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Preventive and therapeutic moderate aerobic exercise programs convert atherosclerotic plaques into a more stable phenotype☆

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ABSTRACT

The mechanisms by which exercise affects atherosclerotic plaque stability remain incompletely understood. We evaluated the effects of two training protocols on both atherosclerotic plaque structure and the signaling pathways involved in plaque rupture.

Methods: Male low-density lipoprotein (LDL) receptor knockout mice were fed a high-fat, high-cholesterol diet (HFD). One group was subjected to moderate exercise using a treadmill for 14 weeks (preventive protocol). The other group started an exercise regimen after 16 weeks of the HFD (therapeutic group). Atherosclerotic plaques within the aorta were evaluated for lipid and collagen contents, as well as for inflammatory markers. Plasma cholesterol and cytokine levels were also determined.

Results: The mice receiving a HFD developed hypercholesterolemia and atherosclerotic plaques within the aorta. The aortas from the animals in the preventive protocol exhibited smaller lipid cores and higher collagen content. These animals also exhibited lower CD40 expression within the plaques. The aortas of the mice in the therapeutic group exhibited higher collagen content, but no differences in either lipid core size or plaque size were noted. No differences in blood pressure, plasma cholesterol, cytokine levels, plaque size or metalloproteinase 9 expression were observed in the trained animals compared with the sedentary animals.

Conclusion: Moderate aerobic exercise modified atherosclerotic plaque characteristics and converted the plaques into a more stable phenotype, increasing the collagen content in response to both exercise programs. Furthermore, moderate aerobic exercise reduced the animals' fat content and decreased the activity of the CD40–CD40L signaling pathway in the preventive group.

1. Introduction

The development of the plaque vulnerability concept has changed the way we view atherosclerotic vessels [1]. Data from epidemiological [2], clinical [3] and experimental studies [4] have reinforced the perception that it is more important to maintain plaque stability than to attempt to decrease plaque size.

Vulnerable atherosclerotic plaques are composed of a rich lipid core, a thin fibrous cap and an inflammatory infiltrate [5]. Although plaques are well characterized from a morphological point of view, the mechanisms that govern plaque stability and the effects of preventive measures on their features are not well understood. It is not known, for example, whether exercise affects plaque composition or structure.

Clinical and epidemiological findings have consistently demonstrated the beneficial role of exercise in both preventing [6] and treating [7] atherosclerotic disease. More than twenty years have passed since the publication of pioneering reports demonstrating that light or moderate physical activity reduces the mortality and the incidence of heart attacks among older men with and without diagnosed cardiovascular disease [8]. Based on the evidence provided by these reports, physical inactivity has been established as one of the major modifiable risk factors for CHD [9].

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Nevertheless, the mechanisms by which exercise affects atherosclerotic plaque formation and development are not completely clear. Several hypotheses have been offered as follows: exercise improves nitric oxide synthase activity [10], decreases oxidative stress on the vessel wall [11], and inhibits inflammation inside the plaque [4]. Exercise also ameliorates the risk factors associated with atherogenesis [12].

Moreover, a systematic review comprising a large cohort of patients demonstrated that exercise produces a short-term inflammatory response, whereas regular physical activity triggers a sustained decrease in serum inflammatory markers [13]. These data suggest that the anti-inflammatory response induced by exercise may contribute to its beneficial effects in the setting of habitual physical activity. These findings have been challenged by other authors, who were unable to detect any effects exerted by exercise on circulating inflammatory mediators such as CRP and CD40L [14].

Until recently, the assessment of plaque instability markers was limited to experimental and post-mortem studies [3]. More recently, with the development of optical coherence tomography (OCT), intravascular ultrasound, and coronary angiography, human protocols have become more feasible [2]. Nevertheless, to evaluate the mechanisms involved in the atherogenic process, animal models remain necessary.

In animal models of atherosclerosis, it is possible to assess some of the characteristic features of plaque stability, including lipid core size, collagen deposition, and inflammatory infiltrates [15,16].

Therefore, our primary objective was to evaluate the effect of moderate exercise on both the formation and the development of atherosclerotic plaques in an experimental model of atherosclerosis. Moreover, we sought to determine the effect of exercise on some of the mechanisms that affect atherosclerotic plaque stability.

2. Material and methods

2.1. Mice

All procedures were performed in accordance to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The study protocol was approved by the Research Ethics Committee of the USP School of Medicine (*Comissão de Ética para Análise de Projetos de Pesquisa do HCFMUSP* — http://www.hcnet.usp.br/adm/dc/cappesq/) (#393/05). LDL receptor-deficient mice (LDLr−/−) were obtained from the Jackson Laboratory (Bar Harbor, ME). After completion of all the protocols, the mice were anesthetized with ketamine (100 mg/kg) and xylazine (50 mg/kg); blood was collected directly from the left ventricle. The mice were then killed via exsanguination, and the aorta was prepared as described below.

3. Study design

3.1. Protocol A

This protocol was designed to determine whether exercise can prevent the development of atherosclerotic plaques.

Sixteen 10-week old male mice were divided into two groups.

Group A1 received a high-fat, high-cholesterol diet (described below) and did not exercise.

Group A2 received a high-fat, high-cholesterol diet (described below) and exercised according to the protocol below.

3.2. Protocol B

This protocol was designed to determine whether exercise modifies the evolution of established atherosclerotic plaques; therefore, exercise was initiated after 16 weeks of a high-fat, high-cholesterol diet, when the atherosclerotic plaques were well established.

Additional sixteen 10-week-old male mice were divided into two groups.

Group B1 received a high-fat, high-cholesterol diet (described below) for 30 weeks with no exercise.

Group B2 received a high-fat, high-cholesterol diet (described below) for 16 weeks; subsequently, exercise was initiated according to the protocol below, and the high-fat diet was maintained during the exercise period (14 weeks).

Twelve additional animals were studied as controls for each of the four groups. The control animals received a chow diet and were subjected to either exercise or no exercise. After the study period (either 14 or 30 weeks), the control animals were sacrificed, and their aortas were subsequently evaluated. Laboratory measurements (lipids and inflammatory mediators) were performed in these animals. Because neither sizable atherosclerotic plaques nor changes in blood markers were observed, we did not include these results in this paper.

3.3. Diet

All mice had access to diet and water ad libitum. The mice were fed either a commercial diet (Nuvital CR1) or a high-fat, high-cholesterol (HFHC) diet containing 20% fat, 1.25% cholesterol, 0.5% cholic acid (Diets, Bethlehem).

3.4. Exercise training protocol

Low-intensity exercise training was performed on a motor treadmill for 14 weeks, 5 days/wk, for 60 min. Both the running speed and the exercise duration were progressively increased to 60% of the animals' maximal speed, which was established during a graded treadmill exercise protocol in the seventh week. This intensity was maintained for the remainder of the 14-wk training period. All untrained mice were subjected to treadmill exercise (5 min) twice a week to become accustomed to the exercise protocol and handling. Exercise training was performed during the animals' dark cycles.

3.5. Body weight and blood pressure

Body weight (W) was recorded weekly. Blood pressure (BP) was determined non-invasively using a computerized tail-cuff system (BP 2000 Visitech Systems). The measurements were performed during the animals' dark cycles.

3.6. Plasma lipoproteins and total cholesterol

Plasma was separated via centrifugation (12,000 rpm, 15 min). The lipoproteins were separated via fast protein liquid chromatography (FPLC) using an HR10/30 Superose 6 column (Amersham-Pharmacia Biotech., Uppsala, Sweden). Lipoprotein profiles were calculated as the areas under the VLDL, LDL, and HDL peaks of the FPLC profile. Plasma total cholesterol and triacylglycerols were each determined via enzymatic methods according to the manufacturer's instructions (Roche Diagnostic GmbH, Mannheim, Germany).

3.7. Real time PCR

Messenger RNA of PGC-1α, CD40 and MMP9 was quantified using real time PCR (RT-PCR). Total RNA was extracted from aortic roots using TRIzol protocol (Invitrogen, Carlsbad, Calif). Real-time PCR was performed in a 15 μl reaction mixture containing 7.5 μl 2× SYBR Green Reaction Mix (Invitrogen), 0.3 μl each primer, 0.3 μl Super Script III RT/Platinum Taq Mix (10 pmol/ μl), 0.15 μl ROX Reference Dye, and 5 μl sample in water. Quantification was performed by 2-DDCT method, using β-2microglobulin (β2M) as housekeeping gene. The sequences