Original communication

AGE CHANGES IN THE TUNICA INTIMA OF THE AORTA IN GOAT (*Capra hircus*)

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SUMMARY

Age changes in the aortic tunica intima may explain the higher prevalence of atherosclerosis among the elderly. Goat is a suitable model for the study of cardiovascular disease but the age changes in its aortic tunica intima are unreported. This study therefore examined structural changes that occur in the tunica intima of its aorta. Six healthy goats three aged over 60 months, and three aged less than 12 months, were used in this study. The animals were euthanized with sodium pentabarbitone and specimens taken from the various segment of the aorta studied by light microscopy and transmission electron microscopy. Materials for light microscopy were fixed 10% formaldehyde solution, processed for paraffin embedding and 7 micron sections stained with Mason's Trichrome and Weigert's Resorcin Fuchsin/Van Gieson stains. Those for transmission electron microscopy were fixed in 3% phosphate buffered glutaraldehyde solution, post fixed in osmium tetroxide and prepared for durcupan embedding. Ultrathin sections were stained with uranyl acetate, counterstained with lead citrate and examined by EM 201 phillips© electron microscope. Observations reveal that aging is characterized by endothelial discontinuities, presence of lymphocytes and dendritic cells in the tunica intima, subendothelial thickening, vacuolation and disintegration of internal elastic lamina. It is concluded that the intimal breaches observed in intimal aging may promote ingression of macromolecules into the vessel wall, and underpin the higher prevalence of atherosclerosis among the elderly. Control of serum atherogenic molecules should be enhanced in this age group.

Key words: Age, Tunica Intima, Aorta, Atherosclerosis

INTRODUCTION

Tunica intima, comprising endothelium, subendothelial layer and internal elastic lamina, is the most involved vascular layer in initiation and progression of atherosclerosis (Ross, 1999; Libby, 2002). This disease tends to occur more commonly among the elderly than in the young and there is interaction between lesion growth and aging (Lee et al., 2008). The anatomical basis for this is important in performing prevention strategies, but is not yet elucidated. The goat is a suitable model for studying vascular disease (Lemson et al., 1999; Zheng et al., 2000). This study therefore aimed at describing the ages changes in the tunica intima of goat aorta.

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MATERIALS AND METHODS

Materials for this study were obtained from 6 healthy male goats purchased from livestock farmers in Nairobi, Kenya. Ethical approval was granted by Ethics and Research Committee of the Kenya Physiological Society. Three animals were aged ≤ 12 months; and another three ≥ 60 months. The animals were euthanized with sodium pentabarbitone and specimens taken from the middle of the infrarenal

aorta. Material for light microscopy were fixed in 10% formaldehyde solution and processed routinely for paraffin embedding and sectioning. 7 µm sections were stained with Mason's trichrome for demonstration of general structural features; and Weigert resorchin fuchsin/van Gieson stains for demonstration of elastic fibres. Those for electron microscopy were fixed in 3% phosphate buffered glutaraldehyde solution, and subsequently post fixed with 1% phosphate buffered osmium tetroxide solution for 48 hours. The post-fixed specimens were rinsed in sodium phosphate buffer and processed for durcupan embedding and sectioning. Ultrathin sections on 200 mesh copper grids were stained with uranyl acetate, counterstained with lead citrate (Glauert, 1965) and examined by EM 201 Phillips© electron microscope.

RESULTS

Aging affects the endothelium, subendothelial layer and internal elastic lamina. In goats older than 60 months, the predominantly flat endothelial cells in the ascending aorta and the aortic arch are separated by wide gaps (Figure 1A,B). Some of the endothelial cells are coated by amorphous material resembling elastin, part of which passes through the gap between endothelial cells (Figure 1C). Other cells are round, with large irregular nuclei, dendrite like extensions, and variable lysosome-like structures (Figure 1D). Some extensions of these cells show unusual pear shaped structures which project towards the lumen. These structures contain rough endoplasmic reticulum (Figure 1D).

The subendothelial zone consistently circumferentially thickens and comprises two layers, namely an inner one consisting of vacuolated loose tissue that stains poorly for elastic fibres, and an outer one containing bundles of elastic fibres (Figure 2A). There are cells of different staining ability, shapes and sizes in both layers of the subendothelial zone (Figure 2B,C). Some of the cells are round with blunt cytoplasmic extensions, and a slightly dented nucleus that almost fills the whole cell (Figure 2C), while others are elongated with extensions (Figure 2C,D). The internal elastic lamina appears fragmented and disrupted (Figure 2C, D).

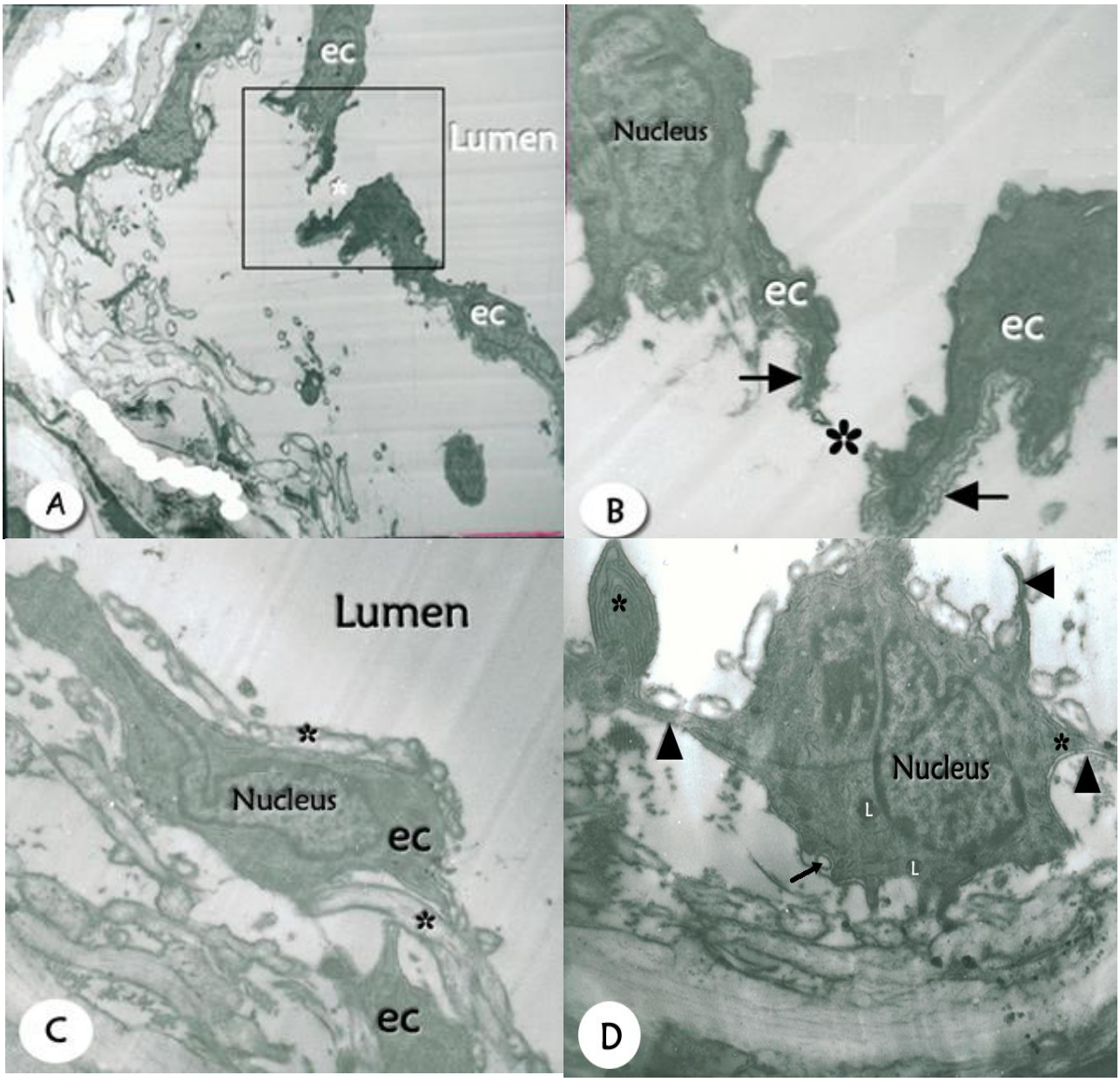

Figure 1A-D: Age related changes in the of aorta of goat endothelium. A: Electronmicrograph of tunica intima of the goat aorta showing discontinuity (star).x1,950 B:Electronmicrograph of tunica intima of the goat aorta showing endothelial discontinuity (star). Note the basement membrane (arrows) closely following the basal contour of the endothelial cell (ec). x8,760. C: Electronmicrograph of tunica intima of the goat aorta showing a gap between endothelial cells (ec) filled with elastin-like material (star). x4000. D: Electronmicrograph of tunica intima of the goat aorta showing a cell in the endothelial layer, with an irregular nucleus, dendritic extensions (arrowheads) containing rough endoplasmic reticulum (star): lysosome-like structures (L) and phagocytic vacuoles (arrow). x8,760.

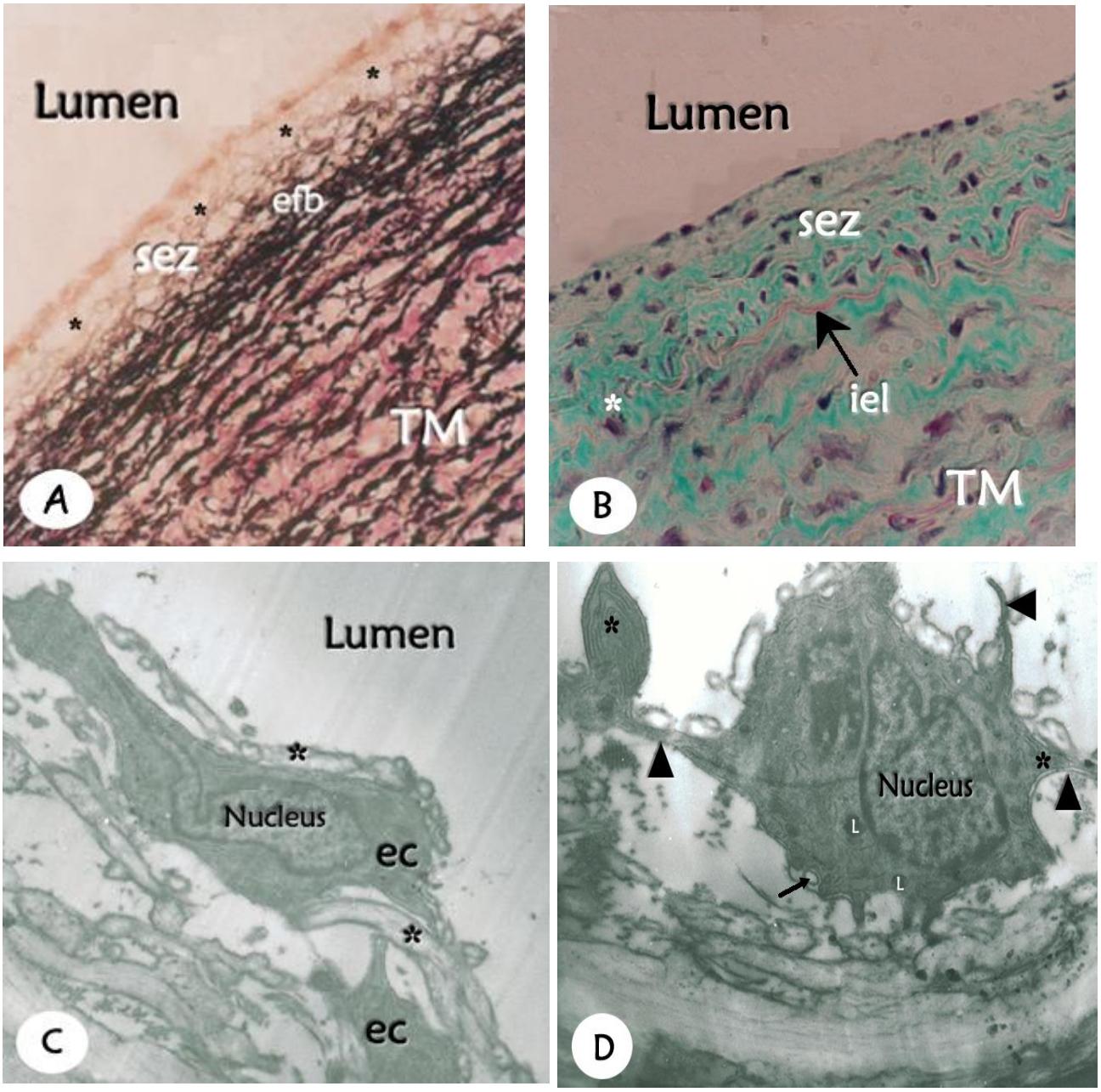

Figure 2A-D: Microscopic Age related changes in the subendothelium of aorta in the goat A: Photomicrograph of tunica intima of aorta in a 7 year old goat showing two layers of the subendothelial zone (sez): an inner vacuolated (stars), and an outer one containing bundles of elastic fibres (efb). Note the fragmentation of elastic lamellae in the tunica media (TM). Weigert elastic stain. x 250. B: Photomicrograph of tunica intima of aorta in a 7 year old goat showing the cellular nature of the subendothelial zone (sez). Note the internal elastic lamina (iel) disrupted in some parts (star) separating intima from the tunica media (TM). Masons trichrome stain. x 250. C: Electronmicrograph of the tunica intima of aorta in a 10 year old goat, showing a subendothelial cell with a dented nucleus that almost fills the entire cell (L), below the endothelial cell (ec). n x4000. D:Electronmicrograph of the tunica intima of aorta in a 10 year old goat, showing a subendothelial cell with an irregular nucleus (Nu) luminal to the internal elastic lamina (iel). Note a disruption in the internal elastic lamina(star). x4000.

DISCUSSION

The endothelial cells of older animals are irregular and heterogenous with large intercellular discontinuities not observed in younger animals. Similar discontinuities of endothelial lining have been demonstrated in aorta of hypercholesterolemic rabbits (Weber et al., 1974). Reduction of endothelial cells with age may be explained by the findings of Kunz and Klein (1975), who demonstrated that in rabbits, there is a decrease in the 3H –thymidine labeling index and in mitotic rate leading to an increase in mean generation time of endothelial cells with advancing age. Further, in aorta of the rhesus monkey, apoptosis of endothelial cells has been suggested to contribute to the reduced cell density (Asai *et al.*, 2000). The discontinuities in the endothelium may result from this reduction in cell density and also from alteration of connexins (Connat *et al.*, 2001a). Such gaps may permit greater ingression of material, including lipids into the vessel wall and contribute to the higher incidence of atherosclerosis among the aged. Pertinent to this suggestion are observations that multiple destruction microfoci in endothelial cells are associated with atherosclerotic lesions (Soloveva and Lysenko, 2010).

Some of the cells in the endothelium are large, irregular and bear lysosome-like structures and dendritic extensions. These are features associated with dendritic cells of the mononuclear phagocytic system. These cells probably perform a phagocytic role, protecting the vessel wall from antigens which may gain access to the vessel wall through the enlarged discontinuities observed in the endothelial lining. The presence of phagocytic cells in the endothelium of the aorta in aged rats and monkeys may be a result of loss of primitive intima which is replaced by a pseudoendothelium made of adhering leukocytes (Aliev *et al.*, 1995; Ye *et al.*, 2000; Connat *et al.*, 2001b).

The subendothelial zone is uniformly thickened, and contains abundant cells, elastic and collagen fibres. These observations are similar to those in other animals (Jayer *et al.*, 1982; Kojimahara, 1985; Wang, 2003; Bonert *et al.*, 2003). The changes may represent a process of intimal remodeling, designed to enable the vessel cope with the increased haemodynamic stress that attends advancing age (Li *et al.*, 1999). Pertinent to this suggestion is the observation that sub-endothelial thickness does increase in hypertension (Gabbiani *et al.*, 1979; Ferrante *et al.*, 1994; Lesauskaute *et al.*, 1999) and that blood pressure is elevated in aged animals (Benetos *et al.*, 2002).

The presence of elastic fibre bundles in the deeper layer of the subendothelium may also constitute a shear stress buffering mechanism. This suggestion is based on the function of elastic tissue which is considered to be that of controlling the rate of change in the form of a structure by reversibly stretching without energy expenditure (Sandberg, 1976; Kielty *et al.*, 2002). Therefore, its presence in the thickened subendothelial zone of aged animals may act to control the longitudinal shearing of the luminal surface of the vessel wall in systole, and its resumption of normal morphology in diastole.

The thickened subendothelial zone contains a heterogenous population of cells, some of which resemble smooth muscle cells and lymphocytes. Increased heterogeneity of subendothelial cells with age has also been found in the aorta in rat and monkey (Haudenschild *et al.*, 1981; Guyton *et al.*, 1983; Aliev *et al.*, 1995; Wang *et al.*, 2003). This cellular composition may enable extracellular matrix remodeling as well as

intima protection by immunosurveillance, as in younger animals.

With age, the internal elastic lamina disintegrates, as shown by Connat *et al.*, (2001b) in rats. This disintegration is part of the general change that occurs in the tunica media, and may permit migration of medial smooth muscle cells and abnormal accumulation of macromolecules to the subendothelial zone, contributing to thickening of subendothelial zone, (Glagov *et al.*, 1993; Ross, 1999; Libby, 2002), and development of atherosclerotic plaques (Ross and Rosenfeld, 1996). Indeed advanced atherosclerotic lesion development in the arterial wall is associated with altered permeability of internal elastic lamina (Lee et al., 2005).

Intimal breaches observed in intimal aging may promote ingression of macromolecules into the vessel wall, and underpin the higher prevalence of atherosclerosis among the elderly. Control of serum atherogenic molecules should be enhanced in this age group.

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