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SHORT COMMUNICATION

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Numerical density of cardiac myocytes in aged rats fed a cholesterol-rich diet and a canola oil diet (*n*-3 fatty acid rich)

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Abstract We studied the myocardium of 45 aged rats fed from 21 days after birth until 15 months of age with a standard rat diet a cholesterol-rich diet (CHO) or canola oil (O). We analysed the cardiac weight (CW) and, using unbiased stereological estimates, studied isotropic, uniform, random sections of the free left ventricular wall to determine the numerical density of the myocytes ($Nv_{[myocyte]}$). The CW was not statistically different between groups A and CHO; it was smallest in animals in group O (21.2% smaller in group O than in group A and 15.3% smaller in group O than in group CHO). $Nv_{[myocyte]}$ was statistically different in all three groups and was greatest in animals in group O. By comparison with rats in group A, group CHO rats had an $Nv_{[myocyte]}$ than was 51.3% smaller and group O, 33.3% greater. Aged rats fed with canola oil diet have a well-vascularized myocardium, which is probably associated with preservation of $Nv_{[myocyte]}$ in the myocardium of these animals.

Key words Aging · *n*-fatty acid · Cholesterol · Myocardium · Stereology

Introduction

Ageing is associated with marked changes in cardiac structure including myocyte loss [13], hypertrophy of the remaining cells [3], and increase in collagen content [4]. These changes may account for the functional characteristics of the aged myocardium, which shows impaired myocardial perfusion [12], altered diastolic compliance [14] and arrhythmia [5].

The *n*-3 fatty acids are long-chained and occur naturally in fish oils and many vegetables, including canola oil [15]. The clinically important *n*-3 fatty acids include eicosapentaenoic acid, docosahexaenoic acid and alpha linolenic acid [7]. Multiple effects are attributed to *n*-3: a protective effect in cardiovascular diseases [9], decrease of both peripheral vascular resistance and blood viscosity [17] and decreases in the risks of myocardial infarction, arrhythmia and thromboses [18].

In a previous study we analysed the influence of different diets on the myocardium of aged rats using stereology [1]. The present work aims to continue that study, observing differences in the numerical density of myocytes in aged rats fed with three different diets (standard rat diet, cholesterol-rich diet and canola oil diet).

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Materials and methods

Forty-five male Wistar rats were divided into three groups of 15 animals each. From 21 days until 15 months of age, the animals received water ad libitum and one of the three different diets: a standard rat diet (Nuvilab) a cholesterol-rich diet and a canola oil diet. (For details of the composition and manner of preparation of the diets see [1].) The study followed the principles of laboratory animal care (NIH publication no. 85-23, revised 1985).

At 15 months of age rats were killed after anaesthesia with ether. The thorax was opened, exposing the heart, this was injected with 1.0 ml of 10% KCl into the left ventricle until diastolic cardiac arrest. Cardiac weight was measured to an accuracy of 0.01 g using Scherle's method [23].

The myocardium is an anisotropic structure, but isotropic sections are necessary for a stereological study. We carried out the estimation by cutting the organ using the orthotrip method [22]. Fragments of myocardium were fixated for 48 h in buffered formaldehyde 4%, pH 7.2, and then embedded in paraffin and sectioned at 5 µm thickness. Haematoxylin-eosin and Sirius red were used to stain the sections. The analysis was based on a video-microscopic system composed of a Leica DMRBE microscope coupled with a Kappa CF 15/5 video camera and a Sony Trinitron monitor.

The optical dissector was used for the calculation of numerical density of the myocytes ($Nv_{[myocyte]}$) defined with two parallel sec-

tions separated by a distance of 3 µm (automatically controlled with the motorized stage of the Leica microscope). The look-up and look-down planes were determined over a frame of 1600 µm^2 [6, 11]. For reasons of efficiency, one nucleus was considered to represent one myocyte. The $Nv_{[myocyte]}$ was determined by 15 random dissector pairs for each specimen [16]. Q_A was the number of the myocyte nuclei seen in focus only in the look-up, when they were partly or totally inside the frame and did not intersect the left or inferior exclusion edges or their extension [10], t is the thickness of the disector and A_T is the test area:

$$Nv_{[myocyte]} = \frac{Q_A^-}{t.A_T} \quad 1/mm^3$$

Differences between groups, two by two, were tested with the nonparametric Mann/Whitney test with the significance level of P=0.05 [26].

Results

Results are summarized in Figs. 1 and 2. Cardiac weight was not statistically different between groups A and CHO. It was smallest in the animals in group O (21.1% smaller in group O than in group A and 15.3% smaller in group O than in group CHO; Fig. 1). The $Nv_{[myocyte]}$ was statistically different in all three groups. It was greatest in the animals in group O. In comparison with in rats group A, in group CHO rats $Nv_{[myocyte]}$ was 51.3% smaller and in group O it was 33.3% greater (Fig. 2).

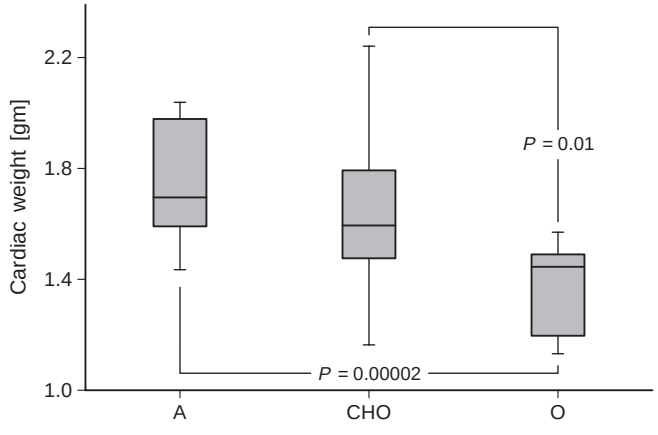

Fig. 1 Box plot of the cardiac weight in aged rats fed a standard diet (*A*), rats fed a cholesterol-rich diet (*CHO*) and rats fed canola oil (*O*). Differences between groups A vs O and CHO vs O were statistically significant

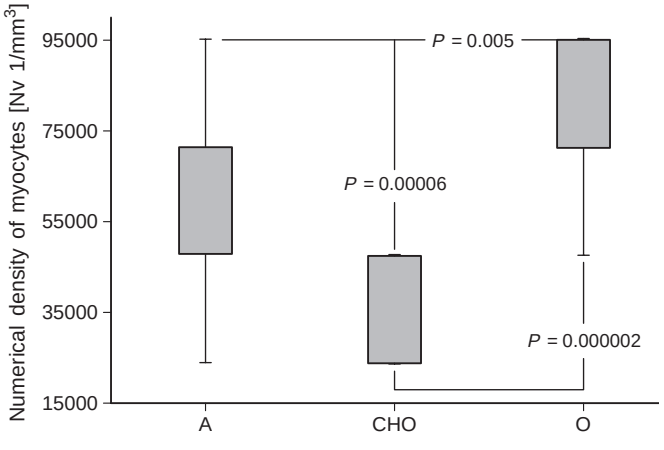

Fig. 2 Box plot of the numerical density of myocytes in aged rats fed a standard diet (*A*), rats fed a cholesterol-rich diet (*CHO*) and rats fed canola oil (*O*). Differences between groups A vs CHO, A vs O and CHO vs O were statistically significant

Discussion

Alpha linolenic fatty acid is an 18-carbon chain fatty acid with three double bonds; in the form of tofu, soybean oil, and canola oil, it is an important plant-based source of the *n*-3 polyunsaturated fatty acids for vegetarians and nonseafood eaters [24].

In humans, the use of *n*-3 fatty acid in the diet has been considered to have a beneficial effect on cardiovascular function, reducing blood pressure levels and diminishing both peripheral vascular resistance and blood viscosity [19, 21].

At 3 and 15 months after birth, experimental Wistar rats are young adults and aged animals, respectively [2, 8]. In this work, after 15 months of experimentation the $Nv_{[myocyte]}$ was greatest in animals fed the canola oil diet and smallest in animals fed the cholesterol-rich diet. In a previous study, aged rats fed a canola oil diet showed the best myocardial vascularization, characterized by increased stereologically estimated length density of blood vessels as well as a high HDL-C and a low LDL-C in the serum [1].

Reduction in myocyte number occurs shortly after sexual maturity in mammals and continues throughout life in the absence of atherosclerosis, diabetes, hypertension and ischaemic heart disease. A significant loss of myocytes is normal in ageing, and in the ventricular myocardium this phenomenon persists longer on the left than on the right side [3]. A healthy 70-year-old man will have lost nearly 30% of the myocyte population as a result of ageing along, regardless of any other cardiac disease state; this detrimental effect precedes the occurrence of ventricular dysfunction [20]. Cell loss decreases the cardiac functional reserve and may constitute a critical variable in the development of ventricular dysfunction and failure in the elderly. Chronic death of myocytes may also form the basis for the depressed capacity of the aged heart to sustain acute and chronic increases in pressure and volume loads on the myocardium [25].

This study has verified that, in rats, diet affects the numerical density of cardiac myocytes. The loss of myocytes was smaller in aged rats fed a canola oil diet (*n*-3 fatty acid rich) for a long period than in rats of similar age fed other diets, mainly the cholesterol-rich diet. As pointed out previously, stereological estimates of numbers of myocardial vessels in rats fed the canola oil diet suggest the maintenance of good myocardial vascularization into old age in these animals. The well-vascularized myocardium of canola oil-fed aged rats probably explains the higher figures for numerical density of myo-

cytes determined in these animals. This is an exciting finding that merits further confirmation with human studies.

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