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Stereology of myocardial hypertrophy induced by physical exercise

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Summary. Twenty young female Sprague-Dawley rats were randomly assigned to 2 groups. Ten animals served as sedentary controls, the 10 experimental animals were subjected to a training program with gradually increasing intensity of 18 weeks duration on a motor-driven treadmill. The rats were fixed by retrograde vascular perfusion via the abdominal aorta under anesthesia. Two transverse and 2 longitudinal sections per animal were selected at random from the left ventricular papillary muscles for light and electron microscopic stereological investigation. Length density and surface density of myocardial cells and capillaries were estimated with correction for partial anisotropy and curvature by means of the mathematical model of a Dimroth Watson orientation distribution. Left and right ventricular weight increased by 20% in the exercise group ($P<0.001$), whereas body weight remained unchanged. Physical training led to a significant increase of heart muscle fiber cross-sectional area by 17% ($P<0.01$). The ultrastructural volumetric composition of the myocardial cell cytoplasm by myofibrils, mitochondria, and sarcoplasmic matrix remained unchanged. Volume density, length density and surface density of capillaries, as well as capillary cross-sectional area and capillary anisotropy parameters were not significantly altered by training. From the data one concludes an increase of the 3-dimensional capillary-fiber ratio by 19% ($P<0.001$). Thus physical training induces mild absolute biventricular cardiac hypertrophy in young female rats, in which capillary proliferation compensates for the increase of mean oxygen diffusion distance resulting from fiber thickening, by supplying each unit of fiber length by more units of capillary length.

Key words: Exercise – Hypertrophy – Morphometry – Myocardium – Stereology

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Introduction

Physical training of sufficient intensity and duration induces cardiac hypertrophy in man and experimental animals. Capillary vascularization deserves particular interest, as recent evidence shows convincingly that coronary vasodilator reserve is impaired in hearts with hypertrophy induced by chronic pressure overload (Holtz et al. 1977; Mueller et al. 1978; O'Keefe et al. 1978; Breisch et al. 1984), but remains unchanged or is even augmented in hearts with hypertrophy induced by physical training (Laughlin et al. 1978; Spear et al. 1978; Scheuer 1982). With respect to rat myocardium quantitative structural studies have been limited to trained male animals, using capillary density and capillary-fiber ratio as indicators of capillarization (Scheuer and Tipton 1977; Scheuer 1982). However, male rats tend not to increase their food intake to cover the energy needs imposed by exercise, and their body weights fail to follow the normal growth curve; female rats have a compensatory increase in food intake, and their body weight follows a normal growth curve (Oscai et al. 1971; Scheuer and Tipton 1977). As heart weight is strongly correlated to body weight, absolute hypertrophy (increased absolute heart muscle mass) is rarely found in trained male rats but is easily induced in female rats. Moreover, capillary density and capillary-fiber ratio (estimated from transverse sections as number of capillary profiles per tissue area and as number of capillary profiles per muscle fiber profile) are less than optimal definitions of capillarization, because they depend not only on the extent of the capillary bed, but also on the three-dimensional capillary arrangement and on the state of muscular contraction (Mattfeldt and Mall 1984). Recently stereological methods have been developed which allow efficient and practically unbiased estimation of more meaningful definitions of capillary characteristics (Weibel 1980; Cruz-Orive et al. 1985). These methods are now standard for studies on skeletal muscle capillaries (Mathieu et al. 1983) and have been introduced by us into heart muscle stereology (Mattfeldt and Mall 1984; Mattfeldt et al. 1985). In the present study using these stereological techniques the hypothesis is tested, that compensatory capillary proliferation is induced in absolute left ventricular hypertrophy in female rats subjected to running exercise. This might account for the unchanged or augmented coronary vasodilator reserve of these hearts. The intellectual basis of the study is rooted in three props: modern quantitative stereology; in the conception of the "myocardial synergide" (Doerr 1971; Doerr and Rossner 1977) which implies that a meaningful evaluation of experimental myocardial changes requires the study of both muscular and non-muscular components of the myocardium; and in the pioneering studies of Linzbach (1947, 1960) who investigated the growth pattern of myocardial cells in the human heart and suggested that counting of muscle fiber profiles should be restricted to the papillary muscles, where sections of fixed orientation can be obtained reproducibly.

Methods

Animal model

Twenty young female Sprague-Dawley rats were randomly assigned to 2 equal groups. Ten animals served as sedentary controls, the 10 experimental animals were subjected to a training program of 18 weeks duration on a motor-driven treadmill with exact speed control (without slope). In the first 14 weeks duration and speed of running were gradually increased; in the last 4 weeks the animals ran 90 min per day at 6 days per week at a constant speed of 32 m/min. All rats were kept in single cages and received tap water and standard laboratory chow ad libitum. At the end of the training period the animals were fixed by retrograde perfusion at a pressure of 120 mm Hg after catheterization of the abdominal aorta. Before fixation the vascular system was flushed with a dextran solution (Rheomacrodex) containing procain-hydrochloride for 2 min which dilates the coronary arteries and their branches and leads to cardiac arrest in diastole. The inferior vena cava was incised to drain the blood 10 s after starting the dextran infusion. This procedure counteracts the collaps of capillaries at low venous pressures. The vascular system was subsequently perfused with 0.2 mol phosphate buffer containing 3% glutaraldehyde for 12 min. The wet weights of the left ventricle (including interventricular septum) and of the right ventricle were measured by a precision balance.

Stereology

Mammalian myocardium is a tissue built up nearly exclusively by a network of tubular structures (muscle fibers and capillaries). Their geometrical properties can be described by the following stereological parameters: V_V, volume density per tissue volume; S_V, surface density per tissue volume; and L_V, length density per tissue volume. The papillary muscles are particularly suitable for a comprehensive stereological analysis of all these variables, because only here can the axis of anisotropy be detected macroscopically (as in skeletal muscle). Whereas the myocardial cells are indeed nearly perfectly parallel cylinders, previous studies have shown that this is not true for the myocardial capillary network; the latter is adaequately described by the stereological model of a Dimroth-Watson orientation distribution, which allows correction of L_V and S_V estimates for partial anisotropy and curvature of capillaries, and provides quantification of the degree of parallel orientation in 3D space by dimensionless constants (Mattfeldt and Mall 1984). Note that the resulting estimates are "automatically" corrected for fixation in heterogeneous phases of the cardiac cycle.

In practice both left ventricular papillary muscles were temporarily embedded in Agar-Agar and allocated randomly to either transverse or longitudinal sectioning. 200 μm thick slices were thus cut parallel or perpendicular to the macroscopic muscle axis by means of a Sorvall tissue sectioner (Mall et al. 1978; Mattfeldt et al. 1980). From both orientations 2 slices were selected for stereological evaluation by random numbers, postfixed in OsO_4, dehydrated, and definitively embedded in Epon-Araldite. For light microscopic evaluation semithin sections of 1 μm nominal thickness were stained with Toluidin-Blue and examined with phase contrast and oil immersion (Fig. 1 and 2). Ultrathin sections from the same blocks were stained with lead citrate and uranyl acetate and analyzed with a Zeiss EM 10 electron microscope (Fig. 3). Stereological analysis was then performed as a multiple stage sampling procedure at 3 levels of final magnification.

At *Stage I* (light microscopy, 2 transverse and 2 longitudinal sections per animal, final magnification 1,000×), systematic subsampling of 10 visual fields per section was performed. Volume densities of myocardial cells, capillaries, and interstitial tissue were estimated by point-counting. Boundary length of capillary profiles per tissue area, $B_A(\alpha)$ (cap/tiss), was estimated for transverse ($\alpha=0$) and longitudinal ($\alpha=\pi/2$) sections with an isotropic test grid (Merz 1967) from the number of intersection counts per test line length, I_L, using $B_A(\alpha)=\pi/2*I_L$. The number of capillary profiles per tissue area, $Q_A(\alpha)$ (cap/tiss), was estimated by using the unbiased counting frame (Gundersen 1977). From the ratios $B_A(0)/B_A(\pi/2)$ and $Q_A(0)/Q_A(\pi/2)$ the anisotropy constants of the capillary surface and length elements, K_S and K_L,

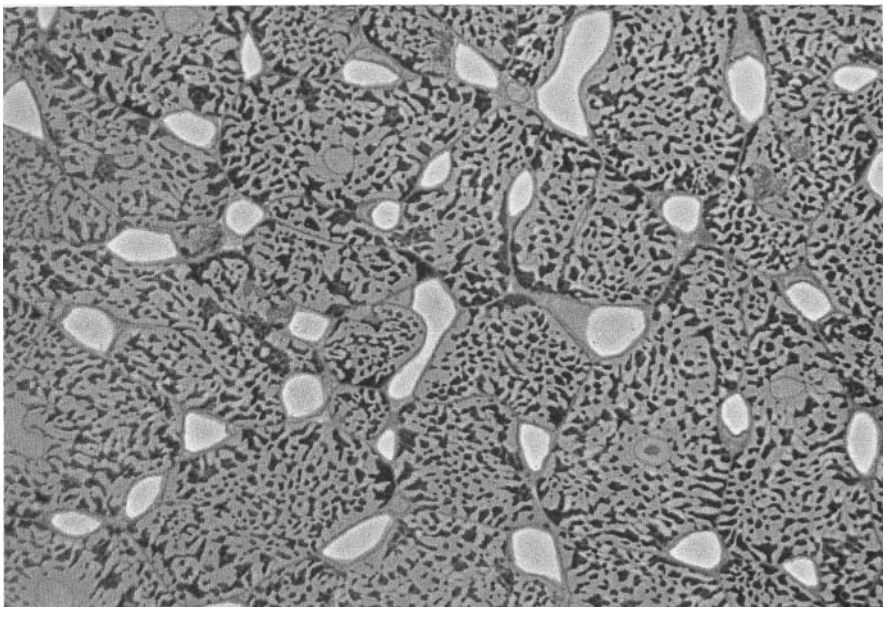

Fig. 1. Light micrograph of a transverse section of a left ventricular papillary muscle. Capillary profiles are easily delineated, whereas muscle cell boundaries cannot be distinguished with certainty. Semithin section, Toluidin-Blue. Final magnification $\times 1{,}250$

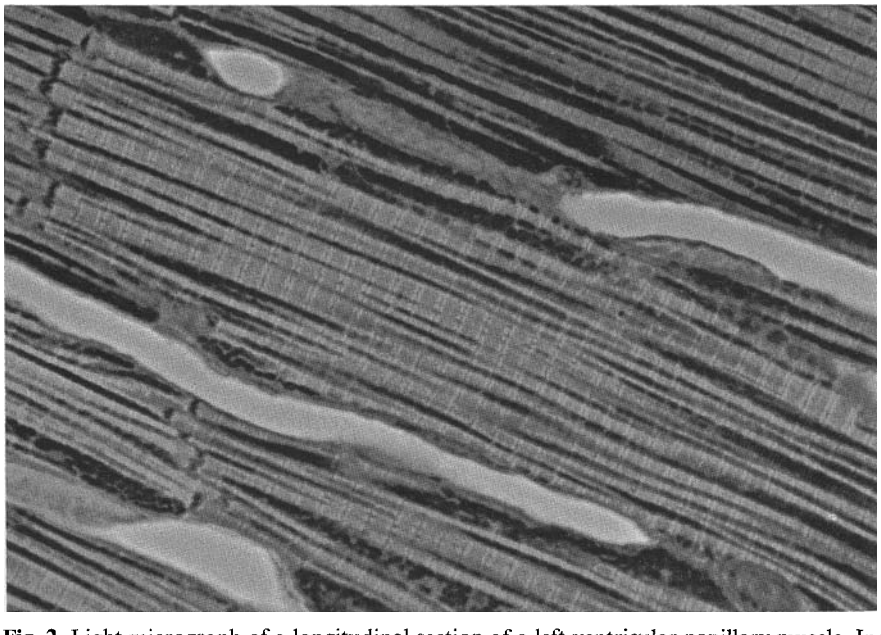

Fig. 2. Light micrograph of a longitudinal section of a left ventricular papillary muscle. Inconspicuous arrangement of capillaries and muscle fibers which show normal sarcomeres and intercalated discs. Note partial anisotropy of capillaries: the tubules are cut in turn longitudinally, obliquely, and nearly transversely. Semithin section, Toluidin-Blue. Final magnification $\times 2{,}000$

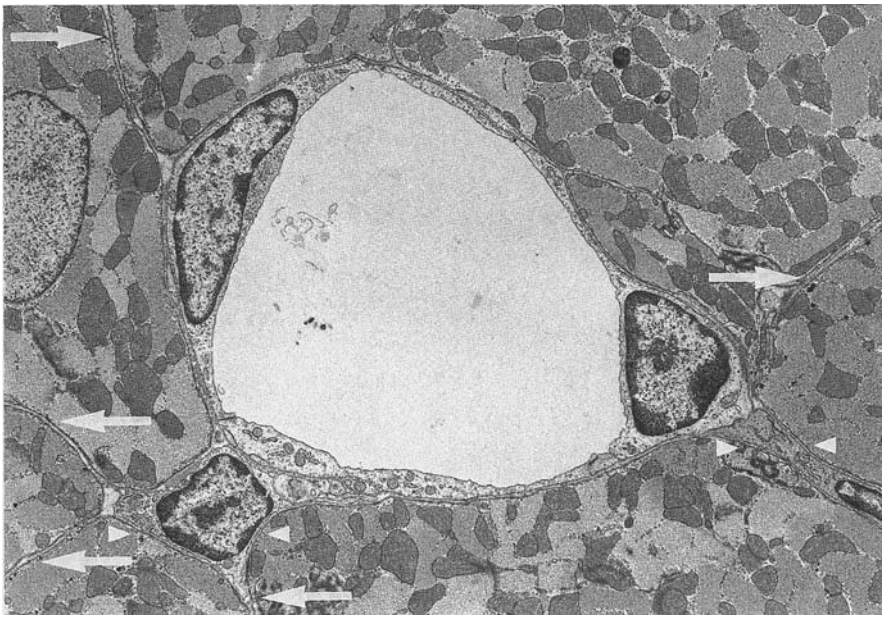

Fig. 3. Electron micrograph of a transverse section of a left ventricular papillary muscle. A capillary with 2 endothelial cells is accompanied by interstitial cells (*arrowheads*). The arrows indicate boundaries between myocardial cells, clearly discernible at the electron microscopic level only. Final magnification $\times 17{,}000$

of the Dimroth-Watson distribution were calculated as previously described in detail (Mathieu et al. 1983; Mattfeldt and Mall 1984). The stereological correction coefficients $c(K_{L,S},0)$ were then calculated for each constant which provided estimates corrected for partial anisotropy:

$$S_V(\text{cap/tiss}) = c(K_S,0) * B_A(0)(\text{cap/tiss}) \quad (1),$$

$$L_V(\text{cap/tiss}) = c(K_L,0) * Q_A(0)(\text{cap/tiss}) \quad (2).$$

At *Stage II* (electron microscopy, 2 transverse and 2 longitudinal ultrathin sections per animal, final magnification $4{,}000\times$) the number of myocardial cell profiles per tissue area was counted on 10 systematically selected visual fields per section. Length density of myocardial cells per tissue volume, L_V(myoc/tiss), was estimated using equation (2). In agreement with other groups we found that electron microscopic resolution is imperative for counting of myocardial cell profiles in hearts fixed by vascular perfusion because the width of the intercellular spaces lies below the resolution of light microscopy (Fig. 3; Loud et al. 1984). As we found that the relative bias due to partial anisotropy was negligible for myocardial cells in the left ventricular papillary muscles ($<1\%$), anisotropy constants are displayed only for capillaries in the tables.

At *Stage III* (electron microscopy, 2 transverse ultrathin sections per animal, final magnification $41{,}460\times$) volume densities of myofibrils, mitochondria, and sarcoplasmic matrix per myocardial cell cytoplasmic volume were estimated by point-counting on 30 systematically selected visual fields per section.

Finally estimates of mean "true" cross-sectional areas $\bar{a}$ of capillaries and myocardial cells were obtained using the relation

$$\bar{a} = V_V/L_V \quad (3)$$

(Mathieu et al. 1983), and "true", 3D capillary-fiber ratios were obtained from the ratio $L_V(\text{cap/tiss})/L_V(\text{myoc/tiss})$. Note the difference between $\bar{a}$ of capillaries and conventional capil-

Table 1. Baseline data

Parameter	Control group $n=10$ Mean ± SEM	Exercise group $n=10$ Mean ± SEM	Level of statistical significance
1. Body weights			
Initial body weight [g]	115 ± 1	114 ± 1	N.S.
Final body weight [g]	284 ± 5	291 ± 5	N.S.
2. Heart weights			
Left ventricle (including septum) [mg]	769 ± 21	924 ± 23	$P<0.001$
Right ventricle [mg]	204 ± 5	239 ± 6	$P<0.001$
Sum of both ventricles [mg]	973 ± 26	1,163 ± 27	$P<0.001$

Table 2. Stereological results obtained by light microscopy

Estimate	Control group $n=10$ Mean ± SEM	Exercise group $n=10$ Mean ± SEM	Level of statistical significance
1. Volume fractions per tissue volume			
Volume density of myocardial cells [%]	86.8 ± 0.4	86.9 ± 0.4	N.S.
Volume density of capillaries [%]	10.6 ± 0.4	10.2 ± 0.5	N.S.
Volume density of interstitial tissue [%]	2.6 ± 0.3	2.9 ± 0.5	N.S.
2. Surface area per tissue volume			
Boundary length of capillary profiles per tissue area in transverse sections, $B_A(0)$ (cap/tiss) [mm/mm^2]	65.7 ± 2.2	65.4 ± 1.4	N.S.
Boundary length of capillary profiles per tissue area in longitudinal sections, $B_A(\pi/2)$ (cap/tiss) [mm/mm^2]	45.5 ± 1.2	42.7 ± 1.5	N.S.
Anisotropy constant of capillary surface area elements, K_S	−3.7 ± 0.9	−6.1 ± 2.0	N.S.
Correction coefficient $c(K_S, 0)$	1.038 ± 0.011	1.022 ± 0.012	N.S.
Surface area of capillaries per tissue volume, S_V (cap/tiss) [mm^2/mm^3]	68.2 ± 1.8	66.8 ± 1.3	N.S.
3. Length per tissue volume			
Number of capillary profiles per tissue area in transverse sections, $Q_A(0)$ (cap/tiss) [mm^{-2}]	3,515 ± 86	3,609 ± 125	N.S.
Number of capillary profiles per tissue area in longitudinal sections, $Q_A(\pi/2)$ (cap/tiss) [mm^{-2}]	687 ± 46	643 ± 40	N.S.
Anisotropy constant of capillary axes, K_L	+4.8 ± 0.6	+5.5 ± 0.9	N.S.
Correction coefficient $c(K_L, 0)$	1.065 ± 0.014	1.054 ± 0.011	N.S.
Length of capillaries per tissue volume, L_V (cap/tiss) [mm/mm^3]	3,744 ± 75	3,803 ± 115	N.S.
Mean "true" capillary cross-sectional area [μm^2]	28.3 ± 1.0	26.9 ± 1.3	N.S.

Table 3. Stereological results obtained by electron microscopy

Estimate	Control group $n=10$ Mean ± SEM	Exercise group $n=10$ Mean ± SEM	Level of statistical significance
1. Myocardial cells			
Length of myocardial cells per tissue volume, L_V (myoc/tiss) [mm/mm^3]	2,995 ± 134	2,543 ± 78	$P<0.01$
Mean "true" myocardial cell cross-sectional area [μm^2]	291 ± 13	340 ± 10	$P<0.01$
3-dimensional capillary-fiber ratio	1.26 ± 0.04	1.50 ± 0.04	$P<0.001$
2. Ultrastructural volumetry of myocardial cell cytoplasm			
Volume density of myofibrils [%]	64.8 ± 1.9	65.2 ± 1.0	N.S.
Volume density of mitochondria [%]	26.5 ± 1.1	27.3 ± 1.0	N.S.
Volume density of sarcoplasmic matrix [%]	8.7 ± 1.0	7.5 ± 0.7	N.S.

lary cross-sectional area which equals merely capillary profile area. Group means were compared by means of Student's *t*-test for unpaired data. A result was considered as statistically significant if the probability of error, *P*, was smaller than 0.05.

Results

There were no deaths and no signs of cardiac failure in either group. The training regimen led to a mild biventricular cardiac hypertrophy in the exercised rats with an increase of absolute heart weight by 20% at unchanged body weight (Table 1). Light and electron microscopic examination disclosed no striking structural abnormalities in the hearts of the control and experimental animals. Likewise, careful electron microscopic scrutiny of the intercellular spaces did not disclose collapsed capillary lumina, indicating that fixation by vascular perfusion had probably opened all capillaries. The stereological results are displayed in Table 2 and 3 and may be summarized as follows: hypertrophy is documented by an increase of mean cross-sectional area of the myocardial cell by 17% ($P<0.01$), as estimated via equation (3) from a decreased length density at unchanged volume density of the myocardial cells. Fiber growth is not accompanied by any alterations of the volume densities of myofibrils, mitochondria, and sarcoplasmic matrix within the myocardial cell cytoplasm. The central finding is the fact that volume, surface area, and length of capillaries per tissue volume, as well as mean "true" capillary cross-sectional area, and 3D capillary orientation pattern remain completely unaltered despite myocardial cell thickening. Consequently 3D capillary-fiber ratio is augmented by 19% ($P<0.001$). Thus capillary proliferation is documented which compensates myocardial cell thickening by supplying each mm fiber length with more mm capillary length.

Discussion

The present study confirms earlier observations that adaptation to a prolonged training program can induce absolute cardiac hypertrophy in young female rats (Oscai et al. 1971; Schaible and Scheuer 1981). Like many previous investigators we found symmetrical hypertrophy of both cardiac ventricles after training (Hort 1951; Van Liere et al. 1965; Lin et al 1977; Hort et al. 1985), indicating that cardiac muscle mass increases due to an enhanced volume load. With respect to intensity we consider our training program to be a mild exercise regimen, since speed and duration of running were increased gradually in small physiological steps, and hypertrophy in the range of 20% within 4.5 months is much less than the high increases in heart weight often brought about by experimental sudden severe pressure overload. In addition there were no signs of cardiac failure.

Restriction of the sampling universe to the left ventricular papillary muscles might be considered to be a possible limitation of the present study. However, it is highly improbable that the growth response of the papillary muscle differs appreciably from that of the remaining myocardium for the following reasons. Volume load, in general, induces harmonic cardiac hypertrophy with proportionate increase of fiber width and length (Hort 1951; Linzbach 1960; Lin et al. 1977; Hort et al. 1985). Assuming pure cell growth without cell division (Claycomb 1975), myocardial cell length density should, at 20% hypertrophy, decrease to $1.2^{-2/3} = 89\%$ of the control value – this is in close agreement with the observed decrease of myocardial cell length density to 85% observed in the papillary muscles. Moreover, studies on capillarization and ultrastructure in cardiac hypertrophy induced by pressure overload have provided very similar results in samples from the papillary muscles as are known to occur in the remaining left ventricular myocardium (Anversa et al. 1980). Finally, it should be kept in mind that the widely acknowledged concept of muscle fiber growth below and above the "critical heart weight" – introduced in the pioneering investigations of Linzbach (1947, 1960), later confirmed by others (Astorri et al. 1977) – was essentially obtained by morphometric analysis of the papillary muscles. Thus the drawback of a limited sampling universe appears to be more than counterbalanced by the unique opportunity of quantifying 3D capillary geometry.

For the first time anisotropic stereology has shown unambiguously, with exclusion of the possible geometrical biases, that exercise conditioning produces capillary proliferation in left ventricular myocardial hypertrophy of young female rats after repeated physical exercise. The data offer a possible microstructural equivalent to the physiological observation that coronary vasodilator reserve is impaired in hypertrophy induced by pressure overload but not in exercise-induced hypertrophy (Holtz et al. 1977; Laughlin et al. 1978; Mueller et al. 1978; O'Keefe et al. 1978; Spear et al. 1978; Scheuer 1982; Breisch et al. 1984). Coronary vasodilator reserve corresponds to the minimal coronary vascular resistance and is measured at maximum coronary vasodilation. After complete relaxation of the muscular vessels,

capillary resistance will most probably represent a major component of total vascular resistance per unit tissue volume. Under this assumption stereology indeed correctly predicts the different functional behaviour of the microcirculation. From the present data one predicts an unchanged coronary vasodilator reserve in exercise-induced hypertrophy, because length density and mean "true" cross-sectional area of capillaries remain unchanged. Moreover, the reduced capillary density found in many morphometric studies on hypertrophy induced by pressure overload (e.g. Breisch et al. 1984) correctly anticipates a reduced coronary vasodilator reserve. As impaired coronary vasodilator reserve in hypertrophy induced by pressure overload is associated with increased size of experimental acute myocardial infarction (Koyanagi et al. 1982), whereas a diminished size of experimental acute myocardial infarction was observed after physical training (McElroy et al. 1978; Tornling 1982), capillary stereology might be a factor with respect to tolerance of ischaemia-induced myocardial damage.

Myocardial capillary proliferation after physical training has been indicated by enhanced planar capillary-fiber ratios or increased capillary densities in a number of studies using various methods of vascular perfusion (Leon and Bloor 1968; Bloor and Leon 1970; Tomanek 1970; Bell and Rasmussen 1974; McElroy et al. 1978; Anversa et al. 1983). It was shown that incorporation of [^{3}H]-thymidine into capillary wall cells is enhanced after physical training (Ljungqvist and Unge 1977; Tornling 1982). Moreover, a recent study provides evidence that endurance exercise stimulates capillary growth in the face of hypertension and ventricular hypertrophy in spontaneously hypertensive rats (Crisman et al. 1985). In general, proliferation of myocardial capillaries and/or capillary endothelial cells can be observed at a variety of experimental conditions, e.g. chronic hypoxia (Friedman et al. 1973), prolonged application of the powerful coronary vasodilator dipyridamole (Tornling et al. 1978; Tornling 1982; Mattfeldt and Mall 1983), chronic ethanol administration to rats and rabbits (Mall et al. 1980, 1982), and chronic bradycardial pacing (Wright and Hudlicka, 1981). The common denominator of all these studies is an enhancement of myocardial blood flow. As each exercise bout leads to a vigorous increase of myocardial perfusion (e.g. Pannier and Leusen 1977), the present study confirms the well-known hypothesis that increased myocardial blood flow is an important stimulus for capillary neoformation in mammalian myocardian (recent reviews: Hudlicka 1982; Tornling 1982). Recently the effect of different training intensities was compared in a series of studies (Anversa et al. 1982, 1983, 1985; Loud et al. 1984). These studies indicate that capillary proliferation might be limited to mild exercise regimens, whereas strenuous exercise fails to evoke capillary neoformation. This discrepancy could be due to a nonspecific stress reaction in strenuous regimens and/or to an inability of the capillary bed to counterbalancing excessive fiber thickening by compensatory proliferation. Moreover, several lines of evidence suggest that the capacity of capillary neoformation may be limited to young animals (Tomanek 1970; Tornling et al. 1978). Thus age, sex and species, as well as the specific exercise regimen, must be properly considered when evaluat-

ing the capillary proliferative response of the myocardium in training programs.

Muscle fiber growth induced by training was not accompanied by any alterations of the volume densities of myofibrils, mitochondria, and sarcoplasmic matrix within the myocardial cell cytoplasm. Consequently the mitochondria-to-myofibril volume ratio, a frequently used defining characteristic of myocardial cell ultrastructure in investigations on cardiac hypertrophy and insufficiency, remained unchanged. This finding confirms the results of other authors (Bozner and Meessen 1969) and is in agreement with the recent observation that exercise training can normalize the disproportionate growth of these organelles in spontaneously hypertensive rats (Crisman and Tomanek 1985). Training facilitates a proportional growth of energy-supplying and energy-consuming organelles. Thus stereological methods support the concept of "physiological hypertrophy" by documenting both capillary proliferation at the light microscopic level and a completely adaequate growth response of the muscle fiber at the ultrastructural level.

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References

Anversa P, Olivetti G, Melissari M, Loud AV (1980) Stereological measurement of cellular and subcellular hypertrophy and hyperplasia in the papillary muscle of adult rat. J Mol Cell Cardiol 12:781–795

Anversa P, Beghi C, Levicky V, McDonald SL, Kikkawa Y (1982) Mophometry of right ventricular hypertrophy induced by strenuous exercise in rat. Am J Physiol 243:H856–H861

Anversa P, Levicky V, Beghi C, McDonald SL, Kikkawa Y (1983) Morphometry of exercise-induced right ventricular hypertrophy in the rat. Circ Res 52:57–64

Anversa P, Beghi C, Levicky V, McDonald SL, Kikkawa Y, Olivetti G (1985) Effects of strenuous exercise on the quantitative morphology of left ventricular myocardium in the rat. J Mol Cell Cardiol 17:587–595

Astorri E, Bolognesi R, Colla B, Chizzola A, Visioli O (1977) Left ventricular hypertrophy: a cytometric study on 42 human hearts. J Mol Cell Cardiol 9:763–775

Bell RD, Rasmussen RL (1974) Exercise and the myocardial capillary-fiber ratio during growth. Growth 38:237–244

Bloor CM, Leon AS (1970) Interaction of age and exercise on the heart and its blood supply. Lab Invest 22:160–165

Bozner A, Meessen H (1969) Die Feinstruktur des Herzmuskels der Ratte nach einmaligem und nach wiederholtem Schwimmtraining. Virchows Arch B [Cell Pathol] 3:248–269

Breisch EA, White FC, Bloor CM (1984) Myocardial characteristics of pressure overload hypertrophy. A structural and functional study. Lab Invest 51:333–342

Claycomb WC (1975) Biochemical aspects of cardiac muscle differentiation. J Biol Chem 250:3229–3235

Crisman RP, Rittman B, Tomanek RJ (1985) Exercise-induced myocardial capillary growth in the spontaneously hypertensive rat. Microvasc Res 30:185–194

Crisman RP, Tomanek RJ (1985) Exercise training modifies myocardial mitochondria and myofibril growth in spontaneously hypertensive rats. Am J Physiol 248:H8–H14

Cruz-Orive LM, Hoppeler H, Mathieu O, Weibel ER (1985) Stereological analysis of anisotropic structures using directional statistics. Appl Statist 34:14–32

Doerr W (1971) Morphologie der Myokarditis. Verh Dtsch Ges Inn Med 77:301–335

Doerr W, Rossner JA (1977) Toxische Arzneiwirkungen am Herzmuskel. Springer, Berlin Heidelberg New York (Sitzungsberichte der Heidelberger Akademie der Wissenschaften 77/4:175–207

Friedman I, Moravec J, Reichart E, Hatt PY (1973) Subacute myocardial hypoxia in the rat. An electron microscopic study of the left ventricular myocardium. J Mol Cell Cardiol 5:125–132

Gundersen HJG (1977) Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. J Microsc 111:219–223

Holtz J, von Restorff W, Bard P, Bassenge E (1977) Transmural distribution of myocardial blood flow and of coronary reserve in canine left ventricular hypertrophy. Basic Res Cardiol 72:286–292

Hort W (1951) Morphologische und physiologische Untersuchungen an Ratten während eines Lauftrainings und nach dem Training. Virchows Arch 320:197–237

Hort W, Frenzel H, Lange P, Tezuka F (1985) Rückbildung der Herzhypertrophie. *In*: Mall G, Otto HF (eds) Herzhypertrophie, Springer, Berlin pp 43–47

Hudlicka O (1982) Growth of capillaries in skeletal and cardiac muscle. Circ Res 50:451–461

Koyanagi S, Eastham CL, Harrison DG, Marcus ML (1982) Increased size of myocardial infarction in dogs with chronic hypertension and left ventricular hypertrophy. Circ Res 50:55–62

Laughlin MH, Diana JN, Tipton CM (1978) Effects of exercise training on coronary reactive hyperemia and blood flow in the dog. J Appl Physiol 45:604–610

Leon AS, Bloor CM (1968) Effects of exercise and its cessation on the heart and its blood supply. J Appl Physiol 24:485–490

Lin H-L, Katele KV, Grimm AF (1977) Functional morphology of the pressure- and the volume-hypertrophied rat heart. Circ Res 41:830–836

Linzbach AJ (1947) Mikrometrische und histologische Analyse hypertropher menschlicher Herzen. Virchows Arch [Pathol Anat] 314:534–594

Linzbach AJ (1960) Heart failure from the point of view of quantitative anatomy. Am J Cardiol 5:370–382

Ljungqvist A, Unge G (1977) Capillary proliferative activity in myocardium and skeletal muscle of exercised rats. J Appl Physiol 43:306–307

Loud AV, Beghi C, Olivetti G, Anversa P (1984) Morphometry of right and left ventricular myocardium after strenuous exercise in preconditioned rats. Lab Invest 51:104–111

Mall G, Reinhard H, Kayser K, Rossner JA (1978) An effective morphometric method for electron microscopic studies on papillary muscles. Virchows Arch [Pathol Anat] 379:219–228

Mall G, Mattfeldt T, Volk B (1980) Ultrastructural morphometric study on the rat heart after chronic ethanol feeding. Virchows Arch [Pathol Anat] 389:59–77

Mall G, Mattfeldt T, Rieger P, Volk B, Frolov VA (1982) Morphometric analysis of the rabbit myocardium after chronic ethanol feeding – early capillary changes. Basic Res Cardiol 77:57–67

Mathieu O, Cruz-Orive LM, Hoppeler H, Weibel ER (1983) Estimating length density and quantifying anisotropy in skeletal muscle capillaries. J Microsc 131:131–146

Mattfeldt T, Mall G, Volk B (1980) Morphometric analysis of rat heart mitochondria after chronic ethanol treatment. J Mol Cell Cardiol 12:1311–1319

Mattfeldt T, Mall G (1983) Dipyridamole-induced capillary endothelial cell proliferation in the rat heart – a morphometric investigation. Cardiovasc Res 17:229–237

Mattfeldt T, Mall G (1984) Estimation of length and surface of anisotropic capillaries. J Microsc 135:181–190

Mattfeldt T, Möbius H-J, Mall G (1985) Orthogonal triplet probes: an efficient method for unbiased estimation of length and surface of objects with unknown orientation in space. J Microsc 139:279–289

McElroy CL, Gissen SA, Fishbein MC (1978) Exercise-induced reduction in myocardial infarct size after coronary artery occlusion in the rat. Circulation 57:958–962

Merz WA (1967) Die Streckenmessung an gerichteten Strukturen im Mikroskop und ihre Anwendung zur Bestimmung von Oberflächen-Volumen-Relationen im Knochengewebe. Mikroskopie 22:132–142

Mueller TM, Marcus ML, Kerber RE, Young JA, Barnes RW, Abboud FM (1978) Effect of renal hypertension and left ventricular hypertrophy on the coronary circulation in dogs. Circ Res 42:543–549

O'Keefe DD, Hoffman JIE, Cheitlin R, O'Neill MJ, Allard JR, Shapkin E (1978) Coronary blood flow in experimental canine left ventricular hypertrophy. Circ Res 43:43–51

Oscai LB, Molé PA, Holloszy JO (1971) Effects of exercise on cardiac weight and mitochondria in male and female rats. Am J Physiol 220:1944–1948

Pannier JL, Leusen I (1977) Regional blood flow in response to exercise in conscious dogs. Eur J Appl Physiol 36:255–265

Schaible TF, Scheuer J (1981) Cardiac function in hypertrophied hearts from chronically exercised female rats. J Appl Physiol 50:1140–1145

Scheuer J (1982) Effects of physical training on myocardial vascularity and perfusion. Circulation 66:491–495

Scheuer J, Tipton CM (1977) Cardiovascular adaptations to physical training. Ann Rev Physiol 39:221–251

Spear KL, Koerner JE, Terjung RL (1978) Coronary blood flow in physically trained rats. Cardiovasc Res 12:135–143

Tomanek RJ (1970) Effects of age and exercise on the extent of the myocardial capillary bed. Anat Rec 167:55–62

Tornling G, Unge G, Skoog L, Ljungqvist A, Carlsson S, Adolfsson J (1978) Proliferative activity of myocardial capillary wall cells in dipyridamole-treated rats. Cardiovasc Res 12:692–695

Tornling G (1982) Capillary neoformation in the heart and skeletal muscle during dipyridamole-treatment and exercise. Acta Pathol Microbiol Scand A (Suppl) 278:1–63

Van Liere EJ, Krames BB, Northup DW (1965) Differences in cardiac hypertrophy in exercise and in hypoxia. Circ Res 16:244–248

Weibel ER (1980) Stereological Methods. II. Theoretical Foundations. London, Academic Press

Wright AJA, Hudlicka O (1981) Capillary growth and changes in heart performance induced by chronic bradycardial pacing. Circ Res 49:469–478

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