Physical Activity and Atherosclerosis: Which Animal Model?

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Abstract/Résumé

Atherosclerosis is a progressive disease that is the most important single contributor to human cardiovascular morbidity and mortality. Epidemiologic studies show that physical activity, or routine exercise, reduces the risk of developing cardiovascular disease. The mechanisms through which exercise may function in primary or secondary prevention of atherosclerosis remain largely to be established. Most studies in humans are performed after the onset of clinical signs when disease is well advanced and the prescription of exercise is based on empirical evidence of benefit in secondary prevention. Animal models permit the study of the initiation and progression of preclinical stages of atherosclerosis. In order to provide information relevant to treatment and prevention, these models should mimic human disease and interactions of physical activity with disease processes as closely as possible. The purpose of this review is to compare animal models of atherosclerosis and to summarize the available data in those models in regard to the effects of exercise.

L’athérosclérose est une maladie évolutive; elle est la cause la plus importante de morbidité et de mortalité cardiovasculaires chez l’humain. D’après les études épidémiologiques, l’activité physique pratiquée régulièrement réduit le risque de maladie cardiovasculaire. On ne connaît pas encore les mécanismes par lesquels l’exercice physique contribue à la prévention primaire et secondaire de l’athérosclérose. La plupart des études sont faites après la manifestation des signes cliniques quand la maladie est bien installée; dès lors, la

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Cardiovascular disease is the leading cause of morbidity and mortality in the de-veloped world and is predicted to become the predominant global disease by 2020 (Lopez and Murray, 1998; Murray and Lopez, 1997). Atherosclerosis is the most important single factor contributing to this disease burden (Libby, 2002). Atherosclerosis was first described by Rokitanski in 1852 and his observations were modified by Virchow in 1856 (Karsch, 1992). The term atherosclerosis derives from the Greek athere, meaning “gruel,” which reflects the accumulation of lipid, and sclerosis, meaning “hardness,” which reflects the accumulation of fibrous tissue and mineral. These morphologic changes occur primarily within conduit arteries; however, there is evidence that important functional differences may occur in resistance arteries in the absence of similar morphologic changes (Kelm, 2001; Laughlin et al., 1996). Stary has recently revised the histologic stages that corre-spond with the natural history and progression of atherosclerosis (Stary, 2000a, 2000b). Current theories in regard to the etiopathogenesis of atherosclerosis focus on an inflammatory response to vascular injury (Fan and Watanabe, 2003; Libby, 2002).

Exercise intolerance is a classic clinical sign of cardiovascular disease in humans and animals. Epidemiologic and clinical studies report that routine physi-cal activity (exercise) and physical fitness correlate inversely with cardiovascular disease morbidity and mortality (Fagard, 2002; Ford, 2002; Lakka et al., 2001; LaMonte et al., 2001; Laukkanen et al., 2002; Rauramaa et al., 1995). Indeed, physical inactivity is one of the risk factors to which the global disease burden is attributed (Murray and Lopez, 1997). The unexplored mechanisms through which exercise exerts protective effects can be examined through the judicious use of animal models that share similarities with human cardiovascular pathophysiology.

Models for Studying Atherosclerosis

GENETIC HYPERCHOLESTEROLEMIA

The link between hypercholesterolemia and atherogenesis is well established (Libby, 2002). The discovery of receptor-mediated cholesterol metabolism facilitated the identification of mutations associated with familial hypercholesterolemia (Brown and Goldstein, 1976). A spontaneous mutation of the low-density lipoprotein re-ceptor (LDLR) in the Watanabe rabbit (Kondo and Watanabe, 1975) provided a model for studying the autosomal dominant form of human familial hypercholes-terolemia (Goldstein et al., 1983; Kita et al., 1981). The identification of mutations
of apolipoprotein-B that impair LDL clearance by LDLR in hypercholesterolemic pigs provided additional support for receptor-mediated cholesterol metabolism (Prescott et al., 1991; Rapacz et al., 1986). These mutations implicated genes of interest when the era of genetic manipulation of laboratory rodents erupted. Low-density lipoprotein receptor (LDLR/⁻) knockout mice have become common models for inducing hypercholesterolemia (Ishibashi et al., 1993). Subsequently, knockout of the apolipoprotein E (apoE/⁻) gene demonstrated a two-receptor model of hepatic lipoprotein clearance and produced severe hypercholesterolemia in combination with reduced plasma levels of high-density lipoprotein cholesterol (HDL) (Ishibashi et al., 1994).

The importance of cholesterol metabolism by peripheral tissues has been emphasized by the recent identification of Tangier disease in which mutation of the adenosine triphosphate-binding cassette transporter A1 (ABCA1) is associated with premature atherosclerosis in the presence of low plasma HDL and LDL (Asztalos and Schaefer, 2003). More than 800 mutations have been reported to date to cause human familial hypercholesterolemia (Civeira et al., 2004). These studies provide the foundation for a vast literature on cholesterol metabolism in genetically manipulated animals. See recent reviews of myriad additional genes that have been overexpressed or knocked out to study specific mechanisms of vascular disease in mice (de Winther and Hofker, 2002; Knowles and Maeda, 2000; Reardon and Getz, 2001; Svenson et al., 2003).

**DIET-INDUCED HYPERCHOLESTEROLEMIA**

Animal models should mimic the pathophysiology of human disease as closely as possible (Moghadasian et al., 2001). Familial hypercholesterolemia accounts for approximately 5% of myocardial infarctions associated with coronary artery disease (Goldstein and Brown, 2001). Modifiable nongenetic environmental factors including diet, body mass, physical inactivity, and others account for more than 80% of the risk for coronary heart disease (Willett, 2002). For this reason models that do not require genetic manipulation more closely resemble most cases of human atherosclerosis.

Humans, pigs, and some primates develop spontaneous atherosclerosis, whereas mice, rabbits, and rats do not (Moghadasian et al., 2001) (Table 1). Most nongenetic models of atherosclerosis are induced by feeding a high fat and cholesterol (HC) diet to susceptible species. Anitschkow in 1913 demonstrated that feeding cholesterol to rabbits produced aortic atherosclerosis (Goldstein et al., 1983). The addition of cholesterol to the diet in primates elevates cholesteryl ester transport protein (CETP) (Fusegawa et al., 2001). Species with well-documented susceptibility to diet-induced atherosclerosis (human, pig, primate, and rabbit) tend to exhibit greater mean CETP activity and lower mean phospholipid transfer protein (PLTP) activity. Conversely, species that are resistant to diet-induced atherosclerosis (dog, mouse, and rat) tend to have low CETP and high PLTP (Cheung et al., 1996; Guyard-Dangremont et al., 1998) (Table 1).

Some reports suggest that CETP activity cannot be detected in pigs (Pussinen et al., 1997); however, the assay of CETP in pigs is confounded by the presence of an HDL-associated peptide that inhibits CETP activity (Cho et al., 1998). The
Porcine CETP gene recently has been mapped using conserved sequences of human and rabbit CETP (Shi et al., 2002). Plasma high-density lipoprotein cholesterol (HDL) concentrations are regulated by and correlate inversely with plasma CETP in susceptible species (Tsutsumi et al., 2001). Maintenance of high plasma HDL tends to exert an anti-atherogenic effect (Kwiterovich, 1998), and species such as the dog, mouse, and rat with normally high relative plasma levels of HDL tend to be resistant to atherosclerosis (Kieft et al., 1991; Moghadasian et al., 2001) (Table 1). A deficiency of hepatic lipase makes rabbits a suitable model for studying the controversial role of hepatic lipase in atherosclerosis (Dugi et al., 2001; Moghadasian et al., 2001). A detailed discussion of HDL metabolism and reverse cholesterol transport is beyond the scope of this review. (For more detail, see Assmann and Nofer, 2003; Asztalos and Schaefer, 2003; Barter et al., 2003; Huuskonen et al., 2001; Jiang, 2002; Redinger, 2003; Stein et al., 2002).

There are qualitative differences in the atherogenicity of various dietary fats (Aguilera et al., 2002). Coconut oil is more atherogenic than other saturated, monounsaturated, and polyunsaturated fats (Goldsmith and Jacob, 1978; Kritchevsky et al., 2003; Pronczuk et al., 1991). The recognition of hyper-triglyceridemia as a synergistic and recently as an independent risk factor for cardiovascular disease (Assmann et al., 2002; Ginsberg et al., 1995; Pearson et al., 2002; Stensland-Bugge et al., 2000) suggests that dietary models of atherosclerosis should include elevation of plasma triglyceride levels. High fat diets incorporating coconut oil produce combined hypercholesterolemia and hypertri-glyceridemia in pigs (Thomas et al., 2002), primates (Pronczuk et al., 1991), and rabbits (De las Heras et al., 2003).

Table 1: Comparative Features of Lipid Metabolism in Animal Models of Diet-Induced Atherosclerosis

<table>
<thead>
<tr>
<th>Species</th>
<th>Spontaneous disease</th>
<th>Dietary susceptibility</th>
<th>CETP</th>
<th>PLTP</th>
<th>HDL</th>
<th>Hepatic lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>+++</td>
<td>+++</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Pig</td>
<td>+</td>
<td>++</td>
<td>&lt;?</td>
<td>=</td>
<td>&gt;</td>
<td>+</td>
</tr>
<tr>
<td>Primate</td>
<td>+</td>
<td>++</td>
<td>=</td>
<td>&lt;</td>
<td>&lt;</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit</td>
<td>no</td>
<td>+</td>
<td>=</td>
<td>&lt;</td>
<td>&lt;</td>
<td>No</td>
</tr>
<tr>
<td>Dog</td>
<td>+/-</td>
<td>Resistant</td>
<td>&lt;</td>
<td>=</td>
<td>&gt;&gt;&gt;</td>
<td>+</td>
</tr>
<tr>
<td>Mouse</td>
<td>no</td>
<td>Resistant</td>
<td>none</td>
<td>&gt;&gt;</td>
<td>&gt;&gt;</td>
<td>+</td>
</tr>
<tr>
<td>Rat</td>
<td>no</td>
<td>Resistant</td>
<td>&lt;</td>
<td>&gt;&gt;</td>
<td>&gt;&gt;</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: + mild; ++ moderate; +++ severe; = approx. equivalent to human; < less than; > greater than; ? contradictory reports?
Comparative Anatomy and Pathophysiology

Although the size and cost of murine models of vascular disease are distinct advantages, there are several advantages to the use of a large animal model for studies designed to determine mechanisms responsible for beneficial effects of physical activity on vascular disease. First, there are dramatic differences between physical characteristics, smooth muscle content, and structural elements that impact the hemodynamic environment of conduit arteries of small mammals such as mice vs. larger mammals. Differences in smooth muscle and elastin content of the aorta and carotid arteries of the mouse, rat, and pig can be appreciated in Figures 1 through 4. Xu (2004) reported that the coronary artery of a mouse contains approximately 3,000 fewer cells than that of a human coronary artery. These differences in artery structure mean that murine arteries are much more compliant than the arteries of large mammals, including humans.

 Compliance of the artery and stretch of the wall are known to be significant signals for control of vascular gene expression (Awolesi et al., 1995; Fisslthaler et al., 2001; Ziegler et al., 1998). Therefore the greater compliance of murine arteries

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**Figure 1.** Sections of abdominal aorta stained with hematoxylin and eosin show relative thicknesses of the tunicae intima and media of the mouse (upper left), rat (upper right), and pig (lower left). Bar graph at lower right shows the differences in the ratio of smooth muscle cells of the tunica media to endothelial cells of the tunica intima (SMC/EC),
Figure 2. Sections of abdominal aorta stained with Verhoeff’s elastic methods show elastin layers (black), the tunicae media of the mouse (upper left), rat (upper right), and pig (lower left). Bar graph at lower right shows the differences in the number of elastic tissue layers.

Figure 3. Sections of common carotid artery stained with hematoxylin and eosin show relative thicknesses of the tunicae intima and media of the mouse (upper left), rat (upper right), and pig (lower left). Bar graph at lower right shows the differences in the ratio of smooth muscle cells of the tunica media to endothelial cells of the tunica intima (SMC/EC).
may influence expression of eNOS and other endothelial genes (Peng et al., 2003), which may have significant impact on progression of vascular disease (Vita and Keaney, 2000; Vita and Loscalzo, 2002). Further, the fact that mice at rest have heart rates nearly an order of magnitude greater than those of large mammals (Gödecke et al., 1998) also confounds the extrapolation of results from mice to large mammals (Schmidt-Nielsen, 1983). The high heart rates and smaller arterial pulse pressures in mice produce dramatically different types of stress across arterial walls than those seen in arteries of large mammals (Schmidt-Nielsen, 1983). These important differences in the cardiovascular systems between large and small mammals have important implications at rest and during exercise. Major and fundamental differences in cardiovascular structure and hemodynamics may preclude the translation of data from murine models to human health and disease.

Second, resting metabolic rate is much greater in small than in large mammals, and the physical demands of providing adequate cardiac output during vigorous exercise are not the same in large and small mammals. As a case in point, consider differences in the effects of exercise on cardiorespiratory function. Mice use over 50% of their maximal ability to transport oxygen at rest, so exercise produces a smaller relative effect on oxygen consumption, cardiac output, heart rate, and coronary hemodynamics in mice than in large mammals that have 10- to 15-fold increases in these parameters during maximal exercise (Schmidt-Nielsen, 1983). It is therefore interesting that exercise training appears to produce substan-
tial changes in eNOS expression in systemic arteries and the coronary circulation of large mammals (Hambrecht et al., 2000; Laughlin, 1985; Laughlin et al., 1996; 2001; Sessa et al., 1994; Wang et al., 1993) and in mice (Davis et al., 2003; Fukai et al., 2000; Kojda et al., 2001). Since there is evidence that the beneficial effects of physical activity on vascular disease are mediated by these protective effects provided by maintaining normal endothelial cell phenotypes, it is important to determine the effects of exercise in both large and small mammals (Vita and Keaney, 2000; Vita and Loscalzo, 2002).

These effects of exercise and physical activity on endothelial phenotype may be the result of the effects of exercise on the hemodynamic forces on the arteries. If this is true and exercise-induced hemodynamic forces on the arterial walls differ in mice and large mammals, then exercise may have different effects on eNOS expression in mice and large mammals. Also, eNOS may have a different level of importance for vascular function during exercise and a different contribution to the vascular protective effects of exercise in large and small animals. Indeed, it is possible that eNOS plays a more important or modified role in the control of vascular function (control of blood flow) and structure in large mammals than it does in rodents.

Results from experiments with eNOS-/- mice indicate that eNOS plays a key role in protecting arteries from the forces that produce vascular disease (Vita and Loscalzo, 2002). For example, Kuhlencordt et al. (2001) reported results from experiments with double KO mice (eNOS-/- and apoE-/- mice) which indicate that eNOS deficiency increases atherosclerosis in apoE-/- animals. Similarly, Yojo et al. (2000) report that neointimal formation was greater in eNOS-/- mice following ligation of the carotid artery than in wild-type mice. Thus, while there is evidence that eNOS plays an important role in protection of murine arteries from the forces producing vascular disease, significant differences between metabolic and vascular anatomic features of mice and human beings raise concern about the application of murine results to humans. In contrast, pigs share important metabolic and anatomic features with humans that make them good animal models of cardiovascular disease (Dixon et al., 1999; Gerrity et al., 2001; Johnson et al., 1999). Pigs develop spontaneous atherosclerosis (Skold et al., 1966) and in response to high fat diets are susceptible to induction of arterial lesions similar to lesions in human arteries (Massmann et al., 1977).

Diet-induced coronary artery disease has been associated with endothelial dysfunction in miniature pigs (Verhamme et al., 2002). In contrast, wild-type mice are highly resistant to atherogenesis (Moghadamian et al., 2001) and require knockout of genes such as apolipoprotein E (apoE-/) or LDL receptor (LDLR-/) to induce atherosclerosis and endothelial dysfunction (de Winther and Hofker, 2002; Knowles and Maeda, 2000; Reardon and Getz, 2001). Thus, results of murine models of vascular disease may not reflect those of humans. Therefore it is important to establish whether similar effects result from alterations in eNOS expression in large mammals, and whether the adaptive responses of large mammalian coronary arteries to the loss of or decreased expression of eNOS are similar to those of mice. Without results from a large animal model, it is difficult to correctly interpret available evidence indicating that eNOS expression is dramatically decreased in hyperlipidemia and atherosclerosis.
Also, hypercholesterolemia impairs brachial artery flow-mediated vasodilation (FMD) in humans (Zhang et al., 2000). Brachial FMD testing is used to assess endothelium-dependent relaxation (EDR), the impairment of which has been interpreted as indicative of endothelial dysfunction (Gokce et al., 2002). Hypercholesterolemia impairs EDR in carotid of apoE KO mouse (d’Uscio et al., 2001), and rabbit aorta (Jiménez et al., 2001), in association with reduced eNOS activity (Blair et al., 1999; Feron et al., 1999) by increased caveolin without effect on eNOS protein and expression: rabbit aorta (Jiménez et al., 2001), pig iliac (Rodriguez et al., 2002), and pig coronary (Wilson et al., 2001). However, neither eNOS mRNA nor protein levels were affected by hypercholesterolemia in apoE−/− mice (Gödecke et al., 2002; Matsumoto et al., 2003). Thus it appears that the effects of hypercholesterolemia on endothelial function are not the same in mice as in humans and large mammals.

No animal model is perfect as a model of human disease. However, available evidence indicates that the pig is an optimal model because of the many important similarities between the metabolism of pigs and humans and between the cardiovascular systems of pigs and humans. Miniature swine have been shown to possess many physiologic characteristics that resemble those of people. These characteristics are as follows:

1. The maximal oxygen/kg body weight of pigs is similar to that of humans (Armstrong et al., 1987a; 1997b; Sanders et al., 1977).
2. Pigs are a good model in which to study lipoprotein metabolism, due to the similarity of the chromatographic profile of cholesterol distribution in pigs with diet-induced atherosclerosis and dyslipidemic humans (Dixon et al., 1999; Kieft et al., 1991; Pussinen et al., 1997) and whole body metabolism (Armstrong et al., 1987a; 1987b; Sanders et al., 1977).
3. Porcine coronary anatomy is similar to that of humans (White and Bloor, 1981).
4. The innate coronary collateral development of pigs is sparse like that of humans (White and Bloor, 1981).
5. Pig arteries are similar to those of humans and these structural similarities allow application of and evaluation of human cath lab procedures in this animal model.
6. Porcine systemic hemodynamic variables such as mean blood pressure, pulse pressure, heart rates, cardiac output, etc., are similar to those of humans.
7. Pigs redistribute cardiac output, favoring blood flow to active skeletal muscle during exercise in a manner similar to humans (Armstrong et al., 1987a; 1987b; Sanders et al., 1977).
8. Miniature pigs exhibit cardiorespiratory adaptations to exercise training that are similar to those reported for humans (Armstrong et al., 1987a; 1987b; Sanders et al., 1977; White and Bloor, 1981; White et al., 1986; 1998).
9. Pigs on a high fat diet develop vascular disease that is similar to that observed in humans (Dixon et al., 1999).
Comparison With Human Disease

LESION MORPHOLOGY

Human clinical syndromes commonly are associated with atherosclerosis of the coronary arteries (myocardial ischemia and infarction), carotid arteries (stroke), and aorta (aneurysm) (Kieffer et al., 2002). In order to provide information relevant to treatment and prevention of these disease manifestations, animal models should mimic the lesions in these vessels as closely as possible.

Pigs fed a high fat (HF) diet develop lesions in all three sites (Dixon et al., 1999; Gass et al., 1979; Gerrity et al., 2001; Johnson et al., 1999; Massmann et al., 1977; Turk et al., 2003, Verhamme et al., 2002; Weinberg, 2002) (Table 2, Figure 5). Primates fed a HF diet also develop lesions in the aorta, carotid, and coronary arteries (Kramsch et al., 1981; Strong et al., 1994; Williams et al., 2003). Rabbits fed a HF diet develop predominantly aortic (Aguilera et al., 2002; De las Heras et al., 2003; Weinberg, 2002) and some coronary lesions (Kitajima et al., 1998), but are resistant to carotid lesions without additional injury such as balloon artery dilatation (Yano et al., 2000). Genetically modified LDLR-/- and apoE-/- mice typically develop lesions primarily in the aortic sinus (Kunjathoor et al., 2002) that are morphologically dissimilar to spontaneous human or porcine disease. Lesions rarely progress to involve the aortic arch (Lutgens et al., 2002), abdominal aorta, and coronary arteries (Calara et al., 2001) in geriatric mice. Much like the rabbit, mice develop carotid lesions only when ligation or other manipulation is superimposed on a HF diet (Chen et al., 2002; Godin et al., 2000; Yogo et al., 2000).

The vascular lesions that develop in pigs are histologically similar to Stary Stages I through VI. Stage I is defined as isolated macrophage foam cells (Figure 6A). Stary Stage II consists of multiple foam cell layers (Figure 6B). In Stary Stage III extracellular lipid pools develop (Figure 6C). The presence of mineralization is consistent with Stage IV (Figure 6D). The development of a fibromuscular “cap” overlying an accumulation of foam cells and lipid occurs in Stage V (Figure 7A and B). The formation of hematomata, surface defects, and thrombosis are consis-

Table 2  Comparative Lesion Distribution in Animal Models of Diet-Induced Atherosclerosis

<table>
<thead>
<tr>
<th>Species</th>
<th>Coronary</th>
<th>Carotid</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Pig</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Primate</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Rabbit</td>
<td>+</td>
<td>injury</td>
<td>+++</td>
</tr>
<tr>
<td>Mouse</td>
<td>+</td>
<td>injury</td>
<td>sinus</td>
</tr>
</tbody>
</table>

Note: + mild; ++ moderate; +++ severe; injury: requires injury in addition to diet; sinus: primarily lesions of the aortic sinus.
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Figure 5.  A: Abdominal aorta and iliac bifurcation of a pig fed a high fat and cholesterol diet stained with Sudan IV showing fatty streaks (red).  B: Common carotid artery of a pig fed a high fat and cholesterol diet stained with Sudan IV showing fatty streaks (red).  C: Cross-section of left anterior descending branch of the coronary artery showing fatty streaks (red).

tent with Stage VI and may be associated with acute clinical syndromes (Figure 7C). Stages I–III may be reversible upon removal of dietary fat, whereas mineralization and Stages IV through VI do not regress (Stary, 2001; Strong et al., 1994).

Effects of Exercise

LIPID METABOLISM

Physical activity and physical fitness in humans generally correlate directly with HDL and inversely with LDL and triglyceride levels in fasting plasma (Berg et al., 1997; Duncan et al., 2003; Herd et al., 2001; Leon et al., 2000; Rauramaa et al., 1995; Sgouraki et al., 2001; Sternfeld et al., 1999; Wei et al., 1997; Zhang et al., 2002). High plasma HDL levels are postulated to be anti-atherogenic by facilitating reverse cholesterol transport (Assmann and Nofer, 2003). Exercise has been reported to elevate HDL and reduce coronary artery disease in young adult male cynomolgus monkeys (Macaca fascicularis) fed a HF diet (Kramsch et al., 1981). Paradoxically, a recent study in adult males of the same species fed a slightly dissimilar HF diet reported no effect of exercise on HDL or coronary artery lesions (Williams et al., 2003). Exercise in pigs has been reported to shift the distribution of HDL3 to the HDL2 fractions (Stucchi et al., 1991) without significant elevation of HDL in pigs on a normal diet (Kist et al., 1999) and pigs on a HF diet (Thomas
Figure 6. A: Cross-section of common carotid artery stained with oil-red-o shows accumulation of small numbers of lipid-laden (red droplets) cells within the intima, Stary Stage I. B: Cross-section of common carotid artery stained with Verhoeff’s elastic method shows multiple layers of lipid-laden foam cells within the intima, Stary Stage II. C: Cross-section of common carotid artery stained with Verhoeff’s elastic method demonstrates central cavitation of lipid-laden foam cells associated with extraction of extracellular lipid pools during tissue processing, Stary Stage III. D: Cross-section of common carotid artery stained with Alizarin red for mineral central multifocal mineralization (arrow) in association with lipid-laden foam cells, Stary Stage IV.

et al., 2002). In pigs from the latter study, we observed lower intimal medial thickness (IMT) in lesions in the common carotid artery and abdominal aorta in exercise trained vs. sedentary pigs (Turk et al., 2002; Turk and Laughlin, 2002).

Exercise training tends to decrease plasma CETP and increase HDL in normal humans (Seip et al., 1993; Wilund et al., 2002a; 2002b). Voluntary exercise has been reported to increase the HDL/total cholesterol and HDL/LDL ratios in mice (Yashiro and Kimura, 1980); however, as discussed above, the mouse tends to be resistant to atherosclerosis due to an absence of CETP and high normal HDL (Moghadasian et al., 2001). The effects of exercise on plasma CETP have not been reported in other species.

Exercise training has been reported to increase lipoprotein lipase activity and reduce plasma triglycerides in humans (Duncan et al., 2003; Herd et al., 2001; Senti et al., 2001; Zhang et al., 2002), pigs (Stucchi et al., 1991), rabbits (Meng and Pierce, 1990), rats (Hamilton et al., 2001; Kusunoki et al., 2002) and mice (Kamei et al., 2003). The effects of exercise on hepatic lipase and HDL are controversial, with both decreased (Bergeron et al., 2001) and increased (Duncan et al.,
hepatic lipase activity having been reported in association with elevation of HDL in humans. Exercise has been reported to have no effect on hepatic lipase activity in pigs fed a HF diet (Thomas et al., 2002). The effect of exercise on hepatic lipase in other species has not been reported.

**VASOMOTOR EFFECTS**

Hambrecht and colleagues have reported that regular physical activity is superior to percutaneous coronary angioplasty for event-free survival (Hambrecht et al., 2004) and improves endothelium-dependent vasodilation in patients with coronary artery disease (Hambrecht et al., 2003). The latter observation was associated with an increase in eNOS protein and Akt-dependent Ser1177 phosphorylation in the internal mammary artery. We have recently reported that exercise increases eNOS protein and preserves endothelium-dependent relaxation in brachial arteries of hypercholesterolemic pigs (Woodman et al., 2003). We also have reported that endothelium-dependent relaxation is preserved in coronary arteries of hypercholesterolemic pigs independent of an alteration in eNOS protein by decreasing the production of a vasoconstrictor and increasing NO-release by NOS and/or NO bioactivity and bioavailability (Thompson et al., 2004; Woodman et al., 2004). In addition, the observation of eNOS protein in macrophage foam cells in coronary

**Figure 7.** A: Cross-section of common carotid artery stained with picrosirius red shows a fibromuscular cap (pink) overlying core foam cells and extracellular lipid, Stary Stage V. B: Same section as A polarized to show birefringent collagen in the fibromuscular cap, Stary Stage V. C: Cross-section of common carotid artery stained with Verhoeff’s elastic method shows central cavitation with hematoma formation consistent with Stary Stage VI.
arteries of hypercholesterolemic pigs (Thompson et al., 2004; Woodman et al., 2004), similar to that reported previously in human atherosclerotic lesions (Wilcox et al., 1997), suggests that the net effect on vasomotor tone of NO generated by endothelial and nonendothelial cells in atherosclerotic lesions remains to be elucidated (Kawashima and Yokoyama, 2004).

INFLAMMATION

The adherence of leukocytes to the vascular endothelium is one of the earliest events observed in the arterial wall of cholesterol-fed animals (Fan and Watanabe, 2003; Libby 2002). Oxidized LDL elicits a pro-inflammatory response, while HDL has antioxidant properties and promotes an anti-inflammatory response (Fan and Watanabe, 2003; Tedgui and Mallat, 2001). Acute exercise is pro-inflammatory (Akimoto et al., 2002; Drenth et al., 1998; Ji, 1999; Suzuki et al., 2002), whereas physical fitness or endurance exercise training in healthy subjects has been reported to decrease markers of inflammation (Church et al., 2002; Ford, 2002; Isasi et al., 2003; LaMonte et al., 2002).

Physiological laminar shear stress is thought to protect endothelial cells against inflammatory activation. Atherosclerosis typically develops at sites of low shear stress or turbulence. Exercise is hypothesized to increase laminar flow and reduce lesion development (Moore et al., 1992). Exposure of endothelial cells in vitro to low or oscillatory shear stress increases the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, and monocyte chemoattractant protein-1 (MCP-1) that promote monocyte adhesion. Exposure to continuous laminar flow downregulates these adhesion molecules (Tedgui and Mallat, 2001; Yoshisue et al., 2002). The use of techniques developed for ex vivo visualization cell adherence to microvascular endothelium may help dissect the postulated effects on laminar flow and endothelial activation (Glinskii et al., 2003).

Plasma C-reactive protein (CRP) is an acute phase reactant that has been proposed as an inflammatory marker of the risk for developing cardiovascular disease (Ridker et al., 2002). Acute severe exercise tends to increase CRP (Akimoto et al., 2002; Fallon, 2001), while endurance exercise training tends to reduce serum CRP (Barbeau et al., 2002; Mattusch et al., 2000; Smith et al., 1999). In healthy adults, more frequent physical activity is independently associated with lower odds of having elevated CRP (Abramson and Vaccarino, 2002), and higher energy expenditures through physical activity were significantly associated with lower serum CRP levels (Manns et al., 2003). Feeding a HF diet elevates serum CRP in pigs (Verhamme et al., 2002). Total cholesterol correlates with CRP in pigs fed a HF diet (Turk et al., 2003).

DOSE EFFECT

There is epidemiologic evidence for a positive dose effect of exercise in protection against human cardiovascular disease (Sacco et al., 1998; Wei et al., 1997). This dose effect remains largely unexplored in animal models. However, reduced aortic atherosclerosis has been reported in rabbits fed a HF and exercised for as little as 10 minutes per day (Kobernick et al., 1957) or daily treadmill exercise to exhaus-
tion (Myasnikov, 1958). Prolonged intense exercise training improves myocardial blood flow in humans with coronary artery disease (CAD) and pigs with coronary stenosis (Hagberg, 1991; Sanders et al., 1977). A reduction in overall mortality rate due to coronary artery disease in humans is more associated with recent activity than past activity (Sherman et al., 1999). Similar studies have not been performed in animal models.

PRIMARY VS. SECONDARY PREVENTION

Most studies of animal models of atherosclerosis do not progress to the development of clinical disease due to the risk of loss of animals that are expensive to develop, maintain, and exercise train. However, such studies are needed to study the mechanisms of secondary prevention models since the effects of exercise in healthy human beings (primary prevention) may differ from those in patients with cardiovascular disease (secondary prevention) (Adamopoulos et al., 2001; Brevetti et al., 2001; Schulze et al., 2002).

Limitations of Animal Models

Most nongenetic animal models of atherosclerosis require feeding a HF diet that elevates not only total cholesterol and LDL but also HDL (Kieft et al., 1991; Thomas et al., 2002). This elevation of HDL by the experimental diet may confound the detection of a potentially atheroprotective effect of exercise to increase HDL. Fat supplementation tends to increase HDL in normal men, but this response is attenuated in men with coronary artery disease (Clifton and Noakes, 2000). When saturated fats are replaced with monounsaturated or n-6 polyunsaturated fats from vegetable oils, primarily LDL decreases. When carbohydrates replace saturated fats in a low-fat diet, LDL and HDL decrease similarly (Sacks and Katan, 2002).

The carbohydrate and fat content of the diet modify the metabolic effects of exercise in humans (Hung et al., 2003; Turcotte, 1999). Exercise prevents the elevation of triglycerides that typically occurs in sedentary humans who consume a high carbohydrate diet (Graham and Adamo, 1999; Hellerstine, 2002; Koutsari et al., 2001). A similar finding has been reported in rats (Zavaroni et al., 1981).

Summary

The epidemiologic literature clearly shows that diet and exercise affect lipid metabolism and cardiovascular inflammation. Despite a dearth of mechanistic data, recent recommendations from the American Heart Association for primary prevention of cardiovascular disease include prescription of at least 30 minutes of moderate-intensity physical activity on most, and preferably all, days of the week (Pearson et al., 2002). The study of animal models such as the pig which possesses a cardiovascular anatomy and pathophysiology similar to that of humans is needed in order to discern the mechanisms underlying the beneficial effects of exercise on lipid metabolism, vascular inflammation, and the primary and secondary prevention of cardiovascular disease. The pig, unlike the mouse, develops spontaneous atherosclerosis (Moghadasian et al., 2001; Xu, 2004).
We have shown that hypercholesterolemia in the pig is associated with elevation of CRP, a marker of inflammation and cardiovascular disease risk in humans (Turk et al., 2003). We also have also shown that exercise preserves endothelium-dependent relaxation in brachial and coronary arteries of hypercholesterolemic pigs independent of an effect on blood lipids (Thompson et al., 2004; Woodman et al., 2003; 2004). For these reasons we propose the pig as a good animal model for studying the mechanisms through which exercise is beneficial in the prevention and treatment of human atherosclerosis.

References


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