially, the role of whole body ponderosity was investigated as a risk factor for coronary artery atherosclerosis. These monkeys ranged in PI from 96 to 202 g/cm, with a mean of 127.5 ± 3.03 (SEM). In terms of ponderosity, this sample (n = 36) was thought to be representative of the population of breeding colony females (n = 82) from which...
colony ($F_{[3,42]} = 7.64, p < 0.001$). Post-hoc Tukey tests confirmed that PI was significantly lower during 1.5 to 2.4 years spent in the colony than during the subsequent three yearly intervals (all comparisons $p < 0.01$). This finding implies an increment in ponderosity with age. However, the effects of age on ponderosity were not analyzed, as age estimates based on dentition of these wild-caught adults were not considered accurate enough for rigorous statistical analysis.

Next, the relationship between ponderosity and the extent of CAA was investigated. As the contribution of risk factors to atherogenesis may occur over a long period of time, mean values of PI (as well as the other risk factors) were computed for each female ($n = 36$) using all samples collected during their time in the colony. The distribution of the mean PI's from each of the sample females was then divided at the mean (127.5 g/cm) into two groups representing high and low ponderosity. The coronary artery intimal area mean was higher in the high PI group, but the difference was not significant ($F_{[1,34]} = 0.93, p = 0.34$) (Figure 3A). Thus, the relative degree of whole body ponderosity had no significant effect on the extent of CAA.

### Regional Subcutaneous Fat Distribution

Regional fat distribution was investigated as a risk factor for coronary artery atherosclerosis. As the location, rather than the quantity, of fat was of interest in the next series of analyses, subscapular and triceps skinfold measurements were adjusted for individual differences in ponderosity by computing a ratio of the skinfold thickness to the PI. In order to determine the stability of these variables, individual measurements of skinfold thickness/PI in a subset of 15 females were compared between four consecutive yearly intervals during time spent in the colony. Repeated measures ANOVA revealed no significant change in subscapular skinfold with years spent in the colony ($F_{[3,42]} = 2.35, p = 0.086$). The relationship of triceps skinfold to time spent in the colony is depicted in Figure 2B. A significant change in triceps/PI ratio was observed with years spent in the colony ($F_{[3,42]} = 3.02, p = 0.040$). Post-hoc comparisons confirmed that triceps skinfold thickness decreased between the first and fourth yearly intervals of time spent in the colony ($p < 0.01$). An index of central to peripheral fat deposition, the ratio of subscapular to triceps skinfold (SS/TRI), which has been used extensively in human studies, was computed and compared between four consecutive yearly intervals of time spent in the colony. No significant change in the ratio of fat deposition was observed ($F_{[3,42]} = 0.90, p = 0.447$).

Next, attention was turned to the relationship between regional fat deposition and CAA. The distributions of mean values of triceps/PI, subscapular/PI, and the SS/TRI skinfold ratio were each divided at the mean into high and low groups and the extent of CAA was compared between these groups for each skinfold ratio. No statistical differences in the extent of CAA were found between the high and low groups of the subscapular/PI (skinfold/PI distribution X = 0.03; $F_{[1,34]} = 0.06, p = 0.80$) (Figure 3B) or the triceps/PI ratio (skinfold/PI distribution X = 0.01; $F_{[1,34]} = 1.94, p = 0.17$) (Figure 3C). When the extent of CAA in the high and low groups of the subscapular/triceps (central/peripheral) skinfold ratio distribution were compared, it was found that the high subscapular/triceps ratio group

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**Figure 3.** Coronary artery atherosclerosis extent, ponderosity, and fat distribution. A. Coronary artery atherosclerosis and ponderosity (mean, SEM) ($n = 36$). B. Coronary artery atherosclerosis and subscapular/PI (mean, SEM) ($n = 36$). C. Coronary artery atherosclerosis and triceps/PI (mean, SEM) ($n = 36$). D. Coronary artery atherosclerosis and subscapular/triceps skinfolds adjusted for chol/cal × days (mean) ($n = 35$).
had significantly more extensive CAA than the low skinfold ratio group (skinfold distribution $X = 2.96; F[1,34] = 6.21, p = 0.018$) (Figure 3D). In fact, the CAA extent was three times greater in the high ratio group than the low ratio group.

However, the high ratio group also lived a significantly longer time in the colony, and thus consumed the atherogenic diet for a longer time than the low ratio group (Low: $X = 3.59 \pm 0.32$ years; High: $X = 4.75 \pm 0.43$ years, $F[1,34] = 4.61, p = 0.039$). Differences between the groups in the duration of diet consumption could cause differences in the extent of CAA. To correct for dietary consumption differences, an index of total cholesterol fed was computed for each female according to the following formula: mg cholesterol/calorie $\times$ days on diet. The relationship between CAA and cholesterol/calorie $\times$ days for the high and low SS/TRI groups is depicted in Figure 4. The index of total cholesterol consumption was used as a covariate in an analysis of covariance (ANCOVA) of CAA extent and it was found that the high skinfold ratio group still had significantly more extensive CAA than the low ratio group ($F[1,32] = 5.23, p = 0.029$) (Figure 3E). Thus, a relatively high central/peripheral pattern of fat deposition, as indicated by the subscapular/triceps ratio, was associated with exacerbated coronary artery atherosclerosis.

Relationship between the Central/Peripheral Skinfold Ratio and Ponderosity

While ponderosity did not aid in the discrimination of the extent of CAA in this study, it has at times been a weak predictor of CAA in human beings. For this reason it is important to examine the relationship between these two variables more closely. The mean values of ponderosity and subscapular/triceps skinfold ratio were positively associated ($r = 0.55, p < 0.001$, one-tailed test). The distributions of PI and the skinfold ratio were each divided at the mean into two groups, and a $2 \times 2$ contingency table was constructed (high ponderosity/high skinfold ratio [$n = 10$], high ponderosity/low skinfold ratio [$n = 4$], low ponderosity/high skinfold ratio [$n = 3$], low ponderosity/low skinfold ratio [$n = 19$]). When the frequency was compared in these four groups, a significant difference was found (Fisher's $p = 0.0013$). A high value of the skinfold ratio was infrequently accompanied by low ponderosity, and a low value of the skinfold ratio infrequently occurred in females with a relatively high PI. Thus a relatively central pattern of fat deposition nearly always occurred in the more ponderous females.

Discussion

In women, a relatively central pattern of fat deposition has been associated with increased risk of coronary heart disease morbidity and mortality. This fat deposition pattern has also been associated with diabetes, serum lipid concentrations, and hypertension.

The results described in the present report are, to our knowledge, the first evidence that female cynomolgus macaques with a high central/peripheral fat distribution ratio have significantly more extensive CAA than those with a low ratio. Further, this is the first report of a regional fat deposition pattern as a risk factor for coronary artery atherosclerosis in a nonhuman primate species. Finally, through use of an animal model, it was possible to quantify CAA extent and show a relationship of disease extent to fat distribution, instead of having to rely on heart disease morbidity and mortality statistics. Cynomolgus macaques have been shown to be a useful animal model for studies of atherosclerosis. The results of this study suggest that $M$. fascicularis may be a useful animal model for the study of the role of fat distribution as a risk factor for coronary artery atherosclerosis.

In recent studies of human beings, a waist/hip circumference ratio has been used as the most sensitive measure of central/peripheral fat deposition in predicting coronary heart disease morbidity and mortality among women. Earlier investigators, using subscapular and triceps skinfold measures, showed that the ratio of these two measures and plasma TG concentrations were significantly associated. Further, subscapular skinfold was found to be positively associated, and triceps skinfold was found to be negatively associated, with coronary heart disease. More recently, the subscapular/triceps ratio, used as a measure of central adiposity, was found to predict differences in serum lipid concentrations in the San Antonio Heart Study. Thus, repeated observations of a relationship between the subscapular/triceps ratio and heart disease and related risk factors have shown the utility and predictive validity of this measure when using human subjects. The present report is the first to describe the utility of this measure in predicting coronary artery disease in a nonhuman primate model. Thus, replications of this finding will be necessary to assure predictive validity in this species. The supra-iliac skinfold measure (which was not used in this study because of the high intra-individual variability) may be a useful descriptor of central fat deposition in macaques. It is uncertain whether a waist/hip circumference ratio would be a better predictor of CAA in cynomolgus macaques.
monkeys than the subscapular/triceps or a suprailiac/tri-
ceps skinfold ratio. This primate species does not vary
greatly in fat deposition in the pelvic region.

Triceps fat deposition declined over time spent in the
breeding colony, whereas, subscapular fat deposition did
not change. Although not statistically significant, the low
triceps/PI skinfold group had more than twice as much
CAA as the high triceps/PI skinfold group, whereas there
was very little difference in CAA extent in the low and high
subscapular skinfold groups. This may indicate that
low levels of peripheral fat contribute most to the sig-
nificance of the ratio of central to peripheral fat as a predictor of the
extent of CAA in this species. This apparent trend toward a
pattern of lower peripheral fat deposition over time oc-
curred in adulthood, along with a significant increase in
whole body ponderosity. It would have been useful to have
a number of anthropometric measurements taken at differ-
et sites to ascertain where fat is being deposited in fe-
nalies in which whole body ponderosity increases during
adulthood.

While mean values of the PI and the subscapular/triceps
ratio were significantly correlated, it was the skinfold ratio,
not the ponderosity index, that predicted CAA. In this
study, a high (above the mean) value of the subscapular/
triceps ratio occurred infrequently among animals with
ponderosity indices that fell below the group mean. Thus,
fat accumulated in adulthood in cynomolgus females is
associated with a relatively central, rather than peripheral,
pattern of fat deposition as in human beings. The close
association between these two variables may account for
the fact that obesity has, at times, been found to be a weak
predictor of coronary heart disease in human beings.

In interpreting the results of this study, there are statisti-
cal considerations to be taken into account. The use of
mean values to represent ponderosity and the skinfold
ratio, which both appear to increase over time, may mini-
mize the extreme degree of ponderosity and central fat
deposition that some females exhibited toward the end of
their life in the colony. As atherogenesis is thought to be a
lifetime process, with risk factor contribution occurring over
long time periods, it was decided to use lifetime mean
values of these variables. Further, this study was retro-
pective, rather than experimental, and the study subjects
represent a nonrandom sample of the breeding colony
population (those that died). In terms of design, this study
is similar to a retrospective epidemiologic study.

The relatively high central fat deposition pattern has
been considered a masculine type of adipose tissue distri-
bution.1,18,22 It is known that female cynomolgus ma-
caque with poor ovarian function have exacerbated coro-
nary artery atherosclerosis.15 The evidence is con-
dictory as to whether estrogen deficiency (due to
oophorectomy or natural menopause) exacerbates coro-
nary artery atherosclerosis in women.23-25 Recent evi-
dence suggests that relatively high androgenic activity is
associated with central fat deposition in women.26 The
findings of the present study suggest that female cynomol-
gus monkeys may provide an animal model to investigate
the role of sex steroids in body fat distribution.

In summary, regional fat distribution was found to be a
significant risk factor for the development of coronary ar-
tery atherosclerosis in female cynomolgus monkeys. Fur-
ther study is needed to clarify changes in the pattern of fat
deposition during adult years. There is also a need to de-
fine the relationship between this risk factor and other tradi-
tional risk factors such as impaired carbohydrate and
lipid metabolism. While these results must be considered
preliminary, they offer the exciting possibility of an animal
model of the health effects of regional fat deposition.

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