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AGING AND ATHEROSCLEROSIS IN HUMAN AND NONHUMAN PRIMATES

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ABSTRACT

Atherosclerosis is a major age-related process and public health problem and its clinical manifestations (coronary heart disease [CHD] and cerebrovascular disease) continue to be responsible for approximately 50% of all deaths occurring annually. In addition, CHD is responsible for over 70 to 80% of deaths among men and women over 65 years old. As our population ages (35 million people over the age of 65 in the U.S. by the year 2030) and because of the increased morbidity and mortality associated with atherosclerosis, an understanding of the role of aging in the development of atherosclerosis is needed.

Multiple risk factors such as smoking, gender, hypertension, and lipids contribute to the development of atherosclerosis. However, these risk factors in combination explain only about half of the individual variability in incidence of CHD, and it has been hypothesized that age-related conditions may play a role. To propectively evaluate the effects of age per se on atherosclerosis progression in humans would require observation over many years. Thus, animal models that are representative of both aging processes and atherosclerosis would be extremely valuable. As such, nonhuman primates have been used extensively in atherosclerosis research. However, studies that will specifically evaluate the role of aging per se in contributing to development of atherosclerosis in nonhuman primates have only recently been initiated.

In this review, the contribution of nonhuman primates to atherosclerosis research will be discussed, as will the development of atherosclerosis in both human and nonhuman primates. In addition, a role for age-related conditions in atherosclerosis development in both human and nonhuman primates will be outlined.

KEY WORDS: Nonhuman primates, atherosclerosis, aging, lipids, obesity, insulin, glycation

INTRODUCTION

Atherosclerosis is a major age-related process and public health problem, and its clinical sequelae (coronary heart disease [CHD] and cerebrovascular disease) continue to be responsible for approximately half of all deaths occurring annually (1,2). CHD accounts for 70 to 80% of deaths among men and women over 65 years old. Furthermore, 50% of all patients hospitalized for acute myocardial infarction are in this age group (3,4). It is projected that by the year 2030, 20% of the U.S. population (approximately 35 million people) will be older than 65 years. Because of the increased morbidity and mortality associated with atherosclerosis in older persons, an understanding of the role of physiologic aging in the development of atherosclerosis is warranted (5,6).

Atherosclerosis is a progressive disease of multifactorial origin in which (in addition to aging) serum lipid concentrations, smoking, and hypertension are considered major risk factors (7-9). Gender is also a risk factor, with the risk for men being approximately twice as high as that for age-matched premenopausal women (7-9). However, a persistent problem in the natural history of atherosclerosis has been that the major risk factors, in combination, explain only about half of the individual variability in extent and severity of atherosclerosis and incidence of CHD. Although the basis for the individual variability may be genetic in nature, an intriguing hypothesis is that age-related conditions may play a role. To prospectively evaluate the role of aging per se on atherosclerosis progression in human beings would require clinical trials of many years' duration. Thus, animal models of both aging processes and atherosclerosis would be extremely valuable. The use of nonhuman primates as models for human atherosclerosis has been well documented (10-12). However, trials designed to specifically evaluate the contribution of aging processes in development of atherosclerosis have only recently been initiated in nonhuman primates (13).

In this review, we will discuss the characteristics of atherosclerosis and cardiovascular risk factors as functions of age in human beings, relate these findings to those observed for nonhuman primates, and report on how age-related processes may affect the development of atherosclerosis in nonhuman primates.

DISCUSSION

Current Concepts of Aging and Cardiovascular Disease

Aging and Traditional Risk Factors: It is well-known that aging is associated with changes in hemodynamic parameters that adversely affect vascular function and accelerate cardiovascular disease in both men and women (14). Specifically, aging is associated with increases in systolic blood pressure and pulse pressure, and the degree to which the pressure is elevated predicts cardiovascular events (15). Elevated blood pressure also frequently coexists with other aging conditions that may also increase CHD risk, including obesity, diabetes mellitus, and hyperlipidemia (16). Further, effective control of hypertension has been shown to decrease both stroke and CHD in the elderly (17).

As with blood pressure, plasma cholesterol, triglyceride, and LDL cholesterol concentrations increase with age (7,8). Plasma cholesterol and triglyceride concentrations increase in men for the first 50 to 60 years of life, plateau, and then begin to decline (7, 18). In women, a similar decline is observed but is delayed by about 10 years. Before the plateau, women have lower cholesterol concentrations, whereas after the plateau, plasma cholesterol concentrations are higher in women compared to men. HDL cholesterol concentrations have less consistent changes with age. However, during the premenopausal years, women have higher HDL cholesterol concentrations than men (18). The changes in lipid concentrations with age have been associated with increased incidence of ischemic heart disease (14,19). However, the relationship between cholesterol and CHD diminishes markedly in elderly men (>70 years) (18). This may be an artifactual decline due to selective mortality.

Other risk factors for CHD, such as obesity, hyperinsulinemia, and diabetes mellitus also increase in the elderly, and will be discussed later in this review.

Natural History of Human Atherosclerosis: Atherogenesis begins in early childhood. Aortic fatty streaks have been observed in neonates and similar fatty streaks are found in coronary arteries about a decade later (10). Fatty streaks are slightly raised intimal areas of fat-filled cells, known as foam cells. Initially these foam cells are derived primarily from macrophages and are rich in cholesteryl esters. With time, smooth muscle cell foam cells and more extracellular lipid are found. At puberty, about 65% of children have substantial accumulations of macrophage and smooth muscle cell foam cells (20). The extent of the fatty streaks increases in the second and third decades of life and then subsequently decreases as more advanced lesions begin to predominate. Atheromas are predominant in the third decade of life and are characterized by large areas of extracellular lipid that may displace the normal cellular architecture (20,21). Although fatty streaks are observed in neonates, it is still unclear if these same fatty streaks progress to advanced lesions with further injury or whether these represent newly formed lesions. However, after age 30, fibrous plaques, which are distinguished from atheromas by their fibromuscular cap, predominate. These plaques begin to form lesion complications, with medial damage and necrosis at the base of the lesion, ulceration, thrombosis, hemorrhage, and mineralization. After the fourth decade of life, the clinical manifestations of atherosclerosis (i.e. myocardial infarction, stroke and gangrene of extremities) may occur and are often due to ruptures or fissures of the plaque, with subsequent plaque hemorrhage and thrombotic occlusion of an artery (22). The natural history of human atherosclerosis is illustrated in Figure 1.

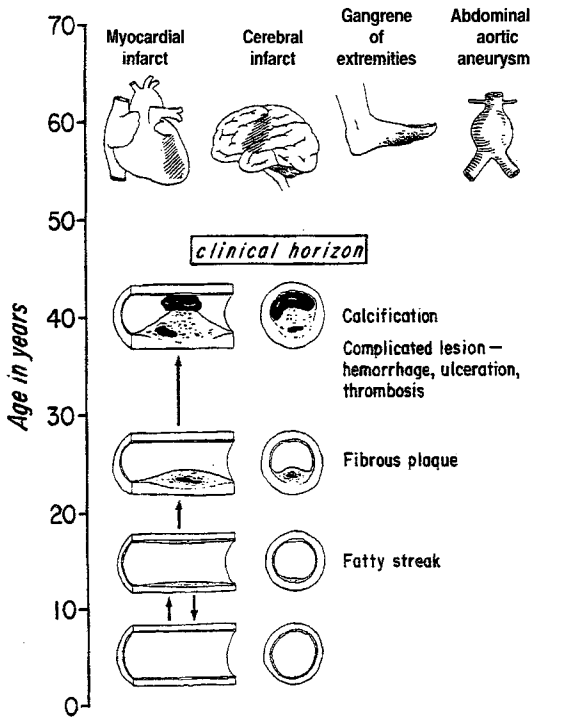

Figure 1. Natural history of atherosclerosis [modified from Strong et al. (20)].

Interestingly, many arteries develop extensive accumulations of atherosclerotic mass (lipids, extracellular matrix proteins, and intimal cells) yet do not develop lumen narrowing (23). This phenomenon, originally referred to as compensation, is now known as remodeling, and allows for maintenance of the lumen area and normal blood flow. The compensatory enlargement of coronary arteries was initially described in nonhuman primates (24) and later documented in humans (25). A more recent comparison of coronary arteries from both human and nonhuman primates found that artery size is increased with atherosclerosis (26). In particular, remodeling was greater in patients with no history of CHD, whereas a failure of remodeling was observed in those with CHD.

Aging and the Cardiovascular System: Arterial aging has been defined as the "age-related structural and functional changes in arteries from the precapillary to aortic level" and includes atheromatous changes (27).

These changes are visible in medium-sized or larger arteries by routine diagnostic studies such as B-mode ultrasonography of the carotid artery. The specific changes include thickening of the intima, plaque formation, and increases in luminal diameter (27). Atheromatous changes, as discussed above, result in development of focal plaques with deposition of lipids and calcium, which may narrow the artery lumen, while age-related arterial changes may result in luminal enlargement (27). Whether remodeling of arteries, which is related to plaque extent (and increases with age), is also affected by aging itself is unknown. Further, age-related changes and atherosclerosis extent and severity in one artery may correlate poorly with events in other arteries (27). Although age-related changes in the artery are considered physiological events independent of atheromatous changes (which are designated as pathological phenomena [27]), it may be difficult to distinguish between the effects of aging per se and the singular or additive effects of co-existing disease on the cardiovascular system (including remodeling). However, given this limitation, the aging process itself has been reported to cause certain functional and structural changes in the cardiovascular system as recently reviewed by Duncan et al (6). Table 1 details these changes.

Table 1: Age-related changes in cardiovascular structure and function*

Structure
Myocardial
Increased myocardial mass
Increased LV wall thickness
Increased deposition of collagen
Valvular
Increased thickness of aortic and mitral leaflets
Increased circumference of all four valves
Calcification of mitral annulus
Arterial
Increased intimal thickness
Increased collagen content
Function
Heart rate
Decreased heart rate at rest
Decreased maximal heart rate during exercise
Decreased heart rate variability
Decreased sinus node intrinsic rate
LV systolic
Unchanged cardiac output
Increased stroke volume index
LV diastolic
Decreased LV compliance
Increased early diastolic LV filling
Myofibril
Unchanged peak contractile force
Increased duration of contraction
Decreased Ca^{++} uptake by sarcoplasmic reticulum
Decreased ß-adrenergic-mediated contractile augmentation
Vascular
Decreased compliance
Increased pulsed-wave velocity

*LV=left ventricular. From (6).

Atherosclerosis in Nonhuman Primates

The ideal animal model for studies of aging and atherosclerosis would be one that gradually develops arterial lesions over the life of the animal and subsequently develops clinical complications of the disease in late-middle and old age, as observed for human beings (11). The characteristics of the disease should be representative of findings in humans, i.e. ranging from minimal abnormalities (such as fatty streaks) to extensive plaques with complications (such as ulceration, necrosis, and thrombus formation). Such an animal model also should demonstrate gender differences, with males developing more severe atherosclerosis.

Atherosclerosis occurs naturally in many species of nonhuman primates; in addition, lesions have been induced experimentally in species where natural lesions have been described as rare (10-12). Atherosclerosis of some nonhuman primates has remarkable similarities to that in humans, such as 1) plasma lipoprotein characteristics and responses to dietary factors such as fat and cholesterol; 2) genetic susceptibility to diet-induced atherosclerosis; 3) the extent to which psychological and social phenomena influence lesion characteristics; and 4) an increased incidence in males compared to females (10). A brief review of the comparative pathology of nonhuman primate atherosclerosis is outlined below (10-12).

The incidence and severity of naturally occurring lesions in nonhuman primate species varies, but in general these lesions are limited to small areas of intimal thickening, due primarily to smooth muscle cells and connective tissue and only small amounts of lipid. The low amount of lipid in these lesions is most likely due to the lack of dietary cholesterol, as most monkeys eat primarily fruits and grains in the wild. However, when fed a cholesterol-containing diet these animals develop hyperlipidemia, and atherosclerotic lesions can be induced to varying degrees in a number of arteries. There appears to be considerable species variability in response to dietary cholesterol; in plasma cholesterol concentrations; and in extent, severity, and location of atherosclerotic lesions.

Both New World and Old World species have been used in studies of atherosclerosis. However, because some species are now endangered and many countries either prohibit or at least limit importation, most studies are now done using only a few species of Old World monkeys.

New World Monkeys: Of New World species, squirrel monkeys (*Saimiri sciureus*) and cebus monkeys (*Cebus albifrons*) have been most often used in atherosclerosis research. Cebus monkeys were used in some of the earliest studies of nonhuman primates. While they demonstrate pronounced age and sex differences in response to dietary cholesterol, this species can no longer be imported. Squirrel monkeys have been used to study genetic differences in response to dietary cholesterol, referred to as hyper- and hypo-responsiveness (28). Hyper-responsive animals are more likely to develop large atherosclerotic plaques, many of which are complicated lesions and may result in congestive heart failure. However, myocardial infarction is generally not

observed (28). Also, unlike people and Old World monkeys, atherosclerosis in squirrel monkeys primarily affects the small intramyocardial artery branches versus the large epicardial coronary arteries. The major disadvantage in using these animals in atherosclerosis studies is the high rate of glomerulonephritis that occurs after feeding the high fat and cholesterol diets (11).

Old World Monkeys: Old World monkeys have been used extensively for atherosclerosis studies. The most commonly used species include two African species (African green monkeys and baboons) and a number of macaque species. African green monkeys (*Cercopithecus aethiops*) fed atherogenic diets have changes in lipoprotein patterns similar to humans (29). Further, atherosclerotic lesions in African green monkeys are frequently fibrotic and have morphometric and biochemical characteristics akin to those of humans (30). African green monkeys have been used in atherosclerosis research to evaluate the pathogenesis of atherosclerosis and nutritional influences on atherogenesis (11,31). Baboons also are representative for the human condition in the magnitude of response in serum cholesterol concentrations as a result of atherogenic diets (32). One advantage of using baboons in atherosclerosis research is their large body size, allowing larger blood and tissue samples and facilitating the use of noninvasive diagnostic testing (11). Unlike humans, however, diet-induced lesions occur primarily in the aorta, with minimal changes reported in the coronary arteries (33). Both African green monkeys and baboons appear to have less hypercholesterolemia in response to a dietary cholesterol challenge than macaque species. Since lesions typically develop in the aorta before the coronary arteries, this may explain the decreased coronary artery atherosclerosis in African green monkeys and baboons.

A number of macaque species have been used in atherosclerosis studies. Stumptail macaques (*Macaca arctoides*) have several characteristics that make them interesting models of atherosclerosis, but have not been used extensively due to problems with availability (11) as well as now being an endangered species. Stumptail macaques become obese with advancing age, and the tendency is greater in females than males (11). Interestingly, females of this species also develop diet-induced atherosclerosis, in contrast to other species of macaques with greater 'female protection' (11). Stumptail macaques that are fed atherogenic diets have a high prevalence of hypertension, which is reported to be related both to the extensiveness of atherosclerosis and degree of obesity (11).

Pigtail macaques (*Macaca nemestrina*) have been more widely used for AIDS research (34) than for the study of atherosclerosis. However, when fed an atherogenic diet, extensive atherosclerosis is observed (11). Also, ethanol treatment results in increased plasma HDL cholesterol concentrations, as in humans (35).

Rhesus monkeys (*Macaca mulatta*) have been extremely popular for the study of atherosclerosis research because of their size, ease of laboratory adaptation, significant rate of myocardial infarction, and similarity of atherosclerotic lesions to those in humans (10,11,36). Although the incidence of naturally occurring atherosclerosis is low in rhesus monkeys (37), the induction of atherosclerosis in this species by feeding a cholesterol-containing diet has been well described (10,11). Although lesion extent varies with the amount of dietary cholesterol and length of time, lesions range from uncomplicated fibrous plaques to more severe lesions, consisting of necrotic foam cells covered by a fibrous cap with occasional mineralization, hemorrhage, or medial destruction (10,38). Lesions are more severe in the coronary arteries and at the carotid bifurcation. In addition, rhesus monkeys fed an atherogenic diet develop clinical complications such as myocardial infarction (10,11,36).

Rhesus monkeys have been used in a number of studies involving regression of atherosclerotic lesions (24,39,40). During regression, foam cells are lost quickly, which correlates with a loss of cholesteryl ester. Extracellular lipids and intimal cells decrease more slowly during regression, with little decrease or increase in extracellular matrix proteins (21). The amount of regression varies, but seems to be related to initial lesion severity and the amount of plasma lipid lowering.

We have found cynomolgus macaques (*Macaca fascicularis*) to be one of the best nonhuman primate models for human atherosclerosis. This is due primarily to the similarities to humans with respect to pathologic characteristics of atherosclerotic lesions (10, 11). While cynomolgus macaques do develop atherosclerotic lesions spontaneously, the process is markedly exacerbated when a cholesterol-containing diet is fed (41). The distribution and morphologic appearance of these lesions are similar to those found in other macaques, although they have been reported to have higher connective tissue and mineral contents (42). Compared to lesions in rhesus monkeys, lesions in cynomolgus macaques have been characterized by more intimal thickening, a greater fibrogenic response, and more extracellular lipid and sterol clefts (39,42).

An interesting observation in cynomolgus monkeys related to aging has been the marked differences in atherosclerosis reported between juvenile and adult animals. As described by Weingand et al (12,43), juvenile (ages 2.5-3.5 years) and adult (6-12 years) monkeys fed comparable atherogenic diets resulting in similar plasma lipid concentrations showed marked differences in coronary artery lesion characteristics and extent. Adult animals developed more extensive lesions characterized as proliferative atherosclerotic plaques, while juveniles had minimal lesions that consisted primarily of fatty streaks. These differences in lesion characteristics and extent were not explained by differences in blood pressure. It was uncertain if these quantitative and qualitative differences in susceptibility to diet-induced atherosclerosis were due to intrinsic age-related changes in the arterial wall and/or related to

the endocrine and metabolic changes associated with puberty (12,43).

Female cynomolgus macaques have a menstrual cycle similar to that of human females and, like human females, they have higher HDL cholesterol concentrations and develop about half the coronary artery atherosclerosis of their male counterparts (44,45). This phenomenon is illustrated in Figure 2. The effect has been shown to be influenced by the animal's estrogenic

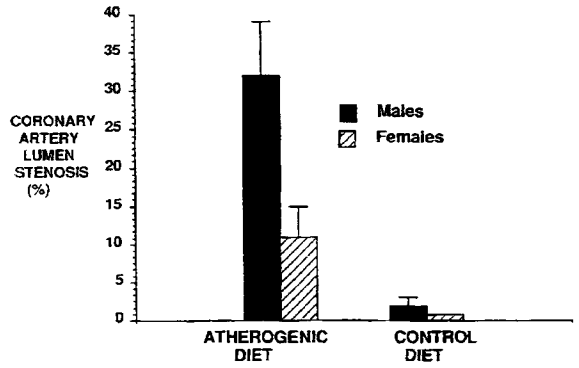

Figure 2: Coronary artery lumen stenosis observed in cynomolgus monkeys fed atherogenic vs control diet. Reprinted with permission from (45).

state. For example, ovariectomized females have plasma estradiol concentrations similar to males and more extensive atherosclerosis than intact females, whereas pregnancy, and the resultant increase in plasma estradiol concentrations, is associated with decreased atherosclerosis (44).

Social status has also been shown to affect atherosclerosis extent in intact female monkeys (44). Cynomolgus macaques in the wild and in captivity form social hierarchies. It is chronically stressful for animals to be subordinate, since they spend most of their time avoiding confrontations with more dominant animals. Subordinate females in social units have decreased plasma HDL cholesterol concentrations and more extensive coronary artery atherosclerosis than their dominant counterparts. Social subordination has since been shown to result in stress-induced impairment of ovarian function and through this mechanism may explain the increased risk of atherosclerosis in subordinate monkeys.

Adams et al (46) showed that ovariectomy resulted in increased extent of coronary artery atherosclerosis, an effect which could be explained, in part, by increased total plasma cholesterol and decreased plasma HDL cholesterol concentrations. In addition, the physiologic replacement of estrogen alone or in combination with progesterone decreased coronary artery atherosclerosis extent by half compared with ovariectomized monkeys. As in women, the beneficial effects of estrogen replacement therapy are only partially explained by changes in plasma lipoprotein concentrations (47). Subsequent studies have suggested that estrogens have direct effects on the artery wall resulting in decreased accumulation of LDL (48) and improvement in coronary artery vascular reactivity (49).

Aging and Atherosclerosis

In aging humans, atherosclerotic lesions may cover an increasing percentage of the luminal surface of coronary arteries (50). The critical question yet to be answered, as put forth by Bowness (50), is whether the increase in atherosclerosis is caused by physiological events (e.g. "arterial aging"), or is due, at least in part, to a time-dependent response to risk factors (e.g., hypertension, dyslipidemia) that can be altered by changes in nutrition and lifestyle. As such, the relationship between aging and atherosclerosis is not clear. As suggested by Masoro (51), "...aging and atherosclerosis may merely share the same time frame, or there may be a causal interaction between them". Although animal studies have shown that intrinsic aging processes may enhance susceptibility to atherosclerosis (40,52), chemical analyses of arteries from specimens indicate that aging changes in extracellular matrix differ from those occurring with development of advanced atherosclerotic lesions (41,50). This suggests that atherosclerosis can be dissociated from the intrinsic aging of the arterial wall (50).

To reliably assess how aging contributes to atherosclerosis, it would be ideal to have a specific biochemical or clinical parameter to assess biologic or chronologic age, or aging rate. Preferably, changes in this "biomarker" would be related to atherosclerotic changes. Such a biomarker could then be used to assess the effectiveness of an anti-aging mechanism on CHD. Baker and Sprott (53) have reviewed the recommended criteria for a parameter to be considered a valid biomarker. Potential uses of biomarkers as suggested by Masoro (51) are summarized as follows:

1) Estimation of the chronologic age of an individual.
2) Estimation of the physiological age of an individual.
3) Prediction of the future occurrence of age-associated diseases.
4) Prediction of impending mortality.
5) Prediction of maximum life span of a species.

Several age-related conditions may be postulated to contribute to this schema. Specifically, insulin resistance and central obesity have been shown to increase with age and may relate to the future occurrence of age-associated disease, e.g. atherosclerosis. Furthermore, advanced glycated end-products (AGEs) have been shown to accumulate with age and are postulated to contribute to atherosclerosis (54). Evaluation of these age-related parameters in nonhuman primate trials may provide valuable information about the role of aging in atherosclerosis.

Although the basis for individual variability in atherosclerosis with age may be genetic in nature, an intriguing hypothesis is that individual differences in coronary artery atherosclerosis can be explained in part by differ-

ences in age-related conditions such as adipose tissue distribution, hyperinsulinemia, and a postulated mechanism such as vascular tissue glycation. For example, cynomolgus macaque males, like men, show much variability in the amount of coronary artery atherosclerosis with specific plasma lipid concentrations. In Figure 3 are data on the relationship between total plasma cholesterol/HDL cholesterol ratio and coronary artery atherosclerosis extent among 152 cynomolgus macaque males (unpublished data). Our hypothesis for aging, as depicted in Figure 3, suggests that more extensive atherosclerosis occurs with high vascular levels of AGE, central obesity, and hyperinsulinemia, while less extensive atherosclerosis occurs in insulin-sensitive and lean states, and decreased vascular AGEs. The relative importance of these risk factors in contributing to atherosclerosis in both human and nonhuman primates is reviewed below.

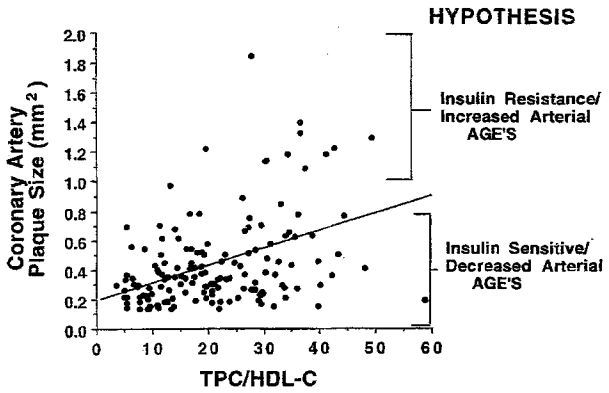

Figure 3: Data from a study of 152 male cynomolgus monkeys fed an atherogenic diet, depicting coronary artery atherosclerosis versus total plasma cholesterol/HDL cholesterol ratio (unpublished observations).

Adipose Distribution and Cardiovascular Disease: The relationship of adipose tissue distribution to cardiovascular disease and cardiovascular disease risk factors was first noticed by Vague in 1956 (55). He described an "android" (central obesity) and a "gynoid" (peripheral obesity) body habitus, and noted that the former was strongly associated with cardiovascular disease, cardiovascular disease risk factors, and, in particular, diabetes. It was noticed in Gothenburg that the distribution of adipose tissue was related to the incidence of cardiovascular disease (56,57). The relationship was independent of other risk factors in women (56) and, although it disappeared after controlling for other risk factors, it was a strong univariable risk factor for cardiovascular disease in men as well (56). The independent variable that categorized adipose distribution in Gothenburg was the waist/hip circumference ratio: a higher waist/hip ratio was associated with a greater likelihood of cardiovascular disease. At the same time, investigators who reanalyzed data from the Framingham Study found a strong association between subscapular skinfold thickness (another measure of central obesity) and incidence of death from all causes, coronary heart disease, and cerebrovascular disease (58). Subsequently, the Paris Prospective Study also reported a relationship of adipose distribution with incidence of CHD (59).

Although the distribution of adipose tissue has been linked independently to cardiovascular events, its linkage to other cardiovascular risk factors has also been investigated. In this regard, the distribution of adipose tissue is also related to diabetes and hyperinsulinemia, dyslipidemia, and hypertension (60-62). An extremely strong association has been noted between central obesity and dyslipidemia, especially derangements in HDL cholesterol (61,62). Ostlund et al (63) have shown that the association between adipose distribution and HDL cholesterol accounts in part for gender-related differences in HDL_2, and that the waist/hip ratio, together with fasting insulin and glucose data, explain over 40% of the variability in HDL_2 cholesterol concentrations.

Studies of adipose distribution initially focused primarily on subscapsular skinfold thickness, or the ratio of subscapular to triceps or other skinfold thicknesses. Alternatively, investigators have focused on ratios of circumferences measured at various sites between the thigh and the lower rib margin. Since it is believed that the metabolically important adipose depot is located within the peritoneal cavity, more recent efforts have utilized computerized tomography (CT) or magnetic resonance imaging (MRI) to quantitate intra-abdominal fat. It has been clearly demonstrated in these reports that males have relatively more intra-abdominal than subcutaneous fat compared to females (64,65). In other studies using CT scanning, a relationship between adipose distribution and other cardiovascular disease risk factors (e.g., insulin, triglyceride, and HDL concentrations as well as blood pressure) has been shown (66).

Insulin Resistance, Hyperinsulinemia, and Atherosclerosis: Past studies have found that aging is associated with a progressive decline in glucose tolerance and development of compensatory hyperinsulinemia (67,68). These phenomena have been attributed to an increased peripheral tissue resistance to insulin action, e.g. insulin resistance (69,70). With regard to cardiovascular disease, epidemiologists have long recognized that insulin is a major risk factor for the development of CHD and that the effect is independent of blood pressure and plasma lipid concentrations. A growing body of experimental evidence has accumulated to support this association (71,72). The major effects of insulin on arterial tissues are summarized in Table 2 (73).

Table 2: Effect of Insulin on Arterial Tissues

Proliferation of smooth muscle cells.
Enhanced cholesterol synthesis and LDL-receptor activity.
Increased formation and decreased regression of lipid plaques.
Stimulation of connective tissue synthesis.
Stimulation of growth factors.

Atherosclerotic plaques are characterized by excessive amounts of lipid and collagen, foam cells, and proliferation of smooth muscle cells (22,74). All of these constituents are postulated to be affected by plasma insulin concentrations.

Given that the clinical and epidemiological evidence consistently implicates insulin, independent of changes in plasma lipid concentrations or blood pressure, in the pathogenesis of atherosclerosis, a mechanism is proposed whereby hyperinsulinemia secondary to insulin resistance (acquired or genetic in etiology) enhances atherosclerosis progression by both direct and indirect effects (72). However, definitive evidence that reducing insulin levels may diminish atherosclerosis development is not available, but is currently being explored in our nonhuman primate trial (13).

Of great interest to gerontologists is the relationship of body composition to the insulin resistance seen in aging. Recent data have suggested the insulin resistance observed with age may be more related to changes in body composition (e.g. an increase in total body fat or central obesity, demonstrated as an elevated waist/hip ratio). In these studies, the waist and waist/hip circumference accounted for most of the variance in peripheral tissue insulin action, whereas age has explained only a small percentage of the total variance when the data were statistically controlled for differences in waist/hip circumference (75,76). These results suggest that insulin resistance may be more closely associated with the abdominal obesity accompanying aging than the aging process per se.

Waist circumference represents two fat depots, subcutaneous fat and intra-abdominal (e.g., visceral) fat. The accumulation of visceral fat is more closely associated with the clinical syndrome of insulin resistance (e.g. glucose intolerance, hyperlipidemia, and hypertension) (77,78). Visceral fat obesity was present in almost 90% of obese patients with ischemic heart disease, and in 40% of non-obese subjects with cardiovascular disease (77).

Determinants of visceral fat accumulation have been suggested to be aging, sex hormone concentrations, excessive sucrose intake, and physical inactivity (78). Therefore, to assess the association of visceral fat accumulation, insulin resistance, and age, we evaluated 60 non-diabetic, community-dwelling subjects (aged 23 to 83 years). Subjects were chosen so that those $\leq$ or $\geq$ 125% of ideal body weight were equally represented in each age decade. Total, subcutaneous and intra-abdominal fat were quantified with an MRI scan at the umbilicus, and insulin sensitivity determined with the modified minimal model. Overall, there was no relationship of age to insulin sensitivity for men or women, nor did weight or body mass index increase with age in this cohort (79). However, intra-abdominal fat increased significantly with age in both men and women. Figure 4 illustrates that in addition, insulin sensitivity was significantly associated with intra-abdominal fat. In multivariate analysis for various combinations of age, sex, and measures of

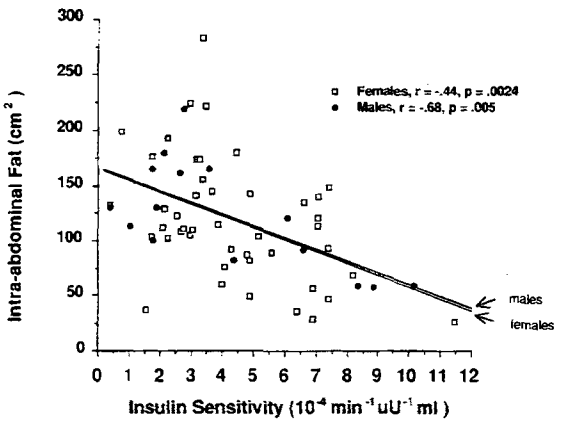

Figure 4: Correlation of insulin sensitivity with intra-abdominal fat in both men and women. From (79).

fat distribution, waist/hip circumference accounted for 29% and intra-abdominal fat for 51% of the variance in insulin sensitivity, whereas age, sex, and interactions of age and sex accounted for only 1% (79). In conclusion, in this study, intra-abdominal fat, not age, was most closely related to the insulin resistance observed with aging.

Glycation Hypothesis of Aging and Its Potential Contribution to Atherosclerosis: It has been proposed that glucose mediates the aging process through the mechanism of glycation of macromolecules (80,81). Non-enzymatic reactions occur between glucose and protein or nucleic acid amino groups to form labile Schiff bases, which rearrange to form stable but chemically reversible Amadori products (e.g. fructoselysine), as shown in Figure 5. This glycation reaction, referred to as the Maillard reaction, is determined by 1) the half-life of the protein in the circulation or tissue, and 2) the degree and duration of hyperglycemia. Thus, extent of glycation of numerous proteins is increased in proportion to the mean blood glucose concentration in diabetes. With time, the Amadori products dehydrate, rearrange and form irreversible structures referred to as advanced glycation end-products (AGEs) (82). The AGE moieties are brown, fluorescent chromophores that can cross-link proteins (83). In collagen samples obtained from the dura mater of normal human subjects, both the fluorescence and absorbance at 340 nm increased linearly with age of the subject (84). However, collagen samples from diabetic subjects had significantly more absorbance and fluorescence than collagen samples from age-matched control subjects (84). Because many of the complications of diabetes are similar to pathologic processes associated with normal aging, the formation of these advanced products is postulated to be the chemical link between the pathophysiology of diabetes and aging by causing age-dependent denaturation and cross-linkage of proteins. In support of this hypothesis, recent immunological studies using anti-AGE antibodies in several human tissues suggest that AGEs may be involved in aging processes, diabetic complications, and atherosclerosis (85).

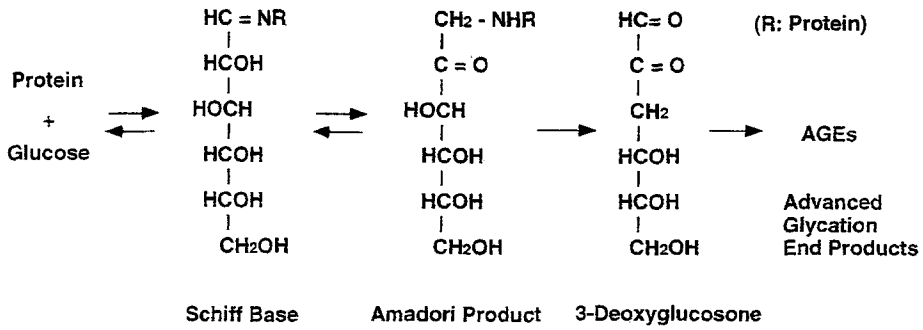

Figure 5: Mechanism for non-enzymatic glycation of protein.

AGEs have been postulated to modulate cellular function through binding to specific cell surface receptors termed RAGE (receptors for advanced glycation end-products) (86). The binding of AGEs to RAGE elicits several responses, such as induction of cytokines, cell growth in macrophages, and enhancement of angiogenesis, and results in induction of cellular oxidant stress (86,87).

Role of AGEs in Atherogenesis: Possible mechanisms by which AGE can contribute to increased atherogenesis are protein trapping by AGE and recognition of AGEs by macrophages (88). These proposed mechanisms are based on current concepts of atherogenesis, which state that atherosclerotic lesions result from two major processes: an accumulation of plasma lipids in the vessel wall, and the proliferation of arterial smooth muscle cells (22,89). The rate at which atherosclerosis develops in any given individual may reflect independent contributions for each of these causal factors. Lipoprotein cholesterol concentrations in the arterial intima correlate positively with the LDL concentrations in the plasma of normal subjects (90). Elevated plasma LDL cholesterol concentrations increase the rate of LDL cholesterol infiltration, and a correspondingly larger amount of LDL cholesterol is deposited in the arterial wall. However, it is postulated that in diabetic subjects and with age, enhanced extracellular trapping of plasma lipoproteins by more rapidly accumulating AGEs on vascular connective tissue components promotes lipid accumulation, even with normal plasma concentrations of LDL cholesterol (88). It has been demonstrated experimentally that at a constant LDL cholesterol concentration, the amount of covalent trapping increases linearly with the extent of nonenzymatic glycation of collagen, whereas at a constant amount of collagen glycation, LDL binding increases as a function of increasing LDL concentration (91). In addition, trapping of proteins in the artery may prevent LDL diffusion out of the intima, and promote formation of AGEs on the LDL particle itself. This leads to a postulated second mechanism: the subsequent recognition and uptake of the AGE-LDL complex by scavenging macrophages could exacerbate atherogenesis by increasing secretion of macrophage-derived growth factor or other macrophage secretory products such as enzymes (86). This postulate is supported by evidence that modifications of proteins by products formed during non-enzymatic glycation result in the specific recognition and uptake of the modified protein by macrophages (85,86). In this situation, AGEs appear to be the primary recognition signal rather than the initial Amadori product. Just as the mechanisms outlined above have been described in tissues of diabetic subjects, equivalent levels of AGEs also have been noted in aging tissue of normal subjects. Increased levels of AGEs observed secondary to aging may contribute to atherogenesis in much the same way as AGEs in tissues of diabetic subjects contribute to accelerated atherogenesis. However, very little direct evidence has been gathered to support this hypothesis. A specific aim of our ongoing nonhuman primate trial is to examine the relationship of AGEs to atherosclerosis by measuring these products periodically in arterial collagen, as well as in skin collagen twice yearly (13).

Nonhuman Primates as Potential Models for Aging Research

Given the natural history of atherosclerosis in humans and the comparative pathology of nonhuman primate atherosclerosis, how can nonhuman primate models help elucidate the relative role of aging per se in atherosclerosis?

Male and female nonhuman primates have patterns of fat distribution and glucoregulatory abnormalities similar to those of human beings. Jen et al (92) evaluated adult (12- to 27-year-old) male rhesus monkeys and reported high correlations between percentage of body weight as fat, midgirth circumference, and abdominal skin fold thickness, demonstrating a predominantly abdominal distribution of fat. Both body weight and percentage of body weight as fat were correlated with basal insulin levels. In addition, Kemnitz et al (93) found that obese male and female rhesus monkeys had excess body fat located predominantly in the abdominal region, and that the abdominal circumference was highly correlated with total body fat. Obese monkeys of both sexes in that study had fasting hyperinsulinemia, greater insulin response to intravenous glucose administration, and marginally impaired

glucose tolerance. In addition, obese males had delayed maximal insulin response to glucose administration, and obese monkeys of both sexes had elevated fasting serum triglyceride concentrations.

Shively et al (94) demonstrated that female cynomolgus monkeys with a relatively high ratio of central to peripheral fat deposition had three times more extensive coronary artery atherosclerosis detected at necropsy than did a control group. This represented the first direct evidence linking regional fat distribution and atherosclerotic lesion size. Furthermore, a high central-to-peripheral fat deposition pattern was associated with hyperglycemia, hypertension, and higher total and LDL lipoprotein concentrations (95). Female cynomolgus monkeys, like women, gain weight postmenopausally, and estrogen replacement therapy prevents the weight gain, primarily by reducing abdominal fat content (96). Thus, it seems clear that nonhuman primates are appropriate models to assess the effects of regional fat deposition on atherosclerosis in human beings.

In addition to becoming obese with age, a number of macaque species develop adult-onset diabetes (97-99). Before the development of diabetes, both cynomolgus and rhesus monkeys are obese and hyperinsulinemic. Associated with diabetes are abnormal plasma lipoprotein profiles and increased glycation of both plasma proteins and lipoproteins (100). Importantly, both spontaneous and chemically induced diabetes are associated with increased severity of atherosclerosis in three species of monkeys (101-103).

As discussed above, a biochemical mechanism implicated in both aging and diabetes that may exacerbate atherosclerosis is non-enzymatic glycation. We determined arterial glycation by measuring fluorescence in a group of ovariectomized female monkeys (96). A significant correlation was found between age and total arterial glycation ($r=0.47$, $p<0.001$). In addition, glucose tolerance was assessed by an intravenous glucose challenge, and animals with higher areas under the glucose disappearance curve had greater amounts of arterial glycation. This suggests that even in nondiabetic animals, both age and variation in plasma glucose tolerance affect arterial glycation.

In addition to total glycation, a specific glycoxidation product of the Maillard reaction [carboxymethyllysine (CML)] also has been quantitated in monkeys. To assess variation among arterial sites, CML levels were determined in iliac arteries from the right and left side of the same animal. As shown in Figure 6, a significant correlation existed ($r=.92$, $p<.001$) (unpublished observations). The highly significant correlation of AGEs in arterial collagen from different arterial sites suggests that similar levels can be quantitated throughout the arterial system. In addition, as with fluorescence, a significant correlation between CML levels in arterial collagen and age of the monkey was found, which is illustrated in Figure 7 (unpublished observations). Finally, evidence *in vivo* has suggested that advanced glycation (as measured by fluorescence) correlates with atherosclerotic extent in human aortas (104).

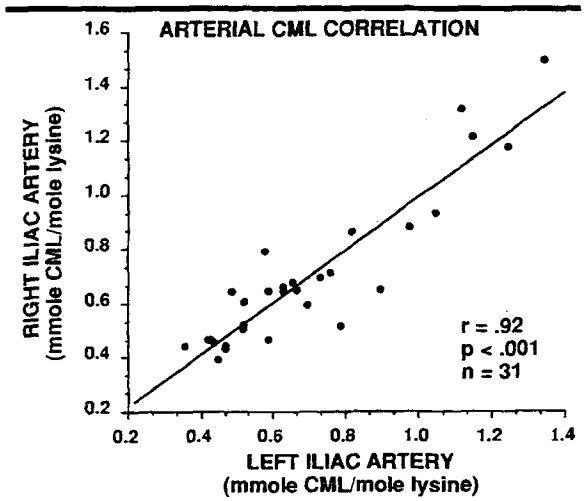

Figure 6: Correlation of carboxymethyllysine (CML) levels in left and right iliac arteries from 31 non-diabetic, ovariectomized cynomolgus monkeys.

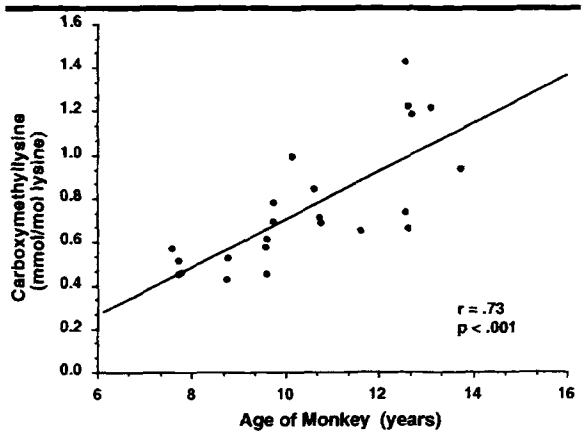

Figure 7: Correlation between glycoxidation products (carboxymethyllysine, or CML) and age of cynomolgus monkeys.

Anti-aging Interventions in Nonhuman Primates: Postulated Role in Atherosclerosis

If age-related conditions as outlined above do contribute to atherosclerosis, interventions that specifically affect the proposed parameters could be postulated to significantly reduce cardiovascular disease. One such anti-aging intervention, i.e. caloric restriction, is currently being evaluated in nonhuman primates (105,106). It has been shown that caloric restriction retards aging processes in rodents (107,108). This observation is supported by evidence that food restriction increases life span, retards age–associated physiological changes, and delays or prevents most age–associated disease. The mechanisms by which calorie restriction exerts its effects are unknown. However, if these findings are to be extrapolated to human beings, it will first be necessary to test varying dietary regimens in some higher species and evaluate the effects on several aging processes and age–related diseases, particularly as they relate to human health. An age–related disease, such as atherosclerosis, would be a valuable endpoint to study, but this goal has been severely hampered by lack of a suitable animal model for both aging and

atherosclerosis. However, as previously described, cynomolgus monkeys have been shown in multiple studies to be an excellent model for study of atherosclerosis and its pathogenesis. Furthermore, caloric restriction can be safely maintained in nonhuman primates without detrimental effects (105,106). Therefore, with this background, we began a clinical trial to evaluate the effect of caloric restriction on the pathogenesis and extent of atherosclerosis in a nonhuman primate model (13).

The specific hypothesis for the trial is that long-term caloric restriction, compared to an ad libitum diet with equivalent cholesterol intake, may reduce the progression of atherosclerotic lesions by decreasing age–associated increases in vascular tissue and blood levels of early and advanced glycated products (i.e. glycated protein and protein cross–links), improve peripheral insulin sensitivity, and reduce intra–abdominal fat. The long–term goal of this study is to assess the independent effect of chronic caloric restriction on coronary artery plaque extent. The role of chronic caloric restriction in modifying cardiovascular risk factors (e.g. insulin resistance, adipose tissue distribution) and glycation/advanced glycation (for both blood and tissue proteins), all of which have been strongly implicated as contributors to cardiovascular risk, will be evaluated. In addition, we plan to assess the relationship of these changes to changes observed in arterial plaque size determined at study completion.

The results from the first year of the trial demonstrate that the decreased caloric intake resulted in a significant decrease in weight (30%), accompanied by a significant decrease in central adiposity as assessed by abdominal CT scans and by anthropometric measurements (13). The diets were designed to provide identical cholesterol intake per body weight regardless of caloric intake. Due to the increased cholesterol content in the calorie-restricted diet, there was no difference in total plasma cholesterol/HDL cholesterol ratios despite a reduction in caloric intake. In addition, there were no detrimental effects of caloric restriction on general chemistry profiles or cardiovascular measurements. At the same time, we have found a significant improvement in insulin sensitivity. No significant change has been observed for glycated proteins at this point.

Our data suggest that caloric restriction is associated with not only a decrease in total abdominal fat mass, but a reduction in intra-abdominal fat mass, the fat depot most significantly linked to cardiovascular disease and cardiovascular risk. Intra-abdominal fat mass showed the most significant change secondary to caloric restriction ($p<.001$), whereas changes in subcutaneous abdominal fat mass were of borderline statistical significance ($p=.07$) in the caloric-restricted animals, and the paraspinous fat mass did not appear to be affected. Interestingly, we have recently reported that intra-abdominal fat mass accumulates with age; this is the fat depot that can most readily explain the variance in insulin resistance with age (79).

In conclusion, atherosclerosis is well described as an age-related event. Yet the relative role of aging per se in contributing to atherosclerosis development has not been adequately explained. Atherosclerosis in nonhuman primates has been studied for years, and much is known about the specific lesions and characteristics. However, only recently have studies been initiated to specifically investigate the effects of age and age-related conditions on atherosclerosis in this species. Definitive conclusions regarding these effects must await the outcomes of ongoing trials.

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