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EFFECTS OF CHRONIC EXERCISE AND AGEING ON REGIONAL ENERGY METABOLISM IN HEART MUSCLE

Heikki Kainulainen

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The effects of chronic running or swimming training and age on the regional distribution of glucose uptake, activities of several glycolytic and mitochondrial enzymes, and regional oxidative capacity were studied in rat hearts. Glucose uptake was measured in Langendorff-perfused hearts or in vivo, and enzyme activities and oxidative capacities were measured from tissue homogenates. Differences of cardiac glucose metabolism between males and females were also studied.

Chronic swimming and running training increase both in vivo and in the isolated perfused heart the subepicardial glucose uptake, which normally is significantly lower than the subendocardial uptake in the hearts of young rats. This redistribution of glucose uptake probably mirrors a similar redistribution of cardiac work load. The distribution of glycolytic enzyme activities is not quite similar to that of glucose uptake, and training does not change these activities as clearly it changes the rate of glucose uptake. The adaptive change of glucose uptake to endurance training was even more clear in female than in male rats: an opposite glucose uptake gradient was found, the subepicardial glucose uptake being higher than the subendocardial uptake. Also, most of the significant changes in enzyme activities were found in the hearts of female rats. Cessation of training restores the adaptive changes in transmural glucose uptake within two weeks.

Glucose uptake measured in vivo increases during rest and exercise as a consequence of training. The increase seems to be independent of the actual work load or supply of major alternative myocardial substrates indicating that physical training increases the preference of glucose as a myocardial energy-yielding substrate.

Regional differences exist in the oxidation capacity of various substrates, the subendocardial capacities tending to be higher than the subepicardial ones. Training enhances oxidation rates of succinate, pyruvate and palmitoylcarnitine in the subendocardium of young rats. On the basis of these results and results concerning glucose uptake, it seems that the myocardium adapts transmurally to chronic exercise by elevating mitochondrial ATP-production in the subendocardium and glycolytic ATP-production in the subepicardium.

Ageing results in abolished gradients in the glucose uptake and in the oxidation rates of substrates, suggesting similar abolishment in the myocardial work load. Training-induced cardiac alterations are absent or small in aged rats.

Key words: Rat heart; chronic exercise; glucose uptake; oxidative capacity; enzyme activities of glycolysis, Krebs cycle, and respiratory chain.

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List of original publications

This thesis is based on the following original articles, which will be referred to by their Roman numerals:

I. Kainulainen, H., Takala, T., Hassinen, I. & Vihko, V. 1985: Redistribution of glucose uptake by chronic exercise, measured in isolated perfused rat hearts. - Pflügers Archiv 403:296-300.

II. Kainulainen, H., Komulainen, J., Takala, T. & Vihko, V. 1987: Regional glucose uptake and protein synthesis in isolated perfused rat hearts immediately after training and later. - Basic Research in Cardiology 82:9-17.

III. Kainulainen, H., Komulainen, J., Takala, T. & Vihko, V. 1989: Effect of chronic exercise on glucose uptake and activities of glycolytic enzymes measured regionally in rat heart. - Basic Research in Cardiology 84:174-190.

IV. Kainulainen, H., Komulainen, J., Takala, T. & Vihko, V. 1989: Transmural distribution of glucose uptake in the left ventricle of aged rats after long-term training. - Medical Science Research 17:373-374.

V. Kainulainen, H., Virtanen, P., Ruskoaho, H. & Takala, T. 1989: Training increases cardiac glucose uptake during rest and exercise in rats. - American Journal of Physiology 257:H839-H845.

VI. Kainulainen, H., Komulainen, J., Leinonen, A., Rusko, H. & Vihko, V. 1990: Regional differences of substrate oxidation capacity in rat hearts: effects of endurance training and hypergravity. - manuscript (submitted).

VII. Kainulainen, H. & Komulainen, J. 1989: Effects of training on regional substrate oxidation in the hearts of ageing rats. - Gerontology 35:289-296.

Abbreviations

ADP = adenosine diphosphate
AMP = adenosine monophosphate
ATP = adenosine triphosphate
ATPase = adenosine triphosphatase
DNA = deoxyribonucleic acid
FFA = free fatty acid
NAD = nicotinamide adenine dinucleotide
NADH = reduced nicotinamide adenine dinucleotide
P_i = inorganic phosphate
RNA = ribonucleic acid

Contents

1. Introduction

Ischemic lesions in the heart are most often localized to the subendocardial region of the left ventricular wall. This vulnerability is suggested to be a consequence of differently distributed mechanical and metabolic activities across the left ventricular wall. There is an increasing amount of evidence that a greater myocardial work load is imposed on the subendocardium than on the subepicardium. The cardiac work load is determined by the wall stress and systolic sarcomere shortening. In general, the empirical data and theoretical models indicate that there is a gradient of stress and systolic movements, a maximum occurring at the endocardial surface, decreasing to a minimum in the epicardial layers. The vulnerability of the subendocardium to ischaemia may also partly be due to the construction of the vascular bed and to the suppression of the capillary circulation in this layer in certain pathological conditions.

Long-term physical conditioning may lead to a lower incidence of death from cardiovascular diseases (Froelicher et al. 1980). Exercise may reduce general coronary risk factors (e.g. serum cholesterol levels and composition), but exercise also enhances cardiac mechanical function and at times leads to a hypertrophic adaptation, i.e. eccentric hypertrophy (Grossman 1980, Wikman-Coffelt et al. 1979). Cardiac adaptation to pathologic pressure overload results in concentric hypertrophy, which is frequently associated with impaired cardiac function. Mostly no adaptation in the oxidative metabolism of cardiac tissue is found after chronic exercise, which has led to an assumption that the myocardium has a sufficient oxidative capacity to meet the increased need for ATP during repeated exercise. This may be an inaccurate assumption, due to the fact that in these measurements the metabolic zonation of the heart has not been considered. In most cases the results have been obtained from the whole heart, from whole ventricles, or from a region not accurately described.

Cardiac glucose metabolism is known to be regionally distributed through the left ventricular wall, the glucose uptake being lowest in the subendocardial layers (L'Abbate et al. 1979, Takala & Hassinen 1981). Effects of training on cardiac glucose

metabolism - especially in connection to regional distribution - is a subject studied hardly at all, although glucose is one of the preferred cardiac fuels.

The purpose of the present work was to study the effect of chronic physical training and age on the regional glucose metabolism and on the regional oxidative capacity in the rat heart. Studies were carried out by measuring regional glucose uptake, regional activities of several enzymes and regional oxidative capacity. Glucose uptake and activities of glycolytic enzymes were compared between males and females. In vitro measurements of glucose uptake were compared with in vivo measurements.

2. Review of literature

2.1. Regional differences of the myocardium

2.1.1. Mechanical work load

The cardiac work load is determined by the wall stress and the systolic sarcomere shortening. Three types of stress - radial, meridional and circumferential - apply to cardiac myocytes (Yin 1981). Several mathematical models have been developed to describe those stresses. Huisman et al. (1980) compared thick-walled models based on linear elasticity suggested by Wong & Rautaharju (1968), Ghista & Sandler (1969), Mirsky (1969) and Huisman et al. (1980). Thick-walled models allow the calculation of wall stress as a function of location. The computed circumferential stress within the left ventricular wall in all these models is highest in the endocardial layers. Calculated meridional stresses exhibit controversial results; three of the models (Wong & Rautaharju 1968, Ghista & Sandler 1969, Huisman et al. 1980) a decrease, and the model of Mirsky (1969) an increase from the endocardium to the epicardium. Circumferential stress is quantitatively the most important of these stresses (Yin 1981). Also, the proportion of fibers oriented circumferentially to those oriented longitudinally is approximately 10:1 (Streeter et al. 1969). Streeter et al. (1970), taking into account fiber orientation within the left ventricular wall, calculated that the peak circumferential stress occurs in the midwall region. Mirsky (1970), considering also the anisotropy and further the effect of heterogeneity of wall material, predicted that maximum circumferential stresses occur within the endocardial layers. In general, the results indicate that there is a gradient of stress, a maximum occurring at the endocardial surface, decreasing to a minimum in the epicardial layers.

The extent of sarcomere shortening during the systole is the other determinant of the mechanical work. Increase in the left ventricular pressure is reflected in a more marked shortening of sarcomeres in the subendocardium than in the outer layers

(Spotnitz et al. 1966). Using ultrasonic crystals LeWinter et al. (1975) showed that the myocardial segment shortening was greater along the midwall of the left ventricle of dogs than in the subepicardium. Also, by measuring the movements of ultrasonic crystals, Sabbah et al. (1981) and Homans et al. (1988) showed that the subendocardium undergoes greater thickening and shortening during systole than does the subepicardium. Greater subendocardial thickening is also observed in canine heart with the use of an epicardial Doppler probe (Bolli et al. 1989).

Mathematical models which take into account the twisting of the heart during contraction show a smaller transmural difference in sarcomere shortening and end-systolic fiber stress than a non-twisting model (Arts et al. 1979). Nevertheless, the subendocardial sarcomeres are subjected to higher strains than the subepicardial sarcomeres (Beyar & Sideman 1986b).

All the above data indicate that the mechanical work load is unevenly distributed across the left ventricular wall, being greater in the subendocardium than in the subepicardium.

2.1.2. Coronary flow, oxygen consumption and metabolic rate

The subendocardium is more susceptible to ischemia than the subepicardium (Allison et al. 1977, Griggs & Chen 1974, Ichihara & Abiko 1977, Levy et al. 1986, Opie 1976). The left ventricular intramyocardial pressure impedes myocardial blood flow during the systole, so that little or no systolic flow occurs in the subendocardium (Archie 1975, Downey et al. 1974, Hess & Bache 1976). The subendocardial to subepicardial flow ratios are usually 1.1 - 1.2 (Hoffman 1978, Hoffman 1987, Hoffman & Buckberg 1978, Holz et al. 1977b, Utley et al. 1974, Yipintsoi et al. 1973). These studies have shown that the subendocardial flow is sufficient to maintain oxygen delivery and aerobic metabolism under basal conditions.

Both tissue pressure and contractile state relate to myocardial oxygen consumption (Graham et al. 1968, Neely et al. 1967, Sarnoff et al. 1958, Sonnenblick et al. 1965). Tissue pressure is higher in the subendocardium than in the subepicardium (Hamlin et al. 1982, Rabbany et al. 1989, Stein et al. 1980) and so is the work load (see chapter 2.1.1). Therefore it is expected that metabolic requirements of the subendocardium exceed those of the subepicardium. A higher oxygen extraction in the inner than in the outer left ventricular layers is suggested because lower oxygen and, in some studies, higher carbon dioxide tensions are found in the subendocardium than in the subepi-

cardium (Flaherty et al. 1978, Gamble et al. 1974, Kirk & Honig 1964, Levy et al. 1986) and also the lowest oxygen saturation is found in the subendocardial capillaries (Holz et al. 1977a, Weiss & Sinha 1978). Monroe et al. (1975) found a similar oxygen saturation gradient in isolated dog hearts, but not in an open-chest model. Similar regionality is found when oxygen saturation of myoglobin (Weiss et al. 1978) and the NAD^+/NADH ratio (Minamidate et al. 1973) are measured.

Dunn & Griggs (1975) calculated the regional metabolic rate using a stop-flow technique to eliminate uneven distribution of blood flow. Their calculations - based on the rate of high-energy phosphate degradation and lactate formation - showed a faster metabolic rate subendocardially than subepicardially. Also Howe et al. (1975) estimated by Krogh analysis, using data on PO_2, hydrogen clearance and intercapillary distance, that the basal endocardial metabolism is 20-30 % higher than the epicardial metabolism. The mathematical model of Beyar & Sideman (1986a), taking into account the transmural distribution of sarcomere lengths, twisting motion of the left ventricle around the long axis, transmural electrical propagation wave and distribution of fiber architecture, shows a transmural metabolic gradient which is characterized by higher values of local oxygen demand in the subendocardial layers than in the subepicardial layers.

2.1.3. Substrate utilization

The myocardium utilizes preferentially free fatty acids, glucose, lactate, ketone bodies and acetate for energy production (Taegtmeyer et al. 1980). There are very few published measurements of regional distribution of cardiac substrate utilization. Camici et al. (1984) found higher pyruvate, palmitoylcarnitine and palmitate oxidation rates in mitochondria isolated from the subendocardium than from the subepicardium of canine heart. Succinate oxidation capacity in mitochondria isolated from the subendocardial layers of horse, bovine and pig hearts exceed that of the subepicardial layers (Tota 1983). Minatoguchi et al. (1984) report no transmural difference in mitochondrial oxidation of succinate in dog heart. Yonekura et al. (1985) did not find any transmural difference in fatty acid incorporation in normal rat heart, studied with the use of a branched chain fatty acid. Uptake of radioiodinated heptadecanoid acid is about 20 % higher in the inner than in the outer left ventricular layers of dog (van der Wall et al. 1983). Myocardial glucose uptake is known to be lower in the subepicardium of the left ventricle than in the subendocardium in some animal species. This was first shown in dogs (L'Abbate et al. 1979) and then in isolated perfused rat hearts (Takala & Hassinen 1981), and later in the hearts of conscious rats (Takala et al.

1983), in hearts of anesthetized and conscious dogs (Breull et al. 1981, L'Abbate et al. 1981), but not in isolated perfused guinea pig hearts (Breull et al. 1981). Also the results of Yonekura et al. (1985) and De Tata et al. (1983, 1986) show higher subendocardial than subepicardial glucose uptake in the resting heart. De Tata et al. (1983) and Matsunami (1982) have reported evenly distributed or higher subepicardial glucose uptake in awake rats and monkeys, respectively. However, these animals were not in a resting state during the measurements. The results obtained by the quantitative deoxyglucose trapping method, developed for myocardium by Takala & Hassinen (1981), show 30 - 60% higher subendocardial than subepicardial glucose uptake (Takala & Hassinen 1981, Takala et al. 1983, Takala et al. 1984a). Up to 30% of the acetyl-CoA oxidized by the tricarboxylic acid cycle is normally derived from glycolysis (Neely & Morgan 1974, Opie 1968). When glucose is the only external substrate available, 70 - 100% of the oxygen consumption can be accounted for by the oxidation of exogenic glucose (Hiltunen & Hassinen 1976, Kobayashi & Neely 1979, Taegtmayer et al. 1980). Addition of lactate, oleate or acetate reduces the total glucose uptake in isolated perfused hearts but does not alter its transmural gradient (Peuhkurinen et al. 1983, Takala et al. 1984b). Acute changes in the cardiac work load such as increased aortic pressure in isolated hearts (Takala et al. 1984b) or acute exercise (Takala et al. 1983) abolish the gradient of glucose uptake. The gradient is also absent during cardiac arrest (Takala & Hassinen 1981) and in spontaneously hypertensive rats (Leipälä et al. 1989). It has been suggested that the disappearance of this gradient is due to the regional changes of cardiac work load in the above situations (Takala et al. 1984a).

2.1.4. Metabolites and enzymes of energy metabolism

As glucose uptake is higher in the subendocardium than in the subepicardium it would be expected that the activities of enzymes participating in glucose metabolism are also higher in the subendocardium. Results concerning this matter are somewhat contradicting. These results are briefly reviewed in Table 1, showing differences in transmural activities in different animal species and contradictions in the activity ratios within species. Nohara et al. (1978) have shown that the heart-type isoenzyme of lactate dehydrogenase (LDH_5) is nearly absent from the atria of canine heart, and that the ratio LDH_5/LDH_4 is greater in the subendocardium than in the subepicardium, which suggests regional metabolic differences. Schultheiss et al. (1981) noted that heart-type isoenzymes increase from the subepicadium to the subendocardium. The most striking result is that the activity of phosphofructokinase, the rate-limiting enzyme of glycolysis, is not elevated in the subendocardium.

Table 1. Summary of comparisons of subendocardial vs. subepicardial activities of enzymes participating in glucose metabolism in the left ventricle.

Reference	Species	Phosphorylase	HK	G6PDH	PFK	Aldolase	GAPDH	PK	LDH
Higuchi et al. 1979[a]	bovine	n.s.		n.s.	↓↓↓	↓	↓		↑
Ichihara & Abiko 1975	dog	>1[b]							
Ichihara & Abiko 1977	dog	n.s.							
Lundsgaard-Hansen et al. 1967	dog		n.s.	↑↑		↓	n.s.	↓	↑↑
Van der Vusse et al. 1990	dog				<1[b]				
Jedeikin 1964	rabbit	>1[b]							
De Tata et al. 1986	rat		n.s.	↓↓	n.s.	n.s.			n.s.
	pig		↓↓	n.s.	↓↓	↓↓			n.s.
	bovine		n.s.	↓↓	n.s.	n.s.			n.s.
De Tata et al. 1983	rat	↓↓							
De Tata et al. 1988a	rat		n.s.	↓↓	n.s.	n.s.			n.s.
De Tata et al. 1988b	rat		n.s.	↓↓ or n.s.[c]	n.s.	n.s.			n.s.
Dowell 1978	rat								n.s.
Kihlström et al. 1989	rat			n.s.					

HK = hexokinase, G6PDH = glucose-6-phosphate dehydrogenase, PFK = phosphofructokinase, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, PK = pyruvate kinase, LDH = lactate dehydrogenase; [a] differences are derived from the activity ratios compared with ratio 1, [b] no figure of probability was given, [c] statistical significance depends on the age of the animals; n.s. = not significant; ↑, ↑↑ $p<0.05$ and <0.01 subendocardial activity being higher; ↓, ↓↓, ↓↓↓ $p<0.05$, <0.01, <0.001 subendocardial activity being lower than the subepicardial activity.

Transmural differences of some metabolites of glucose metabolism are shown in Table 2. In addition to Table 2 Ichihara & Abiko (1982) did not find any transmural differences in concentrations of glucose-1-phosphate, fructose-6-phosphate, dihydroxyacetone phosphate, glyceraldehyde-3-phosphate, 3-phosphoglycerate, 2-phosphoglycerate or phosphoenolpyruvate in normal dog heart. In general, glycogen, glucose6-phosphate and lactate levels tend to be highest in the subendocardium.

Table 2. Summary of comparisons of subendocardial/subepicardial concentration ratios of some metabolites of glucose metabolism in normal hearts.

Reference	Species	Glycogen	Glucose	G6P	Pyruvate	Lactate	L/P
Jedeikin 1964	rabbit	2.3[a]					
	rat	>1[a]					
	dog	>1[a]					
	ox	>1[a]					
Allison & Holsinger 1977	dog	1.23***				1.77***	
Allison & Holsinger 1983	dog	1.21[a]		1.70[a]		2.00[a]	
Allison et al. 1977	dog			1.83**	0.97	1.68**	1.78**
Al Makdessi et al. 1982	dog	n.s.[b]	n.s.[b]			n.s.[b]	
Al Makdessi et al. 1985	dog					n.s.[b]	
Crass et al. 1976	dog	>1*					
Dunn & Griggs 1975	dog					0.96	
Dunn et al. 1978	dog					n.s.[c]	
Dunn et al. 1979	dog					0.99	
Foley et al.1979	dog					0.74	
Griggs & Chen 1974	dog				1.00	1.10	1.10
Griggs et al. 1971	dog			n.s.	n.s	1.09	
Griggs et al. 1972	dog				0.96	1.19	1.24
Griggs et al. 1973	dog					1.06	
Higuchi 1982	dog					1.67[c]	
Ichihara & Abiko 1975	dog	1.3**		1.8**		1.42**	
Ichihara & Abiko 1977	dog	1.30		1.46*		1.07	
Ichihara & Abiko 1982	dog	1.19*		1.63*	1.21	1.43*	1.18
Opie 1976	dog	0.94				1.44	
Pieper et al. 1979	dog	0.94		1.21		1.25	
Todd et al. 1979	dog	1					
De Tata et al. 1983	rat	n.s.					
Takenaka & Higuchi 1974	rat					1.26*	
Takala et al. 1983	rat	n.s.					

G6P = glucose-6-phosphate, L/P = lactate/pyruvate -ratio; [a] no figure of probability was given, [b] no numerical values were given, [c] subendocardial/midwall -ratio ($p<0.05$); n.s. = not significant; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ subendocardium vs. subepicardium.

Transmural differences of high-energy phosphates in diverse studies are presented in Table 3.

Table 3. Transmural differences (subendocardium/subepicardium) of high-energy phosphates in the left ventricle of normal heart.

Reference	Species	CP	ATP	ADP	AMP	Pi
Allison & Holsinger 1983	dog	0.73[b]	0.80[b]	0.93[b]	1.19[b]	
Allison & Holsinger 1977	dog	0.77***	0.81***			
Allison et al. 1977	dog	0.67	0.77***	n.s.[a]	n.s.[a]	
Al Makdessi et al. 1985	dog	0.61	0.93			
Bassenge et al. 1968	dog	0.98	1.00			
Dunn & Griggs 1975	dog	0.93*	1.02			
Dunn et al. 1978	dog	<1*	n.s.[a]			
Dunn et al. 1979	dog	0.88**	0.99			
Foley et al. 1979	dog	0.90	0.97			
Griggs et al. 1972	dog		1.04			
Higuchi 1982	dog	0.91[c]	0.97			1.07
Ichihara & Abiko 1975	dog	0.75*	0.97			
Ichihara & Abiko 1977	dog	0.91[a]	1.20			
Ichihara & Abiko 1982	dog		0.98	0.87	1.00	
Opie 1976	dog	1.01	1.07			0.91
Pieper et al. 1979	dog		0.95**	1.20*	1.35*	
Prinzen et al. 1986	dog	0.9[b]	0.8[b]			
Suzuki et al. 1981	dog		0.92			
Takenaka & Higuchi 1974	rat	0.76**	1.01			1.37***

CP = creatinephosphate; [a] no numerical value was given; [b] no figure of probability was given; [c] ratios of subendocardium/midwall were compared ($p<0.01$); * $p<0.05$, ** $p<0.01$, *** $p<0.001$ subendocardium vs. subepicardium.

In conclusion to Table 3, phosphocreatine stores are lower in the subendocardium and small or no differences are found in the concentrations of ATP, ADP, AMP or Pi. Creatine phosphokinase activity is the same in the subendocardium and in the subepicardium of bovine heart (Higuchi et al. 1979). High-energy phosphate levels do not necessarily reflect differences in energy production and consumption, since turnover rate of ATP may be different in different parts of the heart.

Out of the enzymes of citric acid cycle, citrate synthase is often used as a marker of mitochondrial capacity of tissue. Its activity is lowest in the atria, intermediate in the right ventricle and highest in the left ventricle of the human heart (Lin et al. 1988). Citrate synthase activity in the subendocardium does not differ from that of the subepicardium in human heart (Lin et al. 1988). In rats, no transmural difference (De Tata et al. 1988a) or lower subendocardial activity (Dowell 1978) of citrate synthase activity is found. There are also no transmural differences in citrate concentrations of

dog hearts (Ichihara & Abiko 1982). There are similar contradicting results concerning transmural isocitrate dehydrogenase activity: Lundsgaard-Hansen et al. (1967) did not find any difference in dog heart, but Camici et al. (1984) found higher subendocardial activity. In cows, the activity of succinate - cytochrome c reductase system, as well as the copper concentration, tends to be lower in the subendocardium than in the subepicardium (Tota 1973). No transmural differences are found in the absolute activities of malate dehydrogenase, succinate dehydrogenase, glutamate oxaloacetate transaminase, acyl CoA dehydrogenase, L-3-hydroxyacyl CoA dehydrogenase or β-hydroxybutyrate dehydrogenase (Camici et al. 1984, De Tata et al. 1988a, Higuchi et al. 1979, Lundsgaard-Hansen et al. 1967). Neither is there any transmural difference in the contents of coenzyme Q_{10} in human heart (Lin et al. 1988), nor cytochrome $c + c_1$ or $a + a_3$ in dog heart (Camici et al. 1984). However, as mentioned earlier, canine subendocardial mitochondria have higher respiratory activities than subepicardial mitochondria (Camici et al. 1984). This may be related to different qualitative properties of mitochondia in the inner and outer layers of the left ventricle (Whitty et al. 1978).

Free fatty acid concentrations are a little higher in the subendocardium than in the subepicardium of dog heart (Suzuki et al. 1981), or no difference is found (Al Makdessi et al. 1985, Prinzen et al. 1984, van der Vusse et al. 1982). Triglyceride content of the subepicardium is about 4-fold higher than that of subendocardium in dog heart (Crass et al. 1976). Carnitine content is higher in the subepicardium (Suzuki et al. 1981).

Most of the produced ATP in the myocardium is used for muscle contraction. Myosin possesses this ATPase activity. The myosin molecule is composed of two heavy and several light chains. Two different heavy chains - α and β - are found in most mammalian hearts (Chizzonite et al. 1982, Lompre et al. 1981, Mercadier et al. 1981). Heavy chains form αα-, αβ-, and ββ-dimers, called the respective isoenzymes V_1, V_2 and V_3 (Hoh et al. 1978). Myosin isoenzymic distribution correlates with the speed of myocardial contraction (Schwartz et al. 1981). V_1 isoenzyme has a higher specific ATPase activity (Pope et al. 1980, Schwartz et al. 1983) but is thermodynamically probably less efficient than V_3 (Holubarsch et al. 1985). Schwartz et al. (1980) and Eisenberg et al. (1985) report no transmural differences in myosin isoenzyme pattern in rat and young rabbit, respectively, but in adult rabbit V_1 dominates in the subepicardium. Similarly, Litten et al. (1985) and Leipälä et al. (1988) using electrophoresis, and Sartore et al. (1981) using an immunofluorescence procedure, showed a higher amount of V_1 in the subepicardium than in the subendocardium. Gorza et al. (1981), using immunofluorescence, showed a higher portion of V_3 in the

subendocardium than in the subepicardium. In general, these results indicate a higher myosin ATPase activity in the outer than in the inner layers of the left ventricle.

2.1.5. Other regional features

Protein concentration (Dowell 1977), tissue water content (Griggs & Chen 1974) and protein synthesis (Takala 1981) are evenly distributed across the left ventricle. There are also no transmural differences in DNA or RNA contents (Dowell 1977). Several authors have noted regional differences in myocyte sizes in adult rat heart. Right ventricular myocytes are smaller than the left ventricular myocytes (Bishop et al. 1979, Campbell et al. 1987, Gerdes et al. 1986). Subendocardial myocytes of the left ventricle are larger than subepicardial ones, as has been determined by several different techniques (Campbell et al. 1987, Gerdes et al. 1979, Gerdes et al. 1986, Vliegen et al. 1987, White et al. 1988), although in some studies no statistical differences have been found in rats (Bishop et al. 1979, Loud et al. 1978, Tomanek 1979) or in swine (White et al. 1987). The volume density of myocytes in the subendocardium is higher than in the subepicardium, and correspondingly the interstitial space is smaller (Anversa et al. 1978). Each species is characterized by a typical quantitative composition of the myocardium (Schaper et al. 1985). However, mitochondrial and myofibrillar volume densities do not differ between the transmural layers of the left ventricle of rat (Anversa et al. 1978, Tomanek 1979, Tomanek et al. 1979), cat (Breisch et al. 1984), dog (Schaper et al. 1985) or swine (Singh et al. 1981, White et al. 1987). However, there seem to be two different populations of mitochondria. Whitty et al. (1978) observed a faster sedimentation rate in the mitochondrial population predominating in the subendocardium than in the subepicardium of dog heart, and electron microscopic findings supported this result.

Collagen was previously thought to be more abundant in the subepicardium than in the subendocardium (Buccino et al. 1969, Caspari et al. 1975), but when the epicardium, consisting mainly of collagenous tissue, is removed, no transmural differences are detectable (Bonnin et al. 1981). In the rat left ventricle no transmural differences are found in collagen content with or without the removal of the epicardium (Dowell 1977, Leipälä et al. 1988, Medugorac 1980a, Medugorac 1980b).

Morphometric techniques are mostly used in measurements of the capillary distribution of the heart. Capillary volume density is higher in the left ventricle than in the right ventricle of Wistar-Kyoto rats (Loud et al. 1984). In these same rats the subepicardial capillaries are almost twice as large as the subendocardial capillaries, but there

is no such difference in the number of capillaries per number of myocytes, or in the intercapillary distance (Anversa et al. 1978, 1984). Lund & Tomanek (1978) and Odek-Ogunde (1982) did not observe such a difference in the capillary density of Wistar-Kyoto rats, and neither was there any difference in capillary diameters (Lund & Tomanek 1978). In Sprague-Dawley rats Gerdes et al. (1979) found nearly 40 % more capillaries in the subendocardium than in the subepicardium, but their diameter was approximately 25 % smaller, respectively. Contradicting results are reported by White et al. (1988), who found greater capillary density in the subepicardial than in the subendocardial region. Also in swine (White et al. 1987), the numerical density of capillaries is greater in the subepicardium. No transmural differences in the myocardial capillary densities of cats (Breisch et al. 1980) or young rabbits (Tasgal & Williams 1981) are found. In the rat heart, alkaline phosphatase is histochemically localized primarily in coronary vessels and capillary endothelial cells (Bourne 1954). Using a biochemical approach, Dowell (1977) found higher alkaline phosphatase activity in the subendocardium than in the subepicardium of Sprague-Dawley rats, suggesting a similar distribution of vascular density. However, such difference in alkaline phosphatase activity in Sprague-Dawley was not observed in a recent study (Leipälä et al. 1988). The above results give a confusing view of the regional distribution and size of capillaries, indicating that these are very much dependent on the species or strain of the experimental animals in question.

2.2. Adaptation of the heart to chronic exercise

Chronic exercise results in well-known changes in the cardiovascular system. The most noticeable changes are increased maximal oxygen uptake, a decrease in resting heart rate, an increase in resting stroke volume and cardiac output, a reduced heart rate at submaximal work load, and - less frequently - an increase of the cardiac size (for refs. see the reviews by Blomqvist & Saltin 1983 and by Scheuer & Tipton 1977). The effects of physical training are most extensively studied in man. However, the effect of training on the morphology and biochemical processes of the heart are easier to study in experimental animals. In this chapter the training effects concerning mainly animals are reviewed.

2.2.1. Effects of chronic exercise on heart size and function

The heart may grow by increasing the number of cells or by increasing the size of the cells. Hyperplasia of myocytes is restricted to the prenatal period and to the first weeks after birth (Grossman 1980). Later the myocytes grow in size until they reach their normal size in adulthood (Zak 1973). Under increased work load, the myocytes may grow larger than normally (Wikman-Coffelt et al. 1979). When the heart becomes hypertrophied, endothelial cells and connective tissue cells do not grow, but they proliferate (Oparil 1985). Ceasing the stimulus evoking cardiac hypertrophy is normally reflected as the restitution of cardiac size (Goss 1971), also after an intensive training period (Frenzel et al. 1988). Collagen content is normal or decreased after chronic training (Hickson et al. 1979, Kainulainen et al. 1982, Kainulainen et al. 1983, Kiiskinen & Heikkinen 1976, Masumura et al. 1983, Medugorac 1980a, Medugorac 1980b). Increased cardiac prolyl 4-hydroxylase activity found in running trained mice without a change in the collagen content may indicate an accelerated turnover of collagen (Kainulainen et al. 1983). Possible changes in the proportional amounts of different collagen types after training are not known.

The mechanism increasing work load during endurance-type training is increased cardiac output, i.e. the possibly-resulting hypertrophy is predominantly of a volume overload type (Hickson et al. 1979). Physical training induces cardiac hypertrophy in most dogs (Barnard et al. 1980, Carew & Covell 1978, Reidhammer et al. 1976) but not in beagle dogs (Cohen et al. 1978, Scheel et al. 1981). Training-induced hypertrophy is found in cats (Muntz et al. 1981), in mice (Kainulainen et al. 1982, Kainulainen et al. 1984), in pigs (White et al. 1987) and also in man (Cox et al. 1986, DeMaria et al. 1978). Most studies in male rats have shown that training by running leads to an increase in the heart:body weight ratio, but no increase in the absolute weight of the heart is observed (Harpur 1980). Some studies show a slight increase in the cardiac mass of male rats after swimming (Harpur 1980). In female rats swimming almost always leads to cardiac hypertrophy, but running training only rarely (Harpur 1980). In male rats trained by running, cardiac mass, RNA content, and myocardial fiber size are not different from controls (Dowell et al. 1976b, Nutter et al. 1981) but in female rats myocyte size increases (Frenzel et al. 1988). In female rats cardiac mass and protein content increase during swimming training for 14 days (Hickson et al. 1979). In male mice the development of cardiac hypertrophy results after 10-25 training bouts (1-2 h/day) if the running speed is over 20 m/min, but not at a lower speed (Kainulainen & Vihko 1983, Kainulainen et al. 1982, Kainulainen et al. 1984). The above results show that exercise-induced hypertrophy is dependent on such factors

as type and intensity of training, duration of training, and species and sex of the exercising animal.

Running training in dogs and running or swimming training in rats show many of the common training effects, including bradycardia at rest and at submaximal work load, increased heart rate in maximal work load, and an increase in skeletal muscle oxidative capacity (for refs. see Schaible & Scheuer 1985). Exercise training seems to increase the inotropic state of the heart. Increased left ventricular *dP/dt* of the dog heart is observed during increased heart rate (exercise- or pacing-induced) (Barnard et al. 1980, Rizer et al. 1980, Stone 1977), but in pigs no such improvement is found (White et al. 1987). Trained dogs demonstrate improved pumping ability at similar heart rates compared to controls (Bove et al. 1979). Cardiac mechanisms studied in isolated papillary muscles and trabeculae have produced contradicting results. Several studies have reported increases in isometric tension development and/or isotonic shortening velocity in trained animals with or without cardiac hypertrophy (Molé 1978, Steil et al. 1975, Tibbets et al. 1978, Tibbets et al. 1981). Other studies have reported no change (Grimm et al. 1963) or decrease in cardiac mechanics (Nutter et al. 1981). In isolated perfused working heart, male training rats show increased pumping ability due to enhanced myocardial contractile performance at high left atrial pressures (Bersohn & Scheuer 1977, Giusti et al. 1978, Schaible & Scheuer 1979). This mechanical adaptation is not observed after two weeks of cessation of the training (Giusti et al. 1978). Swimming training enhances the ventricular relaxation rate, but this is not obvious after running training (Schaible & Scheuer 1979, Bersohn & Scheuer 1977). Similar adaptations are found in swimming-trained female rats (Schaible & Scheuer 1981). When rats of both sexes and of same age are trained by running, improved cardiac function is observed only in male rats (Schaible et al. 1981). Swimming training, but not running training, leads to elevated myocardial tissue concentrations of epinephrine and norepinephrine in female rats, which may contribute to the diverse cardiac adaptations after these different training types (Geenen et al. 1988). Results obtained from open-chested rats (heart function studied *in situ*) also show improved cardiac performance after training (Codini et al. 1977, Cutilletta et al. 1979, Dowell et al. 1976a, Fuller & Nutter 1981, Pfeffer et al. 1978). Training elevates sarcolemmal Ca^{2+} pump activity (Pierce et al. 1989) and sarcoplasmic Ca^{2+} transport (Levine & Kinasewitz 1986, Penpargkul et al. 1977), suggesting faster relaxation in the heart. The above results indicate - in general - an increased training-induced contractile performance in experimental animals. Increased cardiac performance can be observed with or without cardiac hypertrophy.

2.2.2. Effects of chronic exercise on cardiac vasculature and flow

Most animal studies show an increase of myocardial vasculature as an adaptation to training (for refs. see Cohen 1983 and Scheuer 1982). In male and female rats, this has been shown as increased weight of coronary vinyl acetate casts (Stevenson et al. 1964, Tepperman & Pearlman 1961), and as increased capillary:muscle fiber ratios (Leon & Bloor 1968, Tomanek 1970). Training also increases the diameter of large vessels in rats (Leon & Bloor 1968) and in dogs (Wyatt & Mitchell 1978). In one study no increase in the relative number of capillaries was found after long-term swimming in rats, but the surface density of capillaries increased at a constant rate, reaching a maximum of 17 % after 180 hours of swimming (Guski 1980). Training tends to increase capillary density more in young rats than in old ones (Bloor & Leon 1970, Tomanek 1970). New growth of capillaries is also suggested after swimming training in rats, based on the autoradiographic evidence of increased ^{3}H-thymidine incorporation to the nuclei of capillary endothelial cells (Ljungqvist & Unge 1973, Mandache et al. 1973). White et al. (1987, 1988) observed that the training-induced increase of capillary density is localized to the subendocardial region. Increased capillary density tends to remain a longer period than other training effect in heart (Leon & Bloor 1976, Tepperman & Pearlman 1961, Tomanek 1970). In the right ventricle, moderate running training results in an increase in the numerical density, luminal surface and total length of capillaries, whereas strenuous exercise has the opposite effect (Anversa et al. 1982, Anversa et al. 1987).

Hearts from trained rats have greater coronary flows, as measured in an isolated working heart model during maximal coronary vasodilatation (Bersohn & Scheuer 1977, Penpargkul & Scheuer 1970, Schaible & Scheuer 1979). During partial vasodilatation increased coronary flow is absent or less profound (Spear et al. 1978, Yipintsoi et al. 1980). Running training in pigs increases the subepicardial blood flow during exercise, but not during rest, the endocardial-to-epicardial flow ratios being 0.82 and 1.20, respectively (Breisch et al. 1986).

2.2.3. Metabolic and subcellular adaptation in response to chronic exercise

2.2.3.1. Energy production in relation to chronic exercise

ATP for the cardiac function and metabolism is mainly derived from the preferred substrates utilized by the heart: free fatty acids, glucose, lactate, ketone bodies and acetate (Taegtmayer et al. 1980). When the nutritional status is normal and especially

after eating, cardiac free fatty acid oxidation is low or absent (for refs. see Opie 1980). When the normal dog heart is presented with a choice of substrates (free fatty acids, glucose, lactate), lactate is the preferred substrate for energy production (Drake et al. 1980). During exercise lactate becomes the most important cardiac fuel (Keul et al. 1965, Keul et al. 1966). One study shows that triglycerides can account for 14 % of the basal oxygen uptake of the human heart (Lassers et al. 1972). Cardiac use of these substrates is greatly dependent on the plasma concentrations of the substrates. The hormonal status also has an influence on the substrate utilization, e.g. insulin enhances glucose utilization (Neely & Morgan 1974).

Acute exercise depletes cardiac glycogen stores (Segel et al. 1975). Training causes supercompensation of glycogen (Lamb et al. 1969, Scheuer et al. 1970, Segel et al. 1975) but this phenomenon is not always observed (York et al. 1975). The hearts of trained animals have reduced levels of triglycerides, and an increased turnover of free fatty acids (Fröberg 1970, Scheuer et al. 1970).

There are very few studies on the effect of training on the rate of glycolysis in the heart. No changes in phosphofructokinase (York et al. 1975) or aldolase (Hearn & Wainio 1957) activities have been found, but the activities of pyruvate kinase (York et al. 1975) and lactate dehydrogenase (Gollnick et al. 1967, Koehler & Medugorac 1980, York et al. 1975) were increased, and changes in lactate dehydrogenase isoenzyme composition were obvious (Ji et al. 1986, Koehler & Medugorac 1980, York et al. 1975). Histochemical studies have shown that regional changes do not occur in lactate dehydrogenase activity (Koehler & Medugorac 1980). Training does not alter the total utilization of glucose in arrested perfused rat hearts (Scheuer et al. 1973).

There is a large bulk of studies which show no training-induced changes in the mitochondrial function of the heart. No elevation in the cardiac activities of cytochrome oxidase, succinate dehydrogenase, citrate synthase or malate dehydrogenase has been observed in rats after swimming or running (Baldwin et al. 1977, Dohm et al. 1972, Gollnick & Ianuzzo 1972, Hearn & Wainio 1956, Hickson et al. 1983, Oscai et al. 1971a, Oscai et al. 1971b, Paniagua et al. 1977, Penpargkul et al. 1978, Scheuer et al. 1974, Walburger & Anger 1970) or in mice (Kainulainen & Vihko 1983, Kainulainen et al. 1984). Walburger & Anger (1970) did not find changes in the activities of glyceraldehyde-3-phosphate dehydrogenase, glycerol-1-phosphate dehydrogenase, creatine kinase, glutamate-oxaloacetate transaminase, mitochondrial isocitrate dehydrogenase, adenylate kinase, 3-hydroxyacyl-CoA dehydrogenase or glutamate dehydrogenase. In some studies no increase has been found in mitochondrial protein concentration (e.g. Baldwin et al. 1977, Oscai et al. 1971a, Oscai et al. 1971b,

Paniagua et al. 1977) or in cytochrome c concentration (Hickson et al. 1979, Oscai et al. 1971a, Oscai et al. 1971b). Running training had no effect on the stereological measures of mitochondria in the apex of mouse heart (Kainulainen et al. 1979) or in rat papillary muscle (Mall et al. 1986). Myoglobin concentration does not change or slightly decreases in mice subjected to different running programs (Kainulainen et al. 1982). In rats, swimming training does not change myoglobin concentration (Hickson et al. 1983). The respiratory function of cardiac mitochondria shows no change in trained dogs (Sordahl et al. 1977) or rats (Arcos et al. 1968) or even a decrease in state 3 oxygen consumption in hypertrophied hearts of female rats with an increase in total mitochodrial protein (Penpargkul et al. 1978). Results like the above have led to a hypothetical conclusion that the heart muscle cells have sufficient pre-existing oxidative capacity to meet the energy demands associated with exercise (Schaible & Scheuer 1985). However, there is a growing amount of evidence showing increased mitochondrial metabolism in response to exercise training. The activity of succinate dehydrogenase increased in mouse heart after running training (Hamilton & Ferguson 1972). Elevated activities of succinate dehydrogenase and glycerolphosphate dehydrogenase (Kraus & Kirsten 1970) and adenylate kinase (Walburger & Anger 1970), as well as increased concentrations of cytochromes, coenzyme Q and mitochodrial protein (Beyer et al. 1984, Kraus & Kirsten 1970, Penpargkul et al. 1978), are found in the hearts of trained rats. Electron microscopic studies have shown increases in the number of mitochondria (Aldinger & Sohal 1970, Guski et al. 1981) and in the volume density of mitochondria (Arcos et al. 1968, Guski et al. 1981, Oscai et al. 1971a, Oscai et al. 1971b, Peterson 1972) in response to training. Examinations of the size distribution of myocardial mitochondria of rats after running or swimming training show an increase in the proportion of small mitochondria (Edington & Cosmas 1972, Guski et al. 1980, Guski et al. 1981), probably due to mitochondrial divisions (Banister et al. 1971). These results indicate increased oxidative function and biogenesis of mitochondria induced by chronic exercise.

2.2.3.2. Energy consumption in relation to chronic exercise

Cardiac contraction uses most of the produced ATP. Increased training-induced contractility is reflected on the subcellular level as enhanced myosin ATPase activity and as changed myosin isoenzyme patterns. Wilkerson & Evonuk (1971) and Bhan & Scheuer (1972) have reported increased myosin and actomyosin ATPase activities in rat hearts after 6-10 weeks of swimming training. Later, similar findings have been reported with or without cardiac hypertrophy (Bhan et al. 1975, Malhotra et al. 1976, Malhotra et al. 1981). Baldwin et al. (1975) did not observe activity changes after 18-

24 weeks' even-speed, moderately heavy running training, but in their later study (Baldwin et al. 1977) a 15 % increase in myofibrillar ATPase activity together with heart enlargement after interval-type running training was observed. The results of Penpargkul et al. (1980) are different. Even-speed running increased actomyosin activity by 10 %, whereas running training at an increased speed did not affect the activity. In dog hearts running training does not increase ATPase activity (Cohen et al. 1978, Dowell et al. 1977). Kainulainen et al. (1984) did not observe any change in the actomyosin ATPase activity in mice subjected to running training, but this is probably due to the fact that mouse ventricular myosin is almost completely of the type V_1 (Lompre et al. 1981). In connection with swimming training, the ATPase activities are generally increased, whereas the results of running training are more conflicting.

Changes in the contractile protein ATPase activity originate in changes in the structure and biochemical properties of myosin molecules, and as a result the amounts and proportions of myosin isoenzymes V_1 and V_3 are changed (Pope et al. 1980). Swimming training leads to a shift towards V_1 in the isoenzymic profile (Jacob et al. 1983, Mercadier et al. 1981, Pagani & Solaro 1983, Rupp 1981, Rupp & Jacob 1982, Scheuer et al. 1982, Schwartz et al. 1983) and therefore to increased ATPase activity.

The diameter of cardiac fibers changes in running trained rats (Dowell et al. 1976b), but after swimming training an increase is observed (Bozner & Meessen 1969).

Increased myofibrillar ATPase activity, changes in myosin isoenzyme profile and growth of the cardiac fibers indicate that an enhanced pumping ability of the heart is clearly reflected on the subcellular level after swimming training, but some inconsistency remains concerning running training.

2.2.4. Factors modulating training effect

2.2.4.1. Sex

When studied in normal perfused heart, intrinsic cardiac function is moderately greater in male rats compared to age or heart weight matched female rats (Schaible & Scheuer 1984). Capasso et al. (1983) have presented opposite results when the cardiac contractility was studied in isolated papillary muscles. Hearts of female rats exhibit 10 to 12 % greater actomyosin ATPase activity than those of male rats (Malhotra et al. 1981, Penpargkul et al. 1980, Scheuer et al. 1982). Sex as a cofactor of training has not

been extensively studied in experimental animals, but cardiac adaptation to training seems to be somewhat different in females than in males. As discussed earlier, in female rats a true cardiac hypertrophy develops, while in male rats this is less common (Harpur 1980). Both sexes show increased heart:body weight ratio, but in males this is connected more to lower body weights after training than to increased heart weight. Also in female rats cardiac enlargement is more common after swimming than running training (Harpur 1980). Male swimming rats without cardiac hypertrophy and female rats with cardiac hypertrophy exhibit enhanced cardiac pumping ability (Schaible & Scheuer 1979, Schaible & Scheuer 1981). However, male and female rats of the same age subjected to the same running program show different adaptations: in males improved function was observed, but in females no improvement was observed (Schaible et al. 1981). Increases in respiratory function seem to be more common in female rats, while improvements in myofibrillar ATPase activities are present in the hearts of both sexes (Bhan & Scheuer 1972, Malhotra et al. 1981). It is not known which factors regulate the seemingly divergent sex-related adaptation to chronic exercise. However, the heart is a target organ for gonadal hormones (Krieg et al. 1978, Stumpf et al. 1977). Androgen receptors have been demonstrated in rat ventricular muscle (Krieg et al. 1978), but estrogen receptors have only been found in rat atrial, not in ventricular myocardium (Stumpf et al. 1977). Moreover, gonadectomy has a larger effect on cardiac function and myosin ATPase activity in male than in female rats (Scheuer et al. 1982). These results indicate that sex hormones, especially androgens, may have influence on the sex-related adaptation to training.

2.2.4.2. Age

Myocardial work capacity (Lakatta & Yin 1982) and oxidative metabolism (Chen et al. 1972, Hansford 1978, Hansford & Castro 1982) deteriorate in ageing animals. Many of the enzymes involved in fatty acid oxidation (Chen et al. 1972, Hansford 1978), the tricarboxylate cycle (Hansford 1978) and oxidative phosphorylation (Abu-Erreisch & Sanadi 1978) show decreased activity in the ageing heart. Concentrations of coenzyme Q (Beyer et al. 1985) and cytochromes decrease by age (Abu-Erreisch & Sanadi 1978, Gold et al. 1968). However, the results of Gold et al. (1968) suggest that in spite of slightly decreased cytochrome oxidase concentration and P/O ratio, there is no decrease in the functional integrity of rat heart mitochondria accompanying senescence, when β-hydroxybutyrate is used as a substrate. Also Manzelman & Harmon (1987) found no decrease in the mitochondrial respiratory properties of aged rat hearts. There is some evidence that glycolytic flux increases in the ageing heart (Abu-Erreisch et al. 1977, Angelova-Gateva 1969, Frolkis & Bogatskaya 1968). Activities of

phosphofructokinase and lactate dehydrogenase show increased activity in the subepicardial layers and decreased aldolase activity in the subendocardial layers of ageing rat hearts (De Tata et al. 1988b). In aged rodents isometric force and rate of force development are preserved, but contraction and relaxation times are prolonged (for refs. see Lakatta 1987). A major determinant of contraction duration is the time course of Ca^{2+}-myofilament interaction. The duration of the myoplasmic Ca^{2+}-transport, measured as a time course of the luminecence of aequorin, is prolonged in old cardiac muscle (Orchard & Lakatta 1985) as well as the rate at which the sarcoplasmic reticulum pumps Ca^{2+} is diminished in the hearts of senescent animals (Froehlich et al. 1978, Narayanan 1981). Also, the transmembrane action potential from right and left ventricles of senescent rats is markedly prolonged compared with that of muscle from young controls (Capasso et al. 1983, Jullien & Verdetti 1988, Jullien et al. 1989, Wei et al. 1984). ATP hydrolysis of contractile proteins tend to decrease in ageing hearts (Bhatnagar et al. 1985, Capasso et al. 1983, Rockstein et al. 1981). Accordingly, the proportion of myosin isoenzyme V_3 progressively increases from under 20 % in early adulthood to over 80 % at the age of 24 months in Wistar rats (Mercadier et al. 1981). The greater proportion of V_3 in ageing heart is accompanied by a reduction in the isotonic shortening velocity (Capasso et al. 1982). Ageing also diminishes the capacity of the heart to adapt to a volume overload (Isoyama et al. 1988).

Starnes et al. (1983) have shown that exercise training improves both work capacity - indicated as increased peak systolic pressure and cardiac output under high work load - and substrate oxidation in hearts of over two-year-old male rats, although not to the level of young sedentary rats. Long-term moderate daily running training prevents characteristic age-related prolongation in contraction duration in 24-month-old rats (Spurgeon et al. 1983) and also enhances isometric twitch parameters of right ventricular papillary muscles (Li et al. 1986). Lifelong mild regular exercise inhibits the age-related decrease of contractile protein ATPase activity in male Fischer rats (Rockstein et al. 1981), but in the study of Farrar et al. (1988) no such improvement after long-term training was found in ATPase activity or in the myosin isoenzyme pattern. In a recent study, Starnes & Rumsey (1988) found improved cardiac performance after training in 24-30-months-old Fischer rats, but not ageing- or training+ageing-induced alterations in the activities of several key enzymes of energy metabolism (phosphofructokinase, citrate synthase, isocitrate dehydrogenase, 3-hydroxyacyl CoA dehydrogenase) or in the content of cytochromes. Only an age-related increase in hexokinase activity was found at the age of 24 months. In several studies no adaptation or even negative adaptation to training has been found in old animals concerning

cardiac blood flow (Bloor & Leon 1970), capillary proliferation (Unge et al. 1979), or various enzyme activities (Chesky et al. 1983, Reznik et al. 1982).

3. Purpose of the present study

Regional differences exist in myocardial parameters such as wall stress, work load, capillary coronary circulation and glucose metabolism. The aim of this thesis was to study the effect of chronic physical activity on the regional energy metabolism in the myocardium of young and aged rats. The specific problems were:

1) to determine the effect of chronic physical training and age on the regional glucose metabolism, referred to as the regional glucose uptake, and the activities of several glycolytic enzymes (I - V);

2) to find out whether there are any differences in the above parameters between male and female rats (III);

3) to study whether the cessation of training results in de-adaptive changes in the myocardial glucose uptake (II);

4) to investigate if glucose uptake after training is different if measured in vitro or in vivo (I, V);

5) to study the effects of different levels of training or age on the regional oxidative capacity of the heart, referred to as oxidation of energy-yielding substrates (VI, VII), and as the activity of some enzymes involved in the oxidative metabolism (III, VI, VII).

4. Materials and methods

4.1. Animals

Male Sprague-Dawley (I,II,III,V) and Wistar (IV,VI,VII) rats or female Sprague-Dawley rats (III), aged 2 months at the beginning of the experiments, were used. The effects of ageing were studied with male Wistar rats, aged 23 months at the beginning of training (IV,VII). The rats were housed under standard cage conditions (room temperature, 12 h/12 h light/dark rhythm) and fed on pelleted rodent chow and tap water ad libitum.

4.2. Training protocols

Swimming or running were used for physical conditioning of the rats. Young rats were trained for 7 - 13 weeks (I-III, V, VI) and old rats for 18 weeks (IV, VII). Training protocols are summarized in Table 4.

4.3. Heart perfusions in vitro

For the glucose uptake measurements in studies I-IV the hearts were perfused using a retrograde aortic perfusion system (Langendorff 1895) with or without recirculation. The perfusion apparatus was constructed as described by Takala (1981). The perfusion fluid reservoirs, connecting glass tubes and heart compartment were thermostated at +37^{o}C. Perfusion pressure was 7.85 kPa (80 cm of water).

Table 4. Training protocols used in the experiments.

Study	Sex	Type of training	Duration in weeks	h/day	Days/ week	Speed m/min	Inclination degrees	Water T °C	Extra load % of BW	Accustoming period (days)
I	male	swimming	8	4	5	-	-	32	-	12
	male	running	9	1	5	28-30	6	-	-	20
II	male	running	10	1	5	36	6	-	-	15
III	male	swimming	8-9	4	5	-	-	32	-	5
	female	swimming	8-9	4	5	-	-	32	-	5
IV,VII	male (aged)	running	20	1	5	20-21	5.5	-	-	15
V	male	swimming	7-13	4	5	-	-	32	-	5
VI	male	running	8	1	5	35	6	-	-	10
	male	running	8	1	5	18-32	6	-	20	10

T = temperature, BW = body weight

The rats were anesthetized with ether and injected with 250 IU heparin (Medica Ltd., Finland) intravenously before excision of the heart. The hearts were perfused with Krebs-Ringer bicarbonate buffer (Krebs & Henseleit 1932) containing 2.5 mM Ca^{2+}, 5 mM glucose, and 10 IU insulin (Insulin Velosulin, Nordisk Gentofte, Denmark) per litre in equilibrium with O_2/CO_2 (19:1). Each heart was preperfused for 10 min without recirculation and paced to a rate of 4.33 Hz (260 beats/min). The perfusion was then switched to recirculation for 30 min, using a medium (50 ml) also containing 3 nM 2-deoxy-D-(^{3}H)glucose (Amersham International Ltd., UK). The heart was then perfused for 10 min without recirculation with the same medium as used for the preperfusion in order to wash any free deoxyglucose out from the tissue (Takala & Hassinen 1981). In studies I and II the recirculating medium also contained ^{14}C-phenylalanine and other amino acids for the measurements of protein synthesis.

In study III some of the hearts and in studies VI and VII all hearts were perfused shortly for 5 min to wash out all blood. These hearts were used for the determination of enzyme activities and substrate oxidation as described later.

4.4. In vivo experiments

To measure the myocardial glucose uptake in vivo (V), catheters for continuous deoxyglucose infusion and simultaneous blood sampling were implanted as described by Takala et al. (1983). The catheters were placed in the aorta and vena cava through the right femoral arteria and vein, respectively. The outer ends of the catheters were passed subcutaneously to the back of the rat's neck, threaded through a thin wire spring anchored to a collar around the neck, and connected further to an infusion swivel. After the surgery each rat was housed individually in a plastic cage (30 x 25 x 15 cm). The catheters were flushed with 0.5 ml of 0.9% NaCl containing 250 U/ml sodium heparin, after operation and a day later. The deoxyglucose tracer experiments were performed 2 days after the surgery, between 8 and 12 a.m. Experiments were done for some animals during rest and for some animals during a swimming exercise. The latter rats were made to swim in a 16-cm diameter cylindrical vessel filled with 32°C water to a depth of 40 cm. 2-(^{3}H)Deoxyglucose in an oxypolygelatine-based plasma expander (Gelifundol, Medipolar, Oulu, Finland) was infused in the following manner: The venous cannula (volume 0.3 ml) was first filled with the deoxyglucose solution, followed immediately by an injection of 1.0 ml of the same solution at swimming time zero. Thereafter the infusion was continued at a rate of 210 µl/min, 100 µl/min and 40 µl/min between time points 0-4 min, 4-8 min and 8-20 min, respectively. The total

infusion volume was 3.0 ml. Arterial samples of 0.6 ml were taken at 0, 5, 10, 15 and 20 min. The mean arterial pressure (MAP) was measured using a Hewlett-Packard 1280 pressure transducer and a Hewlett-Packard 8813A pressure processor. Heart rate was determined using a Hewlett-Packard 8812A pulse recorder. Immediately after the swimming period the rats were anesthetized with intra-arterial sodium pentobarbital, and the hearts were excised and perfused in vitro for 10 min to wash the free extracellular deoxyglucose from the tissue.

4.5. Myocardial sampling

The preparation of the heart and the transmural dissection of the left ventricle in studies I-V proceeded as described by Takala (1981). The atria and the left and right ventricles were dissected free after perfusion, and the atria and the right ventricles were frozen in liquid nitrogen and weighed. The left ventricles were opened, quick-frozen to a sheet between aluminium clamps, weighed and cut in a cryostat (-20 ^{o}C) into 20 μm slices representing concentric layers of the myocardium. The slices were combined to represent 3 equal thicknesses of the cross-section of the muscle. Before slicing and analyzing all the samples were stored at -80 ^{o}C.

In studies VI and VII the atria, outer wall of the right ventricles, and left ventricles were dissected free. The left ventricles were opened and compressed gently to a sheet between aluminium plates and cut into two samples with a razor-blade. These samples represented subendo- and subepicardial layers of the left ventricle. The myocardial samples were weighed and the ventricular samples were immediately homogenized for the assays of oxygen consumption, and the rest of each homogenate was frozen for the enzymatic assays.

4.6. Biochemical methods

4.6.1. Glucose uptake

A portion of each myocardial sample (I-V) was homogenized in 8% $HClO_4$ and de-proteinized using repeated perchloric acid extraction in the presence of 0.1 mM non-radioactive deoxyglucose. The 2-deoxy(^{3}H)glucose radioactivity of the neutralized perchloric acid extracts was measured using Lumagel (Lumac AG, Switzerland) in a

liquid scintillation counter (Wallac Co., Finland). Regional glucose uptake was measured by the 2-deoxyglucose trapping method (Sokoloff et al. 1977). This method is based on the principle that 2-deoxyglucose is taken up in the cells by the glucose carrier and phosphorylated to 2-deoxyglucose-6-phosphate, which is not further metabolized in cardiac muscle (Kipnis & Cori 1959). This metabolite is hydrolyzed only very slowly due to the low glucose-6-phosphatase activity in myocardium (Opie & Newsholme 1967). An operational equation developed by Takala & Hassinen (1981) for the myocardial tissue was used to calculate regional glucose uptake:

$$v = \frac{k_e^2 \cdot C_i^*(t)}{(A \cdot r - k_e \cdot AR_o)(1 - e^{-k_e \cdot t}) + A \cdot r \cdot k_e \cdot t}$$

where v = uptake rate of glucose, k_e = first-order rate constant for deoxyglucose release from the cells, $C^*_i(t)$ = intracellular concentration of deoxyglucose metabolites at time t, R_o = deoxyglucose-to-glucose concentration ratio in the perfusate (I-IV) or plasma (V), $A = V^*_m K_m / V_m K^*_m$, where V_m and V^*_m are the maximal uptake rates and K_m and K^*_m the perfusate or plasma concentrations of glucose and deoxyglucose, respectively, at halfmaximal rates. This equation is used when the deoxyglucose-to-glucose ratio is constant.

4.6.2. Substrate oxidation

Oxidation of energy-producing substrates (succinate, glutamate + malate, pyruvate and palmitoylcarnitine) was estimated using the polarographic method (Estabrook 1967) modified by Starnes et al. (1983) for heart muscle homogenates (VI,VII). Myocardial samples were homogenized with an Ultra-Turrax tissue dispenser and all-glass Potter-Elvehjem homogenizer in 9 vol. ice-cold buffer: 180 mM KCl, 10 mM ethylenediaminetetra-acetic acid and 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.4. Homogenate was diluted for the measurements if necessary. A Clark-type oxygen electrode in a water-jacketed open reaction vessel maintained at 37°C was used. The concentration of dissolved oxygen at 37°C was assumed to be 199 µm. The reaction medium consisted of a 780 µl incubation buffer (250 mM sucrose, 10 mM Tris-HCl, 10 mM K_2HPO_4, pH 7.5.), 50 µl homogenate and 10 µl of one of the following substrates: 1 M succinate, 1 M glutamate + 0.1 M malate, 1 M pyruvate, or 5 mM palmitoylcarnitine. State 3 respiration was initiated by adding 225 nmol ADP (9 µl).

4.6.3 Enzyme activities

Activities of several enzymes were measured to estimate glycolytic and oxidative capacity of myocardial tissue (Table 5). Myocardial muscle samples, stored frozen in -80°C, were homogenized in ice-cold distilled water using an all-glass Potter-Elvehjelm homogenizer (III), or the same homogenates as used for measuring substrate oxidation rates were utilized (VI, VII). Details of the enzyme assays are given in the original articles (III, VI, VII).

4.6.4. Other biochemical methods

Protein was measured using various methods (Table 5). The output of purine compounds (adenosine, inosine, hypoxanthine plus xanthine) was determined in the perfusion studies (I,II) during the recirculation period according to Olsson (1970) in order to detect possible ischaemia during the perfusion. Plasma glucose, free fatty acids and blood lactate were determined using commercial kits (glucose, Mercotest, Merck GmbH, FRG; nonesterified fatty acids, WAKO Chemicals GmbH, FRG; lactate UV-method, Boehringer Mannheim GmbH, FRG) (V). Blood pyruvate was measured according to Bücher et al. (1963) (V).

4.7. Effectiveness of training

In order to reveal the effectiveness of training, citrate synthase activity was measured from the soleus-muscle as described by Vihko et al. (1978)(I-VII). Atrial, left and right ventricular (I-VII), and adrenal (I,II,VI) weights were carefully recorded. Body weights, measured before and after training experiments (I-VII) and changes in the body weights during training, were also followed (II, III, VI). Out of this data total heart weights, ventricular weights and the ratios of myocardial weights-to-body weight were calculated (I-VII).

Table 5. List of myocardial enzyme activities and protein concentrations measured in separate studies with references to the methods employed.

Variable	E.C. number	Method	Paper
Glucose-6-phosphate dehydrogenase	1.1.1.49	Löhr & Waller 1974	III
Phosphofructokinase	2.7.1.11	Wu & Racker 1959	III
3-Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	Bass et al. 1969	III
Pyruvate kinase	2.7.1.40	Penney et al. 1974	III
Lactate dehydrogenase	1.1.1.27	Kornberg 1955	III, VI, VII
Citrate synthase	4.1.3.7	Srere 1969	III, VI, VII
Isocitrate dehydro-genase	1.1.1.42	Bergmeyer 1983	VI, VII
Malate dehydrogenase	1.1.1.37	Ochoa 1955	III
Cytochrome oxidase	1.9.3.1	Whereat et al. 1969	VI, VII
Protein	-	Szarkowska & Klingenberg 1963	I, V
		Lowry et al. 1951	I, II
		Peterson 1977	III, IV, VI, VII

4.8. Statistical methods

Standard procedures were used to calculate means, standard deviations and standard errors. Differences of the means were tested with one-way analysis of variance (I-VII), its randomized block design, and the Student's *t*-test, in which for most of the studies the confidence limit was determined by the Bonferroni method (I, II, IV-VII) (Wallenstein et al. 1980).

5. Results

5.1. Body, heart and adrenal weights

The increase in body weights during the training regimens was significantly lower in training young and aged male rats in some studies (I, II, III, IV) and the absolute weights were also lower in the remaining studies (V, VI, VII) compared to untrained controls. Especially training with extra load reduced the gain of body weight (VI). The body weights of female rats did not change due to training (III).

In male rats, no increase in total heart weight or in the left ventricular weight was observed in any of the present studies (I - VII), but especially after swimming training increased right ventricular and atrial weights were found (I, III, V). The left and right ventricular weights and atrial weights were increased after swimming training in female rats (III). Myocardial-to-body weight ratios were higher in all studies after training (I - VII). Training tended to increase adrenal weights (I, II, VI).

5.2. Regional distribution of glucose uptake

The average left ventricular glucose uptake in isolated beating normal hearts varied between 1.87 ± 0.14 (mean ± SE) and 5.87 ± 1.02 $\mu mol \times min^{-1} \times g^{-1}$ protein (I-III). The average glucose uptake in vivo was 1.7 ± 0.1 $\mu mol \times min^{-1} \times g^{-1}$ protein (V). Training (I-III) or cessation of training did not have any significant influence on the total glucose uptake rates in isolated hearts (I-III), but in vivo a 100% increase was found after training (3.5 ± 0.3 $\mu mol \times min^{-1} \times g^{-1}$, $p<0.001$) (V). Acute swimming did not result in any statistically significant change in the average left ventricular glucose uptake in control or trained rats (V). In ageing hearts training had no effect on the glucose uptake level, measured in isolated perfused heart (IV).

In isolated perfused hearts the subendocardial glucose uptake was about 30% or 12% higher than the epicardial one in the left ventricles of control male ($p<0.05$ or <0.01) (I-III) and female ($p<0.01$) (III) rats, respectively (Figs. 1A and 1B). In vivo

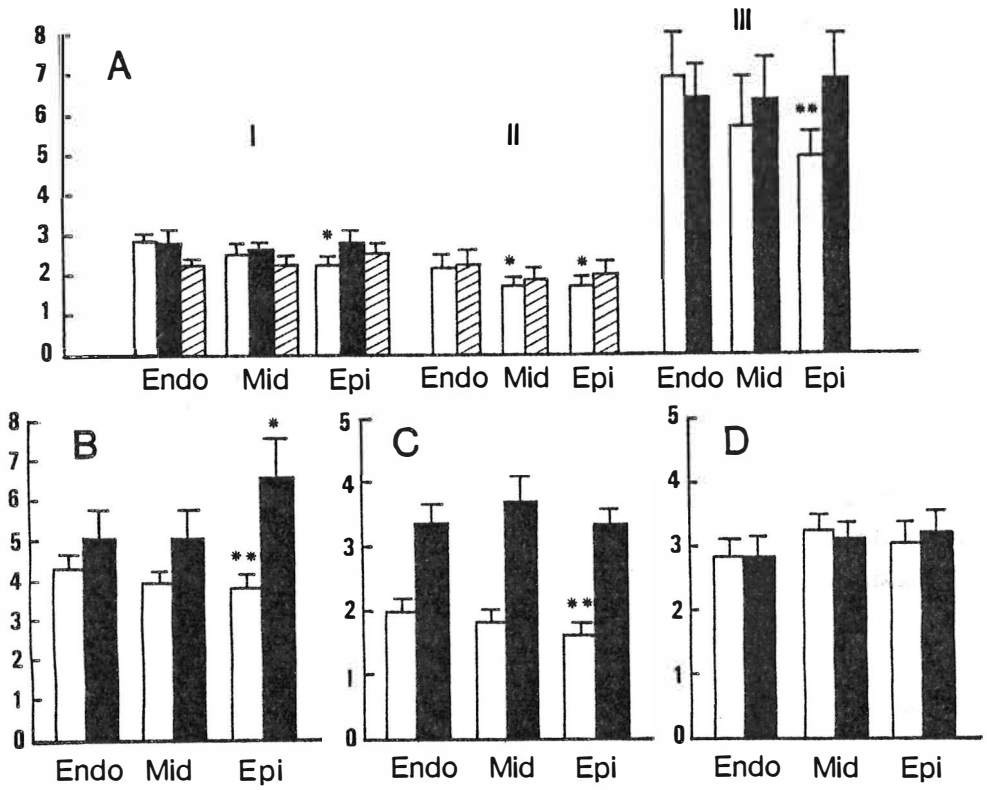

Figure 1. Transmural distribution of glucose uptake in the left ventricles of rats, presented as μmol x min^{-1} x g^{-1} protein (mean ± SE). (A) Langendorff-perfused hearts of young male rats. Roman numerals refer to the original studies. (B) Langendorff-perfused hearts of young female rats (III). (C) Hearts of young male rats in vivo (V). (D) Langendorff-perfused hearts of aged male rats (IV). Blank bars = sedentary controls, hatched bars = running trained, filled bars = swimming trained rats. Endo = subendocardial third, Mid = middle third, and Epi = the outermost third of the of the left ventricular wall. * $p<0.05$, ** $p<0.01$ vs. Endo.

the uptake rate was 25% higher in the subendocardium ($p<0.01$, Fig. 1C) (V). Training abolished this gradient in vitro (I-III) and in vivo (V), and in female rats an opposite gradient was found, the subendocardial glucose uptake being 23% higher ($p<0.05$) than the subendocardial uptake (Figs. 1A-1C) (III). Termination of training restored the gradient in two weeks (II). In ageing control hearts the glucose uptake was evenly distributed through the left ventricle and training had no effect on this distribution (Fig. 1D) (IV).

Right ventricular and atrial glucose uptake rates of control rats were considerably lower (25-46%) than the average left ventricular uptake rates in perfused control hearts (I-III). Training did not influence the atrial glucose uptake rate (II, III) or that of the right ventricle (I, II), except in one study where the right ventricular uptake rates were increased by approx. 52% in males ($p<0.05$) and 60% in females ($p<0.05$) (III).

5.3. Enzyme activities of glycolysis, Krebs cycle and respiratory chain

5.3.1. Myocardial enzyme activities of untrained control rats

Out of the enzymes of the glycolytic pathway phosphofructokinase and 3-phosphoglyceraldehyde dehydrogenase, measured from Langendorff-perfused control hearts, showed a left ventricular gradient. In males the subendocardial activities of phosphofructokinase and 3-phosphoglyceraldehyde dehydrogenase were 50% and 69% higher, respectively, than the activities in the subepicardium (III). In females the respective values were 56% and 14% (III). Pyruvate kinase and lactate dehydrogenase exhibited no left ventricular gradient in perfused hearts (III). In nonperfused hearts the left ventricular gradients of all the above enzymes were similar to those found in perfused hearts. Only phosphofructokinase activity of males did not show a similar gradient (III), and lactate dehydrogenase activity showed a small gradient in one study (III) but not in the other (VI). The mid-myocardial activities of phosphofructokinase and 3-phosphoglyceraldehyde dehydrogenase tended to be higher than the activities in other left ventricular regions (III).

Other enzyme activities measured did not exhibit left ventricular gradients (III, VI) with occasional exceptions of citrate synthase and malate dehydrogenase in non-perfused hearts of female rats (III).

Lactate dehydrogenase, citrate synthase, isocitrate dehydrogenase and cytochrome oxidase activities of the right ventricles were on the same level as the left ventricular activities (VI). The right ventricular and atrial activities measured in study III are not quite comparable to the left ventricular activities due to the possible influence of differences in the specimen handling (see details in III).

Perfusion per se had a peculiar effect on enzyme activities. The activities of phosphofructokinase, lactate dehydrogenase and citrate synthase were higher in the left ventricles of perfused hearts than in nonperfused ones (III). There were no changes due to perfusion in the other left ventricular enzyme activities. The right ventricular and especially the atrial activities of the enzymes mentioned above were lower in the perfused hearts than in the nonperfused ones. Again there were no changes in the activities of other enzymes (III).

5.3.2. Effects of training and age on myocardial enzyme activities

Training-induced changes in the regional activities of enzymes in the myocardium of young rats (III, VI), compared to the respective control values, are presented in Table 6.

In aged hearts citrate synthase, isocitrate dehydrogenase and cytochrome oxidase activities were lower in the subendocardium than in the subepicardium ($p<0.01$, $p<0.05$ and $p<0.05$, respectively)(VII). The right ventricular activity of citrate synthase was higher than the subendocardial activity ($p<0.05$) and right ventricular activities of isocitrate and lactate dehydrogenase activities were lower than the subepicardial activities ($p<0.05$)(VII). Training abolished all regional differences of enzyme activities compared with the respective values of sedentary animals, but had no other effects (VII).

5.4. Oxidation of substrates

The oxidation rates of succinate and palmitoylcarnitine were higher in the subendocardium than in the subepicardium of the left ventricles of the sedentary

Table 6. Training-induced changes in the regional enzyme activities of young, perfused or nonperfused rat hearts compared to the respective activities of control hearts (III, VI).

Enzyme	ENDO	MID	EPI	RV	A
Phosphofructokinase					
-nonperfused male	0	0	0	*	**
-perfused male	0	0	0	0	0
-nonperfused female	0	0	0	*	0
-perfused female	0	0	*	0	**
3-phosphoglyceraldehyde dehydrogenase					
-nonperfused male	0	0	0	0	**
-perfused male	0	0	0	0	0
-nonperfused female	0	0	*	0	*
-perfused female	*	*	*	0	**
Pyruvate kinase					
-nonperfused male	*	*	0	0	0
-perfused male	0	0	0	0	0
-nonperfused female	*	*	0	0	*
-perfused female	*	0	0	0	0
Lactate dehydrogenase					
-nonperfused male	0	*	0	*	0
-perfused male	0	0	0	*	0
-nonperfused female	0	0	0	*	0
-perfused female	0	0	0	***	0
Glucose-6-phosphate dehydrogenase					
-nonperfused male	0	0	0	0	*
-perfused male	0	0	0	*	0
-nonperfused female	0	0	0	0	*
-perfused female	0	0	0	0	0
Citrate synthase					
-nonperfused male	0	0	0	0	0
-perfused male	0	0	0	0	0
-nonperfused female	0	0	0	0	*
-perfused female	0	0	0	0	*
Malate dehydrogenase					
-nonperfused male	0	0	0	0	0
-perfused male	0	0	0	0	0
-nonperfused female	0	0	0	0	*
-perfused female	0	0	0	*	**
Isocitrate dehydrogenase					
-nonperfused male	0		0	0	
Cytochrome oxidase					
-nonperfused male	0		0	0	

ENDO, MID and EPI are subendocardial, midwall and subepicardial regions of the left ventricle, respectively, RV = right ventricle, A = atria; 0 indicates no statistical difference, *, ** and *** indicate increased activities vs. control value ($p<0.05$, <0.01 and <0.001, respectively).

young animals (24.4 ± 4.8 vs. 17.1 ± 2.0, n.s., and 14.4 ± 1.7 vs. 10.2 ± 1.3 nmol O_2 x min^{-1} x mg^{-1} protein, $p<0.05$, respectively; means ± S.E.) (VI). These differences were even more pronounced after training (34.7 ± 2.8 vs. 25.5 ± 1.3, $p<0.01$, and 22.8 ± 2.7 vs. 12.8 ± 2.1 nmol O_2 x min^{-1} x mg^{-1} protein, $p<0.05$, respectively). The oxidation rates of glutamate+malate and pyruvate were similar in the subendocardium and the subepicardium of sedentary or trained rats (VI). Training increased the oxidation of succinate in the subepicardium ($p<0.01$) and that of palmitoylcarnitine and pyruvate in the subendocardium ($p<0.05$) compared with the respective layer of the sedentary animals. Training had no effect on the substrate oxidation in the right ventricles (VI). Extra weight increased the subendocardial succinate oxidation ($p<0.05$ compared with the subendocardium of controls and $p<0.001$ compared with the subepicardium of same hearts). Extra weight also increased the oxidation of palmitoylcarnitine in the subendocardium, so that a more clear transmural gradient was found than in the controls ($p<0.001$). Training + extra weight increased the endocardium oxidation of succinate ($p<0.01$ compared with the subepicardium of the controls), this increase being similar to the trained rats (VI). Oxidation rates of all substrates were slower in the right ventricles than in the left ventricles of the sedentary rats, controls carrying extra weight, or trained rats. Training + extra weight increased the oxidation of glutamate+malate and pyruvate in the right ventricles because no difference was found compared with the left ventricular value of the same heart after the experiment (VI).

Oxidation rates of the substrates were evenly distributed through the left ventricles of sedentary and trained old rats (VII). Oxidation of palmitoylcarnitine and glutamate+malate were lower in the right ventricles than in the subepicardium of the left ventricles of the sedentary rats ($p<0.05$ and $p<0.01$, respectively). After training only glutamate+malate oxidation of the right ventricles was slower than that of the Endocardium and subepicardium of the left ventricles ($p<0.05$ and $p<0.01$, respectively). There were no statistical differences in the oxidation rates between sedentary and trained animals in any region of the aged heart (VII).

5.5. Other results

Heart rate, mean arterial pressure and their product was at the same level in trained and control rats at rest and were increased approximately similarly in both groups during 20 minutes of swimming exercise (V).

Blood lactate and pyruvate concentrations and plasma FFA concentrations were similar at rest in trained and control rats (V). During 20-min exercise the increase of the blood concentrations of lactate and pyruvate were lower and transient in trained rats compared with untrained ones, whereas in plasma FFA concentrations one change was observed: in the exercising control group 20-min value was ~30 % lower ($p<0.01$) than the resting value (V).

Citrate synthase activity of the soleus muscles increased significantly after every training regimen, indicating enhanced oxidative capacity of skeletal muscle (I-VII).

6. Discussion

6.1. Effects of training and extra load on weight parameters

The training-induced body and cardiac weight changes showed similar patterns as in most studies in which young rats are chronically exercised (Harpur 1980). In male rats, both running and swimming reduced the gain of body weight, but in female rats such a reduction was not observed. The weights of cardiac compartments did not change due to training in male rats; the only exceptions were increased right ventricular and atrial weights after training by swimming. In female rats, swimming training increased weights of all cardiac compartments. Elevated heart-to-body weight ratio has been considered as a measure of cardiac hypertrophy (Harpur 1980). Both in young males and females the calculated heart-to-body weight ratios were higher after training, but only in female rats was this index higher due to the increase in absolute cardiac weight. Male rats restrict their food intake during training periods, but in females such behavioural change does not occur (Harpur 1980). Therefore heart-to-body weight ratio can not be regarded as a reliable index of cardiac hypertrophy in male rats. Increased atrial weights after swimming training - but not after running training - may be a consequence of unintentional drinking of water while swimming, leading to increased blood volume and renal function. Water excretion of kidneys is partly regulated by atrial natriuretic peptide, which is a product of endocrine function situated in the atria of the heart (Genest et al. 1987). Increased blood volume may invoke the growth of the atria in order to produce a sufficient amount of atrial natriuretic peptide during long periods of swimming training in rats.

Placing of extra weight evokes stress, indicated as a drop in body weight. This stress is probably transient in non-training rats, as later the gain of body weights was nearly similar to control rats. The combined stresses of training and extra weight lead to a permanent reduction in the growth rate.

6.2. Effects of training on myocardial glucose uptake and on glycolytic enzymes

6.2.1. Total glucose uptake

In perfused hearts with glucose as the sole external energy substrate, no change in the left ventricular glucose uptake is observed due to training, but in vivo during rest the glucose uptake is doubled. A somewhat less-pronounced difference is seen during exercise between trained and sedentary rats. This increase is not mediated through the actual work load during the experiments, because there were no differences in the indices of the left ventricular work load between trained or sedentary groups. Neither were there differences in the concentrations of the blood lactate and pyruvate or plasma FFA between resting groups, so that the availability of these cardiac fuels cannot be the reason for the increased training-induced glucose uptake. The total left ventricular oxygen consumption, and thus the total substrate oxidation, may also depend on the myosin isoenzyme pattern and the myofibrillar ATPase activity, the V_1 isoenzyme being probably thermodynamically less efficient than V_2 and V_3 isoenzymes (Holubarsch et al. 1985). The known increase in the V_1 isoenzyme of myosin by swimming training (Rupp 1981, Takeda et al. 1985) may thus contribute to the increase in glucose uptake by training as a fraction of the increase in the total fuel consumption.

Our results are in accordance with those of Yonekura et al. (1985), who found that chronic pressure overload in terms of salt-induced hypertension in hypertension-prone rats causes simultaneous increase in myocardial glucose uptake and a decrease in fatty acid uptake. Although Yonekura et al. (1985) did not measure the concentrations of glucose, FFA or other competitive cardiac fuels, their results, together with the present results, suggest that chronic elevation in myocardial oxygen consumption increases the preference of glucose as a myocardial energy-yelding substrate. In principle, enhanced glucose uptake could be mediated through altered hormonal and/or neural responses and/or other adaptive responses in the myocardium. Insulin and muscle contraction regulate glucose uptake in skeletal and heart muscle (Berger et al. 1975, Neely & Morgan 1974). These mechanisms seem to be quite distinct and cumulative at least in the skeletal muscle (Nesher et al. 1985, Wallberg-Henriksson & Holloszy 1985). Glucose uptake is accelerated by insulin binding, which is known to increase in at least some skeletal muscles after training (Bonen et al. 1986, Grimditch et al. 1986). Recent studies by Zaninetti et al. (1988a, 1988b) show that insulin, work load and glucose itself stimulate glucose transport by increasing the number of glucose transporters on

the sarcolemma and changing, albeit by different mechanisms, the functional properties of glucose transporters. So far, six different glucose transporters have been identified. These can be divided into two groups: insulin-regulated and insulin-independent transporters (Baly & Horuk 1988). Insulin regulates glucose uptake by changing the number of glucose transporters on the cardiac cell membranes (Watanabe et al. 1984), the first step being the formation of an insulin-insulin receptor complex, so that it is actually the number of receptors and/or their affinity for insulin that regulates the glucose uptake. The increase in serum catecholamines occurring during exercise may increase insulin binding through β-adrenergic receptors in skeletal muscle (Webster et al. 1986). Catecholamines may also increase the number of glucose transporters in the myocardial sarcolemma (Keely et al. 1977) and increase phosphofructokinase activity (Clark & Patten 1981). Also, the tissue concentrations of epinephrine and norepinephrine increase in the myocardium due to training (Geenen et al. 1988).

The recent studies by Kostreva et al. (1985) and Herman & Kostreva (1986) suggest that autonomic responses may also have a direct effect on the cardiac glucose uptake. There is evidence that parasympathetic activity increases at rest after training, causing bradycardia, but various studies performed during exercise have given conflicting results (for refs., see Blomqvist & Saltin 1983). In the present study, no significant differences in heart rate were observed in the four experimental groups. The stress responses to the implantation of the arterious and venous catheters may also modify the heart rate and blood pressure responses.

The total left ventricular glucose uptake was unaffected by acute swimming in both untrained and trained groups, in spite of an increase in the myocardial oxygen consumption, which is known to be closely coupled with the total fuel consumption. The reason for this is probably that the elevation in external lactate, as observed here during swimming, increases the myocardial lactate uptake and decreases the proportional glucose uptake (e.g. Taegtmayer et al. 1980). Similar observations of an increase in the lactate/glucose uptake ratio have been observed previously during and after physical exercise (Gertz et al. 1988, Keul et al. 1965). A smaller blood lactate/pyruvate ratio also suggests more aerobic metabolism in the exercising-trained than in the exercising-sedentary animals (e.g. Saltin & Gollnick 1983).

6.2.2. Regional glucose uptake and glycolytic enzyme activities

The mechanical work load is the major determinant of oxygen consumption in the heart (Neely et al. 1967) and a close positive correlation exists between glucose uptake and mechanical work load during constant concentrations of exogenous substrates (Crass et al. 1970, Hassinen & Hiltunen 1975, Kobayashi & Neely 1979, Taegtmayer et al. 1980). Therefore the greater glucose uptake in the subendocardium than in the subepicardium of young sedentary rats - both in vivo and in isolated perfused hearts - probably reflects similar gradients in the cardiac work load and oxygen consumption (for references, see chapters 2.1.1. and 2.1.2.). This gradient of glucose uptake is not affected by contemporaneous availability of such substrates as acetate, oleate or lactate (Peuhkurinen et al. 1983, Takala et al. 1984b), even though these serve to reduce the total glucose uptake. The removal of the mechanical work load by potassium-induced cardiac arrest (Takala & Hassinen 1981) and increased pressure work load in isolated hearts (Takala et al. 1984b) eliminate the transmural gradient of glucose uptake, suggesting further that mechanical work load is the major determinant of the distribution of the glucose uptake. It should also be noted that the right ventricle and the atria, which work less than the left ventricle, have accordingly lower glucose uptake rates. The recent results by Takala et al. (unpublished) show that elevation of coronary pressure in isolated rat hearts leads to elimination of the transmural glucose uptake gradient, and that this is not due to the increased intraventricular pressure. The elevation of the intramyocardial pressure by higher coronary pressure may, according to Laplace's law, result in a greater increase in the diastolic wall stretch and by the Frank-Starling mechanism to a greater increase in the work load in the subepicardium than in the subendocardium (Feigl 1983). The observed elimination of the glucose-uptake gradient during exercise in untrained rats may be a consequence of this "garden hose effect" of increased coronary pressure.

The present results show that repeated swimming or running exercise in young rats results in a permanent change in the transmural glucose uptake, suggesting a similar change in the oxygen consumption. Vanishing of the glucose uptake gradient is observed both in isolated hearts at coronary pressure of 80 cm of water and in vivo during rest. Therefore this change is not due to a transient or sudden change in regional cardiac work load, but is a consequence of a structural or functional training-induced change in the myocardium. Long-term swimming and running training increase myocyte size in the subepicardium, but not in the subendocardium (White et al. 1988).

In sedentary rat heart there seems to be transmural differences in the myosin isoenzyme pattern (Litten et al. 1985, Leipälä et al. 1988, Sartore et al. 1981, Gorza et al.

1981). Intermittent training leads to a shift in this pattern towards the high cross-bridge turnover isoenzyme V_1 in the rat (Rupp 1981, Jacob et al. 1983, Schwartz et al. 1983). Physical training may change the myosin isoenzyme distribution pattern by redistributing work and tension. In principle, changes in the myosin isoenzyme pattern are capable of inducing a permanent change in energy expenditure.

As discussed in Chapter 6.2.1., hormones regulate glucose uptake in heart muscle. The transmural gradient observed in glucose uptake could principally be caused by gradients in insuline receptors and glucose transporters in the sedentary animals, and chronic exercise may increase the number of insulin receptors or their affinity for insulin predominantly on the subepicardial plasma membranes. Prostaglandins are known to exhibit an insulin-like effect on glucose transport (Olefsky 1977). Furthermore, contractile function during prolonged exercise increases skeletal and heart muscle phospholipase A_2 activity, suggesting increased prostaglandin formation (Federspil et al. 1987). Therefore, regional distribution of cardiac work load may partly regulate the regional glucose uptake through the kallikrein-kinin-prostaglandin system.

The fact that the transmural gradient in glucose uptake reappears 2 weeks after the cessation of training, at a time when the mechanical adaptations to conditioning are no longer observable (Giusti et al. 1978), may indicate that glucose uptake is modulated by sporadic changes in the contractile activity of the myocardium.

One of the aims of this study was to see if there are any changes in the activities of some enzymes participating in glucose metabolism along the changes in the glucose uptake rate. It should be noted that the enzyme activities between the left ventricles on the one hand, and the right ventricles and atria on the other hand, are not quite comparable, due to the possible influence of differences in the handling of the tissue specimens (cryostate cutting of left ventricles, see Methods). The activity of phosphofructokinase, the rate-limiting enzyme of glycolysis, and that of 3-glyceraldehyde-phosphate dehydrogenase expressed left ventricular gradients, the subepicardium activity being the lowest and the midwall activity the highest in most of the measurements. This indicates that the transmural distribution of glycolytic enzyme activities is not quite as similar as the transmural distribution of glucose uptake. Phosphofructokinase was most active in the midwall region. Phosphofructokinase is known to have different isoenzyme patterns and, consequently, different activities in the left and right ventricles and in the atria (Dunaway & Kasten 1985). According to our results, it is possible that phosphofructokinase isoenzymes show different patterns in the subepicardial, midwall and subendocardial regions. Lundsgaard-Hansen et al. (1967)

found transmural gradients in the activities of glucose-6-phosphate dehydrogenase, fructose-1,6-bisphosphate aldolase, pyruvate kinase, lactate dehydrogenase and α-hydroxybutyrate dehydrogenase in mongrel dog hearts. Out of these, the fructose-1,6-bisphosphate aldolase and pyruvate kinase activities showed opposite left ventricular gradients, compared to the glucose uptake gradient found in this study and in dog hearts (Breull et al. 1981, L'Abbate et al. 1981). We did not find any gradient in the pyruvate kinase activity. The lactate dehydrogenase activity of sedentary males expressed a similar gradient to that found by Lundsgaard-Hansen et al. (1967), but the activity values between subepicardium and subendocardium were small. Dowell (1978) did not find any gradient in the activity of lactate dehydrogenase in male rat hearts. The heart-type lactate dehydrogenase isoenzyme (LDH_5) is nearly absent from the atria of canine heart, and the ratio LDH_5/LDH_4 is greater in the subendocardium than in the subepicardium, suggesting regional metabolic differences (Nohara et al. 1978).

As mentioned earlier, there are very few studies on the training and glycolytic metabolism of the heart. No changes in phosphofructokinase (York et al. 1975) or aldolase (Hearn & Wainio 1957) activities have been found, but pyruvate kinase (York et al. 1975) and lactate dehydrogenase (Gollnick et al. 1967, Koehler & Medugorac 1980, York et al. 1975) activities were increased, and changes in lactate dehydrogenase isoenzyme composition were obvious (Koehler & Medugorac 1980, York et al. 1975). Histochemical studies have shown that regional changes do not occur in lactate dehydrogenase activity (Koehler & Medugorac 1980). It should also be noted that physical exercise results in increased blood lactate concentration (Takala et al. 1983), which is known to lead to an increase in lactate uptake (Gertz et al. 1988) and a fractional decrease in the uptake of competitive substrates, e.g. glucose (Takala et al. 1984). Training does not alter the total utilization of glucose in arrested (Scheuer et al. 1973) or beating perfused hearts, as shown in the present study. The regional glucose consumption is, however, changed in vivo during exercise (Takala et al. 1983) and in perfused heart after training. Similar changes could be expected in the activities of glycolytic enzymes, but this seems not to be the case. However, training slightly increases the phosphofructokinase and 3-glyceraldehydephosphate dehydrogenase activities in the subepicardium of female rats - the site where the glucose uptake is also most prominently elevated. The changes in enzyme activities are not so clear as the changes in glucose uptake. Training also increases the phosphofructokinase activity in right ventricles, but this enhancement could only be found in the nonperfused hearts. Elevated activities of pyruvate kinase and 3-glyceraldehydephosphate dehydrogenase (the latter in females only) were found in the subendocardium and midwall regions, which is not in concert with the changes in glucose uptake. However, the possible

compartmentalization of glycolytic ATP production (Pierce & Philipson 1985, Westrin & Backman 1983) may be changed due to training, and this cannot be shown by the methods used in this study. Increased lactate dehydrogenase activity, especially in right ventricles, may be related to the enhanced ability of trained rats to use lactic acid as a myocardial energy fuel during exercise. Lactate is known to be one of the preferred substrates of the heart for ATP production (Taegtmayer et al. 1980).

6.3. Regional oxidative substrate utilization and oxidative enzyme activities

As reviewed above (see chapter 2.1.) the subendocardial metabolic rate seems to be greater than the subepicardial rate in the normal heart. Supporting this suggestion we found that palmitoylcarnitine ($p<0.05$) and also succinate (n.s.) are oxidized more rapidly in the crude homogenates of the subendocardium than in those of the subepicardium of young rats. After training these differences are even more clear. However, we did not observe any transmural differences in the left ventricular oxidation of glutamate+malate or especially in that of pyruvate, which is in contradiction with the results of Camici et al. (1984), who found higher pyruvate oxidation rates in mitochondria isolated from subendocardium of canine heart. It is also worth noting that in the right ventricle, which works less than the left ventricle, the oxidation of all substrates is lower than in the left ventricle of the young rat.

Exercise training improves the myocardial work capacity of young animals, measured as mechanical performance (Bersohn & Scheuer 1977, Molé 1978) or biochemical properties of contractile proteins (Bhan & Scheuer 1972, Rupp 1981) but most frequently no changes are found in the activities of enzymes connected to the oxidation of energy substrates (Baldwin et al. 1975, Baldwin et al. 1977, Kainulainen et al. 1984, Oscai et al. 1971a, Oscai et al. 1971b, Penpargkul et al. 1978), in the mitochondrial oxygen consumption (Arcos et al. 1967, Penpargkul et al. 1978) or in the concentration of cytochromes (Hickson et al. 1979). However, several contradicting results have been published; e.g. enhanced mitochondrial enzyme activities (Kraus & Kirsten 1970) and increased concentrations of cytochromes, coenzyme Q and mitochondrial protein (Beyer et al. 1984, Kraus & Kirsten 1970, Penpargkul et al. 1978). Endurance exercise induces a 3-fold increase in carnitine palmitoyltransferase activity in rat heart (Guzman & Castro 1988). Sex differences in the response to physical conditioning are not well known (Schaible & Scheuer 1985), but they may partly explain the contradicting results. Another explanation may be the myocardial sampling site used in various studies. In most studies the whole or at least

large parts of the ventricular tissue - without any specification of the regional sampling site - have been used for biochemical determinations, and transmural aspects have not been taken into consideration, in which case the regional differences in the adaptation to the physical training may not be detected. The present results clearly show that regional differences exist in the oxidation of various substrates, and that training enhances the oxidation rates of succinate, pyruvate and palmitoylcarnitine in the subendocardium of young rats, which can be seen as steeper regional differences and greater oxidation rates compared with the subendocardium of controls. The subepicardial oxidation of succinate also increases considerably. Training-induced increase in palmitoylcarnitine oxidation suggests an enhanced ability to use fatty acids, and that of pyruvate an enhanced ability to use glucose or lactate as energy-yielding substrates. The last-mentioned alternative is supported by the increased activity of lactate dehydrogenase in the subendocardium. Training enhances in vivo the total cardiac glucose uptake, and both in vivo and in the isolated heart especially the subepicardial glucose uptake. On the basis of these results, it seems that the left ventricle adapts to chronic increase in the cardiac work by elevating mitochondrial ATP-production in the subendocardium and glycolytic ATP-production in the subepicardium. Chronic exercise tends to affect the right ventricular activities of enzymes, especially that of lactate dehydrogenase, more than the left ventricular activities.

As the measurements of oxidative rates in this study were performed from crude homogenates, it remains speculative if the regional differences are a consequence of quantitative or qualitative differences of mitochondria. The study of Camici et al. (1984), showing higher oxidation rates of substrates in isolated subendocardial mitochondria, supports the qualitative alternative. They did not find regional differences in concentrations of cytochromes or in activities of mitochondrial enzymes except in that of isocitrate dehydrogenase (higher in subendocardium). The fact that we did not find any regional differences in mitochondrial enzyme activities (including isocitrate dehydrogenase) and that the oxidation of glutamate+malate did not respond to training as that of other substrates, further suggests that the regional differences in the oxidation rates of energy-yielding substrates are due to qualitative properties of mitochondria and not merely to the different number of them.

Simulated hypergravity conditioning increases the performance and enhances the enzymatic capacity of skeletal muscles (Bosco 1985, Rusko et al. 1987). The present results suggest that hypergravity *per se* increases the myocardial oxidation of substrates in the same manner as training. Combined running training and hypergravity increases especially the right ventricular oxidation rates of pyruvate and

glutamate+malate, indicating that such exercise forces the heart to work regionally more efficiently. However, the possibly-increased stress in this group (indicated as reduced growth rate and increased weight of adrenals) may have its own influence on the results. Also, there is no literature concerning myocardial energy metabolism and hypergravity conditioning, so no far-reaching deductions can be made.

6.4. Sex and age as modulators of training effects

In the hearts of young female control rats, the glucose uptake gradient was less steep than in the male control rats. The myocardial adaptation to endurance training was even more clear in female than in male animals: an opposite gradient was found, the subepicardial uptake being higher than the subendocardial uptake. This indicates, that there may be differences in the myocardial work distribution between both sexes. Also, as reviewed above (see chapter 2.2.4.1.), functional adaptations to exercise training are different between male and female rats. Similarly, the enzymatic adaptations seem to be different, as in the present study most of the significant changes in enzyme activities were found in the hearts of female animals.

The present results indicate that ageing redistributes the left ventricular glucose uptake. This could be explained by the redistribution of the myocardial work load with ageing. Training does not seem to affect this distribution further. Interestingly, and supporting the view that ageing results in an evenly-distributed cardiac work load, the regional differences in the oxidation rates of succinate, glutamate+malate, pyruvate and palmitoylcarnitine were absent in old hearts; only the right ventricular oxidation rates of glutamate+malate and pyruvate were lower than the left ventricular rates. The study of Starnes et al. (1983) showed that exercise training improves both working capacity - indicated as increased peak systolic pressure and cardiac output under high work load - and substrate oxidation - indicated as increased glutamate+malate, palmitoylcarnitine and succinate oxidation rates - in the hearts of 25-month-old male rats, although not to the level of young sedentary rats. In the present study, however, no enhancement was found in the oxidation rates after training (except in the right ventricular oxidation of palmitoylcarnitine), suggesting that no enhancement in the mechanical performance was achieved. The reason for this contradiction is unclear. The training programmes and the ages of rats were nearly identical: in our study the rats were two months older and the training programme was somewhat heavier than in that of Starnes et al. (1983). The strains of rats were different: we used Wistar rats and Starnes et al. Sprague-Dawley rats. Significant strain differences have been found

between Wistar and Sprague-Dawley rats, e.g. in the activities of myocardial glycolytic enzymes (Migler & Cascarano 1982). Age may explain the contradiction, because no adaptation or even negative adaptation to training, demonstrated as decreases in various enzyme activities or blood flow, has been found after a certain age (Bloor & Leon 1970, Chesky et al. 1983, Reznik et al. 1982, Unge et al. 1979). In the present study citrate synthase activity seems to decrease, and lactate dehydrogenase and cytochrome oxidase activities to increase compared with young animals. The decrease in the activity of citrate synthase (the marker enzyme of tricarboxylate acid cycle activity) is in accordance with results showing depressed oxidative metabolism with age. Increased cytochrome oxidase activity with age is a phenomenon known earlier, and may be a response to decreased oxygen delivery in old age; increased cytochrome oxidase activity may allow more efficient use of the oxygen available (Emerson & Wingo 1983).

A great variability exists in the results presented in literature concerning ageing and training considered separately or together. Physiological ageing of animals is a complex phenomenon depending e.g. on nutrition, living conditions and hereditary factors. Comparison of the results of diverse studies remains highly speculative, because there is no common measure for the physiological or biological age of the animals.

6.5. Effect of perfusion on enzyme activities

The perfusion protocol conducted in the present study had a peculiar effect on enzyme activities. The activities of phosphofructokinase, lactate dehydrogenase and citrate synthase were higher in the left ventricles of perfused hearts than in nonperfused ones. There were no changes due to perfusion in the other left ventricular enzyme activities. The right ventricular and especially the atrial activity values of the enzymes mentioned above were lower in the perfused hearts than in the nonperfused ones. Again there were no changes in the activities of other enzymes measured. The perfusion media used contained insulin, which is known to regulate glycolysis by increasing the membrane transport of glucose (Watanabe et al. 1984) and the activity of several enzymes, e.g. pyruvate dehydrogenase (Czech 1977). Enzyme activities are probably regulated by an intracellular insulin-sensitive mediator of peptide nature (Stevens & Husbands 1985). There is also evidence for the possibility that phosphofructokinase-2 (E.C. 2.7.1.105), which catalyses the synthesis of fructose-2,6,-bisphosphate, which in turn is a potent positive effector of phosphofructokinase, is activated by insulin in rat

heart (Rider & Hue 1984). The method used here to measure the activity of phosphofructokinase actually measures the combined activities of phosphofructokinase and phosphofructokinase-2. This means that the perfusion-induced enhancement of the left ventricular phosphofructokinase activity may be the consequence of an enhanced activity of phosphofructokinase-2. However, an increase in the activity of phosphofructokinase would be expected even in the right ventricles and the atria, unless the myocardial activity of phosphofructokinase-2 varies regionally. A possible explanation for the low atrial activities of phosphofructokinase, lactate dehydrogenase and citrate synthase might be inadequate perfusion of the atria during the Langendorff procedure, which could lead to inactivation of these enzymes. This explanation is opposed by the fact that regional distribution of glucose uptake during perfusion is similar to in vivo distribution (Takala et al. 1983). Perfusion may also influence the binding of glycolytic enzymes on the filamentous or membranous structures of the myocardial cells (Pierce & Philipson 1985, Westrin & Backman 1983) or affect possible factors regulating enzyme activities.

7. Conclusions

The heart muscle adapts to long-term physical training by increasing its performance, which can normally be seen as changes in the physiological measures and the biochemical markers of the contractile system. Changes in glucose and/or oxidative metabolism are only rarely observed. Results are, however, obtained usually in whole hearts or whole ventricular walls so that possible regional responses can not be seen. In the present work, the effects of chronic exercise and ageing on the regional distribution of glucose uptake, the regional oxidative capacity, and the regional activities of several enzymes of glycolysis, Krebs cycle, and respiratory chain were studied in rat heart. The present results show:

1. Chronic swimming and running training increase both in vivo and in the isolated perfused heart the subepicardial glucose uptake, which normally is significantly lower than the subendocardial uptake in the hearts of young rats. This redistribution of glucose uptake probably mirrors a similar redistribution of cardiac work load, although the reciprocal redistribution of alternative substrates could also principally explain the redistribution of myocardial glucose uptake. The distribution of glycolytic enzyme activities is not quite similar to that of glucose uptake, and training does not change these activities as clearly it changes the rate of glucose uptake.

2. The myocardial adaptation to endurance training was even more clear in female than in male rats: an opposite glucose uptake gradient was found, the subepicardial glucose uptake being higher than the subendocardial uptake. Also, most of the significant changes in enzyme activities were found in the hearts of female rats.

3. Cessation of training restores the adaptive changes in transmural glucose uptake within two weeks.

4. Glucose uptake measured in vivo increases during rest and exercise as a consequence of training. The increase seems to be independent of the actual work load or supply of major alternative myocardial substrates, indicating that physical training increases the preference of glucose as a myocardial energy-yelding substrate.

5. Regional differences exist in the oxidation capacity of various substrates. Training enhances the oxidation rates of succinate, pyruvate and palmitoylcarnitine in the subendocardium of young rats. On the basis of these results and results concerning glucose uptake, it seems that the myocardium adapts transmurally to chronic exercise by elevating mitochondrial ATP-production in the subendocardium and glycolytic ATP-production in the subepicardium.

6. Ageing leads in abolished gradients in the glucose uptake and in the oxidation rates of substrates, suggesting similar abolishment in the gradient of myocardial work load. Training-induced cardiac alterations are absent or small in aged rats.

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Selostus

Pitkäaikaisen fyysisen harjoittelun ja iän vaikutukset sydänlihaksen alueelliseen energia-aineenvaihduntaan

Sydänlihas mukautuu pitkäaikaiseen fyysiseen harjoitteluun parantamalla toimintaansa. Sydämen toiminnan paraneminen havaitaan fysiologisin mittauksin ja sydämen supistumiskoneiston biokemiallisina muutoksina. Vain harvoin havaitaan liikunnan aiheuttamia sydämen oksidatiivisen tai glukoosiaineenvaihdunnan muutoksia. Energia-aineenvaihduntaa koskevat tulokset on kuitenkin useinmiten saatu käyttäen materiaalina koko sydäntä tai sen kammioita, jolloin mahdolliset aineenvaihdunnan alueelliset muutokset jäävät huomaamatta. Tässä työssä tutkittiin pitkäaikaisen harjoittelun ja ikääntymisen vaikutuksia rotan sydämen alueelliseen glukoosinottoon ja oksidatiiviseen kapasiteettiin. Lisäksi mitattiin useiden glykolyysin, sitruunahappokierron ja hengitysketjun entsyymien aktiivisuudet sydämen eri osissa. Tulokset osoittavat seuraavaa:

1. Pitkäaikainen uinti- ja juoksuharjoittelu lisäävät sekä in vivo että eristetyssä sydämessä vasemman kammion seinän uloimman vyöhykkeen glukoosinottoa. Normaalisti nuorilla rotilla solujen glukoosinotto on nopempaa vasemman kammion seinän sisäosissa kuin ulommissa kerroksissa. Glukoosinoton jakauman muutos saattaa kuvastaa samanlaista sydämen tekemän työn jakauman muutosta. Glykolyysin entsyymien aktiivisuuksien jakauma ei ole aivan samanlainen kuin glukoosinoton jakauma, eikä harjoittelu vaikuta entsyymiaktiivisuuksiin yhtä selkeästi kuin glukoosinottoon.

2. Naarasrotilla harjoittelun aiheuttama glukoosinoton muutos oli selkeämpi kuin uroksilla: vasemman kammion seinän ulkokerrosten glukoosinotto oli merkitsevästi suurempaa kuin sisempien osien glukoosinotto. Naarasrotilla myös glykolyysin entsyymien aktiivisuusmuutokset olivat selvempiä kuin uroksilla.

3. Harjoittelun lopettamisen jälkeen palautuu glukoosinoton jakauma normaaliksi kahdessa viikossa.

4. In vivo sydämen glukoosinotto lisääntyy harjoittelun seurauksena kaksinkertaiseksi sekä levossa että fyysisen kuormituksen aikana. Lisäys ei näytä olevan riippuvainen sydämen työmäärästä tai muiden energialähteiden tarjonnasta. Tämä osoittaa, että harjoittelun seurauksena glukoosin osuus sydämen energialähteenä kasvaa.

5. Sydämen kyvyssä hapettaa energiaa tuottavia substraatteja on suuria vyöhykkeittäisiä eroja. Harjoittelu parantaa sukkinaatin, pyruvaatin ja palmityylikarnitiinin polttokapasiteettia vasemman kammion seinän sisäkerroksissa. Näiden ja glukoosinottoa koskevien tulosten perusteella sydän näyttää mukautuvan pitkäaikaiseen harjoitteluun lisäämällä vasemman kammion sisäkerrosten mitokondriaalista ATP-tuottoa ja ulkokerrosten glykolyysiin perustuvaa ATP-tuottoa.

6. Ikääntymisen myötä sekä glukoosinoton, että substraattien polttonopeuden alueelliset erot katoavat viitaten samanlaiseen sydämen tekemän työn jakauman muutokseen. Harjoittelu ei aiheuta suuria muutoksia vanhojen rottien sydänten energia-aineenvaihdunnassa.

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ORIGINAL PAPERS

I

REDISTRIBUTION OF GLUCOSE UPTAKE BY CHRONIC EXERCISE, MEASURED IN ISOLATED PERFUSED RAT HEARTS

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II

REGIONAL GLUCOSE UPTAKE AND PROTEIN SYNTHESIS IN ISOLATED PERFUSED RAT HEARTS IMMEDIATELY AFTER TRAINING AND LATER

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Heikki Kainulainen, Jyrki Komulainen, Timo Takala & Veikko Vihko

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III

EFFECT OF CHRONIC EXERCISE ON GLUCOSE UPTAKE AND ACTIVITIES OF GLYCOLYTIC ENZYMES MEASURED REGIONALLY IN RAT HEART

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Heikki Kainulainen, Jyrki Komulainen, Timo Takala & Veikko Vihko

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IV

TRANSMURAL DISTRIBUTION OF GLUCOSE UPTAKE IN THE LEFT VENTRICLE OF AGED RATS AFTER LONG-TERM TRAINING

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Heikki Kainulainen, Jyrki Komulainen, Timo Takala & Veikko Vihko

V

TRAINING INCREASES CARDIAC GLUCOSE UPTAKE DURING REST AND EXERCISE IN RATS

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VI

REGIONAL DIFFERENCES OF SUBSTRATE OXIDATION CAPACITY IN RAT HEARTS: EFFECTS OF ENDURANCE TRAINING AND HYPERGRAVITY

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VII

EFFECTS OF TRAINING ON REGIONAL SUBSTRATE OXIDATION IN THE HEARTS OF AGEING RATS

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