

Age-dependent changes in myosin composition correlate with enhanced economy of contraction in guinea-pig hearts

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1. The composition of myosin heavy chains (MHCs) was investigated in young (1- to 8-week-old) and mature (9- to 26-week-old) guinea-pigs using two monoclonal antibodies directed specifically against α -MHC and β -MHC. In addition, maximum force and the rate of ATP consumption during isometric contraction were measured in chemically skinned trabeculae taken from the same hearts.
2. An age-dependent shift in the MHC composition was found. The α -MHC fraction decreased from 0.17 ± 0.02 (mean \pm s.e.m.; $n = 24$) in young to 0.04 ± 0.01 ($n = 43$) in mature hearts. This shift was correlated with a decrease in tension cost (i.e. ATP consumption per second per trabecula volume/force per cross-sectional area) from 4.1 ± 0.2 mmol kN⁻¹ m⁻¹ s⁻¹ ($n = 23$) in young to 2.5 ± 0.1 mmol kN⁻¹ m⁻¹ s⁻¹ ($n = 57$) in mature hearts.
3. From the results it follows that the slow β -MHC isoform, which predominates in hearts of mature guinea-pigs, is about 5 times more economical than the fast α -MHC isoform. Calcium sensitivity of force and ATP consumption decreased with age, but stabilized within a few weeks after birth. The pronounced dependence of cardiac energetics on MHC composition should be taken into account in long-term studies of cardiac overload.

In the heart the expression of different proteins of the contractile apparatus changes during development and under pathological conditions (Swynghedauw, 1986). Such changes in protein expression have important consequences for cardiac energetics. Usually these changes are adaptations to new functional demands of the heart. For instance, during cardiac hypertrophy, an improved economy of contraction (Alpert & Mulieri, 1982) and a decrease in maximal velocity of shortening (Schwartz, Lecarpentier, Martin, Lompré, Mercadier & Swynghedauw, 1981; Ebrecht, Rupp & Jacob, 1982) and in the rate of tension recovery after quick stretch (Ventura-Clapier, Mekhfi, Oliviero & Swynghedauw, 1988) have been observed.

Myosin is one of the main proteins of the contractile apparatus. Together with actin, myosin takes part in the mechanism by which chemical energy of adenosine triphosphate (ATP) is converted to mechanical work. Hoh, McGrath & Hale (1977) have shown that two different myosin heavy chain isoforms exist in the heart: α -myosin heavy chain (α -MHC) and β -myosin heavy chain (β -MHC). The MHCs carry the site for the ATPase activity. In association with the myosin light chains, the two MHC isoforms give rise to three different isomyosins: the homo-

dimers V1, composed of two α -MHCs, and V3, composed of two β -MHCs, and a heterodimer of α -MHC and β -MHC, named V2. It has been observed *in vitro* that V1 has the highest myosin ATPase activity and V3 the lowest. V2 has an ATPase activity intermediate between V1 and V3 (Pope, Hoh & Weeds, 1980).

In adult rats V1 is present almost exclusively in the heart ventricles. During cardiac hypertrophy, a reduced ATPase activity is found, which is correlated with a shift from the fast isomyosin V1 to the slow isomyosin V3 (Mercadier *et al.* 1981; Gorza, Pauletto, Pessina, Sartore & Schiaffino, 1981).

We have studied the ventricular MHC composition, maximum force and rate of ATP consumption (i.e. ATPase activity) during isometric heart contraction in guinea-pigs, because the MHC composition in their hearts is similar to the MHC composition in human hearts. The distribution of α -MHC and β -MHC was examined in both young (1- to 8-week-old) and mature (9- to 26-week-old) guinea-pigs using two specific monoclonal antibodies directed against α -MHC and β -MHC in an enzyme-linked immunosorbent assay (ELISA). To relate the MHC isoforms expressed with the economy of contraction, the maximum isometric force and the rate of ATP consumption were measured in

chemically skinned trabeculae isolated from right and left ventricles. In the method used, the resynthesis of ATP is enzymatically coupled to the oxidation of reduced nicotinamide-adenine dinucleotide (NADH), which can be quantified photometrically. An advantage of this method is that it allows determination of the contractile and energetic properties simultaneously. Moreover, by standardization of the conditions (i.e. composition of the intracellular medium and sarcomere length) disturbing factors present in the intact heart (i.e. hormonal factors and variable calcium concentrations) are minimized. Since the heart normally works under submaximal circumstances the calcium sensitivity of force production and ATPase activity were also determined.

The age-dependent changes observed are important, not only because they might alter cardiac performance and interfere with the interpretation of changes in cardiac performance after cardiac overload, but also because they reflect a steep relation between energetic properties and MHC composition, which might be partly responsible for conflicting findings in the literature on myosin ATPase activity of human myocardium *in vitro* (e.g. Alpert & Gordon, 1962; Mercadier *et al.* 1983; Alousi, Grant, Etzler, Cofer, Van der Bel-Kahn & Melvin, 1990).

METHODS

Preparations

The adult guinea-pigs (Dunkin Hartley, female) studied were divided into two groups: young (150–450 g, 1–8 weeks old; $n = 16$) and mature (500–900 g, 9–26 weeks old; $n = 27$). The guinea-pigs were anaesthetized with xylazine (5 mg kg⁻¹ i.m.), ketamine (60 mg kg⁻¹ i.m.) and pentobarbitone (50 mg kg⁻¹ i.p.), and the hearts were rapidly excised. After excision, the heart was immediately perfused with Tyrode solution (mM: NaCl, 128.3; KCl, 4.7; CaCl₂, 1.36; MgCl₂, 1.05; NaHCO₃, 20.2; NaH₂PO₄, 0.42; D-(+)-glucose monohydrate, 11.1) containing 20 mM 2,3-butanedione monoxime. Unbranched trabeculae were carefully dissected from the walls of the right and left ventricles (RVs and LVs, respectively). After dissection, the trabeculae were transferred to a dish containing standard relaxing solution to which 1% (v/v) Triton X-100 was added in order to chemically permeabilize the preparation. The composition of this standard relaxing solution was (mM): Na₂ATP, 6.13; MgCl₂, 6.56; EGTA, 20; phosphocreatine, 10; Bes, 100; pH 7.1, adjusted with KOH; ionic strength, 200 mM, adjusted with potassium propionate. The preparations were left in this solution for 2 h at about 5 °C to allow solubilization of virtually all membranous structures. Next, the skinned trabecula was attached to aluminium T-clips and mounted in the experimental set-up.

The dimensions of the preparations from young animals were 1.68 ± 0.11 and 1.51 ± 0.11 mm in length, 239 ± 18 and 181 ± 14 μm in width, and 241 ± 18 and 172 ± 14 μm in thickness (means \pm s.e.m.) for, respectively, RVs ($n = 11$) and LVs ($n = 12$), measured at a resting sarcomere length of 2.2 μm. The dimensions of the right ($n = 24$) and left ($n = 33$) ventricular preparations from mature animals were, respectively, 2.01 ± 0.12 and 1.76 ± 0.11 mm in length, 215 ± 10 and 211 ± 10 μm in width, and 203 ± 10 and 200 ± 10 μm in thickness.

Experimental apparatus

The skinned trabecula was attached to a force transducer element (AE 801; SensoNor, Horten, Norway) at one end, and to a fixed hook at the other end. The natural frequency of the force transducer was about 2 kHz. The muscle preparation could be transferred manually between several baths to expose the trabecula to various solutions. The measuring bath used for the ATPase assay (volume, 30 μl) had thin glass windows to allow transmission of near-UV light (340 nm) for the measurement of NADH absorbance. The contents of this bath were stirred continuously by motordriven vibration (frequency about 3 Hz) of a membrane positioned at the bottom of the bath. The temperature inside the bath was kept at 20 ± 1 °C. The absorbance and force signals were filtered at 2.5 Hz (-12 dB per octave) and recorded on an Olivetti M280 personal computer after A/D conversion at a sampling frequency of 5 Hz. The sarcomere length of the preparation was measured in relaxing solution by means of He-Ne laser diffraction (Model 1125; Uniphase).

Measurement of ATP consumption

ATP consumption of the skinned trabeculae was measured by an enzyme-coupled assay as described in detail previously (Ebus, Stienen & Elzinga, 1994). After ATP hydrolysis into adenosine diphosphate (ADP) and inorganic phosphate (P_i), the ADP formed was resynthesized to ATP. This reaction was enzymatically coupled to the oxidation of NADH to NAD⁺. This reaction sequence was catalysed by pyruvate kinase and lactate dehydrogenase. The breakdown of NADH was determined photometrically by measuring the absorbance of 340 nm near-UV light obtained from a 75 W xenon lamp, which was projected through the bath just beneath the preparation. Calibration of the NADH absorbance signal was carried out by injecting 0.05 μl of 10 mM ADP (i.e. 0.5 nmol) into the measuring bath.

Solutions

Three solutions were used, a relaxing solution, a pre-activating solution with low-calcium buffering capacity, and an activating solution, which contained, respectively (mM): MgCl₂, 8.13, 7.69 and 7.57; Na₂ATP, 5.82, 5.82 and 5.94; EGTA, 20, 0.5 and 0; HDTA, (hexan-diaminotetraacetic acid), 0, 19.5 and 0; CaEGTA, 0, 0 and 20; KOOCH₂CH₂CH₃ (potassium propionate), 39.4, 40.3 and 40. CaEGTA was made by dissolving equimolar amounts of CaCO₃ and EGTA. In addition, all solutions contained 0.9 mM NADH, 100 mM Bes, 5 mM sodium azide, 10 mM phosphoenolpyruvate, 4 mg ml⁻¹ pyruvate kinase (500 U mg⁻¹), 0.24 mg ml⁻¹ lactate dehydrogenase (870 U mg⁻¹), 10 μM oligomycin B, and 0.2 mM P¹,P⁵-di(adenosine-5')pentaphosphate. The experiments were performed at pH 7.0 (adjusted with KOH) and 20 °C. The compositions were calculated as described by Fabiato (1981). The free Mg²⁺ and MgATP concentrations were 1 and 5 mM, respectively. Solutions with lower free Ca²⁺ concentrations were obtained by appropriate mixing of the activating and relaxing solutions, assuming an apparent stability of the CaEGTA complex of 10^{6.58}. All chemicals were of the highest purity available (Sigma).

Experimental protocol

During the experiments, the trabeculae were incubated in relaxing solution for at least 3 min, in pre-activating solution for at least 3 min, in activating solution for 1–5 min, and then returned to the relaxing solution. Before the first activation-relaxation cycle the sarcomere length of the preparation, as measured in relaxing solution, was adjusted to 2.2 μm. Then, following a first activation at saturating calcium concentration (pCa (-log[Ca²⁺]), 4.5), the sarcomere length was readjusted to 2.2 μm if necessary, and the length of the trabecula between the clips as well as the width and

the thickness were measured (at $\times 50$ magnification). It was found that after this readjustment, the resting sarcomere length remained stable throughout the experiment. The second contraction served as a first force and ATP consumption rate reference at a pCa of 4.5. The next two to three contractions were carried out at different pCa values (> 4.5). These measurements were followed by a control measurement at saturating Ca^{2+} concentration. This procedure was repeated until the full force–pCa and ATPase–pCa curves were obtained or until the isometric force of a control measurement was less than or equal to 80% of the first control contraction. Usually eight to fifteen contractions could be performed. To correct for the relatively small deterioration of force development and ATPase activity, values were normalized to the nearest control values.

ATPase activity was derived from linear regression analysis of the absorbance signal. To correct for bleaching of NADH under the intense UV light and contaminant ATPase activity in the enzymes used, the slope of the absorbance signal measured after the trabecula had been returned to the relaxing solution was subtracted from the slope of the absorbance signal measured during the contraction. The Ca^{2+} -activated ATPase activity was corrected for basal ATPase activity measured in relaxing solution (pCa 9) (Ebus *et al.* 1994). Basal activity amounted to about 20% of the maximal ATPase activity.

Quantification of α - and β -myosin heavy chains in guinea-pig heart ventricles

Guinea-pigs were weighed on the day of heart removal. After dissection of the trabeculae, the atria and ventricles were separated and subsequently freeze dried. Dry weights were determined and the tissues were stored at -80°C . Heart weights were normalized to body weight.

In rat ventricular tissue the fast (α) and slow (β) MHC isoforms can be separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and quantified by subsequent laser densitometry. However, as found by others (Lompré *et al.* 1981), this separation was not successful for guinea-pig. To determine the amount of α - and β -MHC in guinea-pig ventricular tissue, an ELISA was performed, using two monoclonal antibodies directed specifically against α - and β -MHCs (mAb 249-5A4 and mAb 169-1D5, respectively). The production of these antibodies has been described previously (De Groot, Lamers & Moorman, 1989).

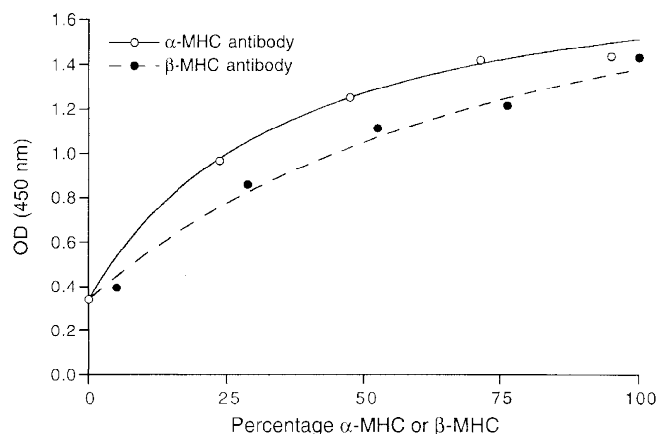
ELISA. Ninety-six-well plates (Greiner) were coated overnight at room temperature with homogenized ventricular tissue ($1\ \mu\text{g well}^{-1}$), diluted in $100\ \mu\text{l}$ phosphate-buffered saline (PBS; 146 mM NaCl, 8 mM Na_2HPO_4 and 2 mM NaH_2PO_4 ; pH 7.35) containing 3%

bovine serum albumin (BSA). After coating, the plates were incubated for 1 h at 37°C with PBS–BSA to reduce non-specific binding. Next, the plates were incubated for 1 h at 37°C with the monoclonal antibodies ($100\ \mu\text{l}$). All samples were applied in duplicate for incubation with the antibody directed against α -MHC (1:50, diluted in PBS–BSA) and also with the antibody against β -MHC (1:100). Following this step, antibody binding was detected using goat anti-mouse–HRP (horseradish peroxidase, Sigma; $100\ \mu\text{l}$; 1:800, diluted in PBS–BSA). Again the incubation took place for 1 h at 37°C . Between each step in the protocol the plates were washed with PBS–Tween 20 (0.05% v/v). The most sensitive chromogenic substrate for detection of HRP-labelled antibodies is tetramethylbenzidine (TMB). Thus, to visualize the immunocomplex formed in the wells, the plates were incubated with $100\ \mu\text{l}$ of $0.1\ \text{mg ml}^{-1}$ TMB (Sigma) and 0.01% hydrogen peroxide in 0.11 M sodium acetate (pH 5.5) for 30 min in the dark. The colour reaction was stopped by adding $50\ \mu\text{l}$ 1 N H_2SO_4 . The plates were read at 450 nm relative to a blank (PBS–BSA added to the wells instead of the antigen in the first step; following steps were the same). The duplicated results were reproducible within 5%. Averaged results were used in the analysis.

Calibration of the ELISA was performed using hypothyroid and euthyroid rat ventricles. The samples were a kind gift from Dr P. P. de Tombe (Chicago, IL, USA). Rats were made hypothyroid by adding $0.8\ \text{g l}^{-1}$ propylthiouracil (PTU) to the drinking water for a period of at least 6 weeks, while euthyroid control rats were housed under the same conditions without PTU. MHC composition in the rat ventricles was determined by SDS–PAGE and subsequent densitometry, as described below. Hypothyroid rat myocardium proved to be 100% β -MHC. The euthyroid control animals contained a mixture of 95% α -MHC and 5% β -MHC. The calibration curves for the ELISA were obtained from the optical density (OD) readings from hypothyroid and euthyroid tissue and from mixtures of these two samples (see Fig. 1). From the curves fitted to the data points the amounts of α - and β -MHC present in the guinea-pig ventricles were calculated. The non-linearity of the relations obtained most probably reflects the binding characteristics of the primary antibodies to the myosin isoforms, because the curvature observed was similar to that found when the total protein concentration of the samples applied was varied. The degree of curvature of the relation between OD and total protein concentration from rat and guinea-pig were very similar. This strongly suggests that the binding characteristics of the two specific antibodies with the MHCs were the same for rat and guinea-pig. Using this method, the α -MHC and β -MHC content could be determined as a fraction of the total MHC content.

Figure 1. Calibration curves for the ELISA

Hypothyroid (100% β -MHC) and euthyroid (95% α -MHC and 5% β -MHC) rat heart samples were mixed in the same proportions as in Fig. 2B to obtain standard samples with known percentages of α - and β -MHC. The percentages of α - and β -MHC in the hypothyroid and euthyroid rat hearts were quantified by separating the MHCs in SDS–PAGE and subsequent densitometric analysis of the bands (see Methods). Hyperbolic relations were fitted to the data points as described in the text.



SDS-PAGE. SDS-PAGE was performed as described previously (Giulian, Moss & Greaser, 1983), using an acrylamide to bis-acrylamide ratio of 200:1 in the separating gel (12% acrylamide; pH 9.3) and of 20:1 in the stacking gel (3.5% acrylamide; pH 6.8). A Protean II xi cell was used (Bio-Rad). The gel dimensions were:

width, 16 cm; length, 20 cm; and thickness, 0.75 mm. The stacking gel was 2.5 cm in length. Samples of the ventricles were dissolved in sample buffer containing 62.5 mM Tris (pH 6.8), 15 mM dithiothreitol, 0.1 mM phenylmethylsulphonyl fluoride, 0.5 mM leupeptin, 1% (w/v) SDS, 0.01% (w/v) bromophenol blue and 15% (v/v)

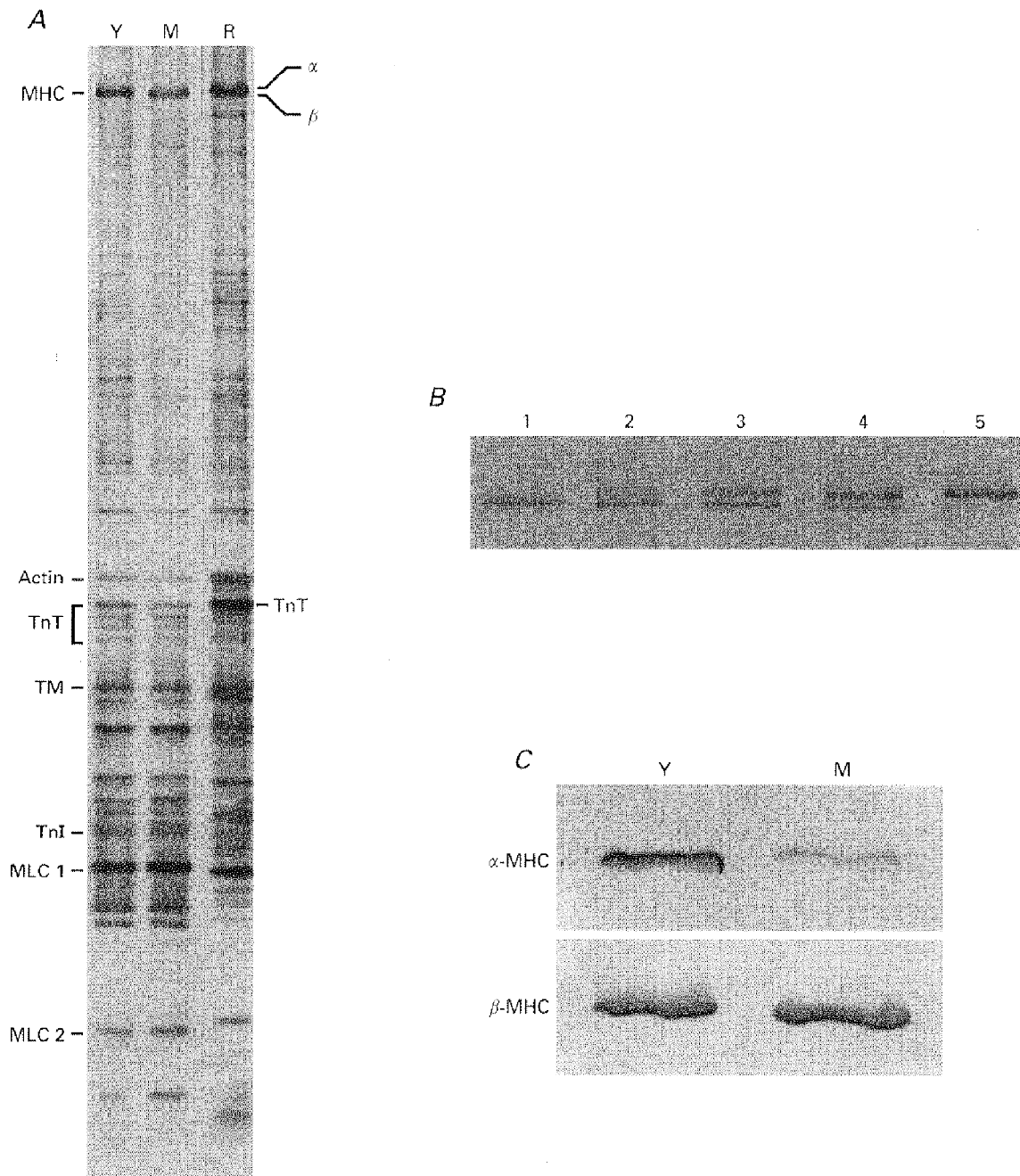


Figure 2. Protein analysis of guinea-pig and rat samples

A, silver-stained SDS-polyacrylamide gel from young (Y) and mature (M) guinea-pig hearts and from euthyroid rat heart (R) (0.8 μ g protein per lane). Contractile proteins were identified by Western immunoblotting with specific antibodies, as indicated in the text: MHC, myosin heavy chain; TnT, troponin T; TM, tropomyosin; TnI, troponin I; MLC, myosin light chain. *B*, separation of the two MHC isoforms in rat heart samples. Lane 1, hypothyroid; lane 5, euthyroid; lanes 2-4, 3:1, 1:1 and 1:3 mixtures of hypo- and euthyroid rat samples, respectively. *C*, although in guinea-pig ventricular muscle the α - and β -MHCs were not separated by SDS-PAGE (*A*, lanes Y and M), the presence of α -MHC and β -MHC in young and mature guinea-pig hearts could be visualized with Western immunoblotting using two monoclonal antibodies directed specifically against α -MHC and β -MHC (0.8 μ g protein was loaded in each lane).

glycerol. Comparable protein amounts ($0.8 \mu\text{g}$) were loaded in each gel lane. The samples were run at constant current (24 mA) for a total of 5 h (approximately 1800 Volt hours). Silver staining was performed as described by Giulian *et al.* (1983). In Fig. 2, examples are shown which indicate that in rat myocardium the α - and β -MHCs were nicely separated, whereas, as has been found by others (Lompré *et al.* 1981), in guinea-pig ventricular muscle the separation was not sufficient to allow a quantitative analysis.

Western immunoblotting. The presence of α - and β -MHC in guinea-pig hearts was also confirmed in Western immunoblots. This technique was also used to determine troponin T (TnT) and troponin I (TnI) content and to investigate TnT and TnI isoform distribution. After electrophoresis, the proteins were transferred onto a nitrocellulose membrane ($0.45 \mu\text{m}$ pore size) using the Bio-Rad TransBlot Cell. After transfer, the nitrocellulose membrane was stained with Ponceau S to demonstrate protein bands. After destaining the membrane in PBS-Tween 20 (0.5%), the nitrocellulose membrane was incubated in 3% BSA in PBS-Tween 20 for 1 h, washed 3 times and incubated for 1 h with various primary antibodies, diluted in BSA-PBS-Tween 20. After removing the primary antibody and washing 3 times, the primary antibody binding was detected using alkaline phosphatase-labelled goat anti-mouse antibody (Promega), diluted 1:7500 in BSA-PBS-Tween 20. Finally, nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Sigma) were used to visualize the specific immunocomplex formed on the nitrocellulose membrane. The primary MHC antibodies used were the same as those used for ELISA, i.e. α -MHC antibody (mAb 249-5A4, 1:50) and β -MHC antibody (mAb 169-

1D5, 1:100). To detect TnT and TnI, TnT antibody (clone JLT-12, 1:200; Sigma) and TnI antibody (mAb 1691, 1:200; Chemicon, Temecula, CA, USA) were used. For further identification of the protein bands on the gels actin antibody (clone C4, 1:100; Boehringer Mannheim), myosin light chain antibody (clone MY-21, 1:200; Sigma) and tropomyosin antibody (clone TM311, 1:400; Sigma) were used.

Data analysis

Sigmoidal force-pCa and ATPase-pCa relations were fitted by a non-linear fit procedure to a modified Hill equation:

$$P(\text{Ca}^{2+})/P_0 = [\text{Ca}^{2+}]^{n_H} / (K^{n_H} + [\text{Ca}^{2+}]^{n_H}),$$

where P is steady-state force (or ATPase activity). P_0 denotes the steady isometric force (or ATPase activity) at saturating Ca^{2+} concentration, n_H represents the steepness of the relationship, and K (or pK) represents the Ca^{2+} concentration (or pCa) at which force (or ATPase activity) = $0.5 \times P_0$, i.e. the mid-point of the force-pCa (ATPase-pCa) relation. P_0 was determined from the first reference activation at a pCa of 4.5. The calibration curves for the ELISA were fitted by means of a hyperbolic function:

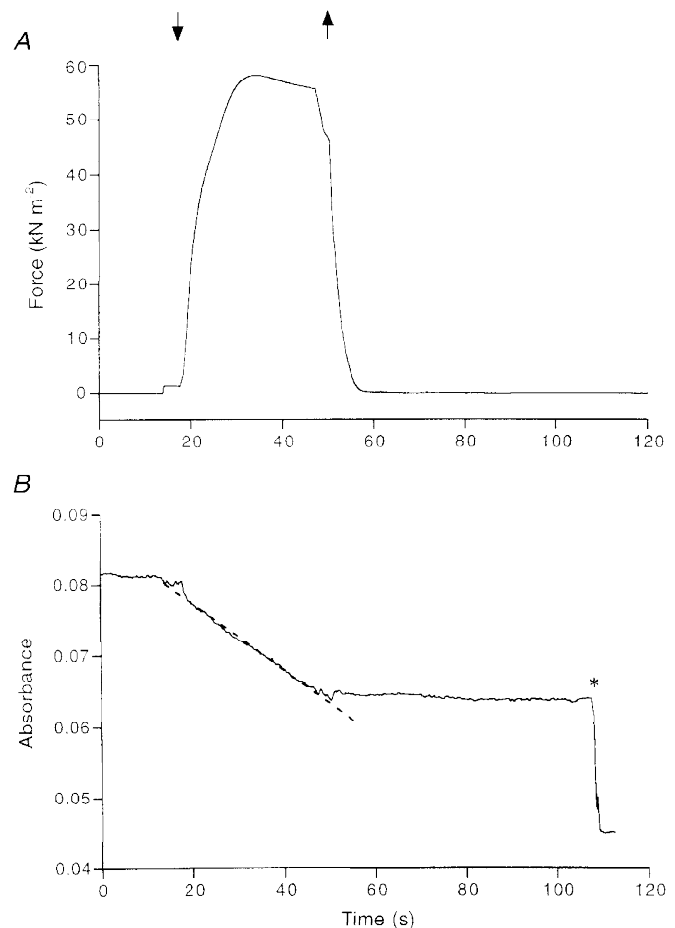
$$\text{OD} = ax/(b + x) + c,$$

where x is the percentage of α - or β -MHC of the total MHC content and a , b and c are constants.

Values are given as means \pm S.E.M. of n experiments. Differences were tested by means of Student's t test at a 0.05 or 0.001 level of significance ($P < 0.05$, $P < 0.001$). The linear regression lines shown in the figures were calculated treating the parameter plotted

Figure 3. Recordings of force production (A) and NADH absorbance (B) during a contraction-relaxation cycle

When the trabecula was transferred to the activating solution (downward arrow), active isometric force developed, and the NADH absorbance signal started to decrease. The rate of decline is a measure of ATP consumption. ATP consumption was determined by calculation of the slope of the dashed regression line fitted to a selected part of the absorbance signal. After returning the trabecula into the relaxing solution (upward arrow), the NADH absorbance baseline was recorded. At the time indicated by the asterisk, 0.5 nmol ADP was injected into the measuring bath to calibrate the absorbance signal. The zero level of the absorbance signal was arbitrarily chosen.



along the x -axis as an independent variable. Differences between the slopes of regression lines, obtained with results from left and right ventricles, were tested using a multiple slope comparison (Sokal & Rohlf, 1981).

RESULTS

ATP consumption and force production in chemically skinned trabeculae were measured during isometric (fixed ends) contraction. Original recordings obtained during a contraction-relaxation cycle are shown in Fig. 3. In this figure, the isometric force production (*A*) and ATPase activity (*B*) at a sarcomere length of $2.2 \mu\text{m}$ are shown. Following transfer of the trabecula to the activating solution, active isometric force developed and the NADH absorbance started to decrease. After the isometric contraction, the trabecula was returned to the relaxing solution. Recording of NADH absorbance in the measuring bath was continued for some time to allow determination of the background decline in NADH absorbance.

The Ca^{2+} sensitivity of force and ATPase activity

The Ca^{2+} sensitivity of the contractile apparatus of the hearts of very young (1- to 2.5-week-old; $n = 7$) and mature (9- to 19-week-old; $n = 5$) guinea-pigs was determined by exposing the trabeculae to different Ca^{2+} concentrations and measuring the isometric force and ATPase activity. The mean force-pCa and ATPase-pCa relationships are shown in Fig. 4*A* and *B*, respectively. The isometric force and ATPase activity values were fitted to the Hill equation. The steepness of the

force-pCa and ATPase-pCa relations did not differ significantly between very young (respectively, 3.26 ± 0.49 and 2.96 ± 0.37) and mature (respectively, 4.01 ± 0.57 and 3.43 ± 0.34) guinea-pigs. The pK values representing the mid-points of the force-pCa and ATPase-pCa relations were considerably higher (0.16 pCa units) in very young guinea-pigs (respectively, 5.74 ± 0.02 and 5.73 ± 0.02 pCa units) than in mature animals (respectively, 5.58 ± 0.02 and 5.57 ± 0.01 pCa units), indicating a decreased calcium responsiveness in mature guinea-pigs. In a separate study (second group), the calcium sensitivity of isometric force and ATPase activity in young guinea-pigs (4-8 weeks old; $n = 9$) and mature guinea-pigs (9-26 weeks old; $n = 22$) were compared. No differences were found between these groups. This indicates that the decline in calcium responsiveness stabilizes quickly after birth.

It should be noted that the results from the first group (very young and mature animals) and from the second group (young and mature animals) were obtained with two different batches of EGTA. Although a correction was made for differences in EGTA purity, the results of calcium sensitivity in the mature groups differed slightly, probably due to minor differences in the free calcium concentration. However, this does not affect the results obtained at saturating calcium concentration presented below.

Since the difference in calcium sensitivity between very young and mature guinea-pigs might be due to changes in the troponin complex (Godt, Fogaça & Nosek, 1990; Reiser,

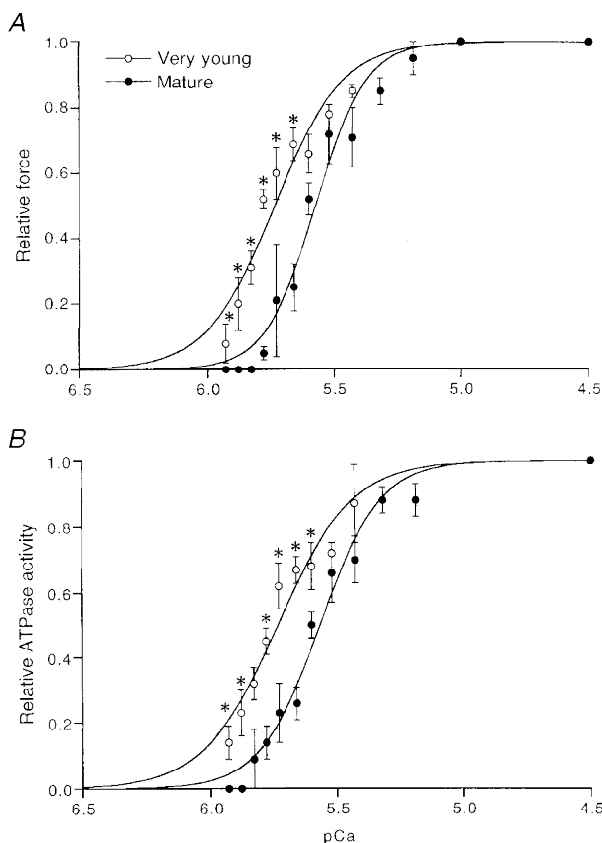


Figure 4. Isometric force and ATPase activity at different free Ca^{2+} concentrations

Isometric force (*A*) and ATPase activity (*B*) for each trabecula are normalized to the control force and ATPase activity found at saturating Ca^{2+} concentration (pCa = 4.5). Mean values are shown \pm s.e.m. * $P < 0.05$, significantly different from mature guinea-pigs. The Hill curves were fitted to the data points as indicated in the text.

Westfall, Schiaffino & Solaro, 1994), the composition of TnT and TnI were compared in very young and mature guinea-pigs, using SDS-PAGE and Western immunoblotting. Actin was used as an internal standard in these determinations. The silver-stained gels and immunoblots both showed that the molecular weights and ratios of TnT and TnI to actin did not vary significantly between very young and mature guinea-pigs. The ratios of the different TnT isoforms, TnT1, TnT2, TnT3 and TnT4, and of TnI to actin were, respectively, in very young guinea-pigs: 0.49 ± 0.02 , 0.20 ± 0.01 , 0.16 ± 0.01 , 0.10 ± 0.01 , and 0.36 ± 0.01 , and in mature guinea-pigs: 0.54 ± 0.02 , 0.21 ± 0.02 , 0.15 ± 0.02 , 0.10 ± 0.01 and 0.44 ± 0.05 .

Force and ATPase activity versus body weight

For all trabeculae measured in this study, maximum force per cross-sectional area and ATPase activity per trabecula volume were calculated. To determine whether these parameters are age dependent, results were presented in Fig. 5 as a function of body weight. Body weight is a reliable index of age because the growth curves of the animals show

little individual variation. Since the number of suitable trabeculae per heart on which measurements could be performed varied, the results of all trabeculae were plotted as a function of body weight. Plotting the mean data as a function of body weight yielded very similar results. Both force per cross-sectional area and ATPase activity per volume gave rise to a scattered pattern of values. Part of this scatter may be due to inaccuracy in the determination of the cross-sectional area of the trabeculae. The regression coefficients (*r*) for the relation between ATPase activity per volume and body weight were 0.65 and 0.47 for LV and RV, respectively (Fig. 5A). The slopes of both regression lines were both significantly different from zero ($P < 0.001$). The regression coefficients for the relation between maximum force per cross-sectional area and body weight for LV and RV were 0.27 and 0.17, respectively (Fig. 5B); the slopes of both regression lines were not significantly different from zero. Tension cost was calculated for each trabecula by dividing the ATPase activity per volume by the force per cross-sectional area. The tension cost informs us about the

Figure 5. Dependence of ATP consumption, isometric force and tension cost on body weight

A, rate of ATP consumption per volume; B, isometric force per cross-sectional area; C, tension cost. BW, body weight; LV, left ventricular trabeculae; RV, right ventricular trabeculae.

Regression lines shown: LV: ATPase activity per volume = $(0.21 \pm 0.02) - (14.0 \pm 2.3) \times 10^{-5} \times BW$ ($r = 0.65$; $P < 0.001$), force per cross-sectional area = $(35.4 \pm 4.9) + (14.0 \pm 7.6) \times 10^{-3} \times BW$ ($r = 0.27$), tension cost = $(5.65 \pm 0.35) - (4.4 \pm 0.6) \times 10^{-3} \times BW$ ($r = 0.78$; $P < 0.001$). RV: ATPase activity per volume = $(0.16 \pm 0.02) - (8.2 \pm 2.7) \times 10^{-5} \times BW$ ($r = 0.47$; $P < 0.001$), force per cross-sectional area = $(34.5 \pm 4.5) + (6.8 \pm 6.9) \times 10^{-3} \times BW$ ($r = 0.17$), tension cost = $(4.44 \pm 0.27) - (2.6 \pm 0.4) \times 10^{-3} \times BW$ ($r = 0.74$; $P < 0.001$).

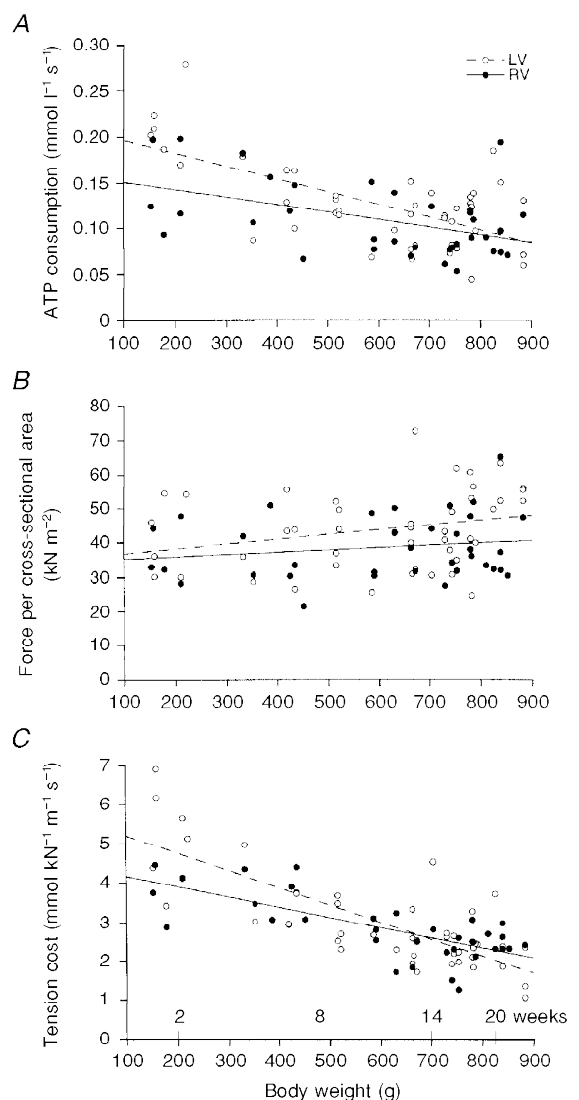


Table 1. Differences between young and mature adult guinea-pigs

Parameter	Young	Mature
ATPase activity per volume ($\text{mmol l}^{-1} \text{s}^{-1}$)		
LV + RV	0.16 ± 0.01 (23)	0.10 ± 0.01 (57)*
LV	0.18 ± 0.02 (12)	0.11 ± 0.01 (33)*
RV	0.14 ± 0.01 (11)	0.10 ± 0.01 (24)*
Force per cross-sectional area (kN m^{-2})		
LV + RV	38.3 ± 2.1 (23)	42.9 ± 1.5 (57)
LV	40.5 ± 3.1 (12)	45.0 ± 2.0 (33)
RV	35.9 ± 2.7 (11)	39.9 ± 1.9 (24)
Tension cost ($\text{mmol kN}^{-1} \text{m}^{-1} \text{s}^{-1}$)		
LV + RV	4.1 ± 0.2 (23)	2.5 ± 0.1 (57)*
LV	4.4 ± 0.4 (12)	2.5 ± 0.1 (33)*
RV	3.8 ± 0.2 (11)	2.4 ± 0.1 (24)*
Heart weight per body weight (mg (100 g)^{-1})	58.1 ± 0.9 (11)	52.6 ± 1.6 (22)†

ATPase activity per volume, force per cross-sectional area and tension cost are given for all trabeculae studied and for LV and RV trabeculae separately. Mean values are given \pm S.E.M. * $P < 0.001$, † $P < 0.05$, significantly different from young guinea-pigs. The number of observations is given in parentheses.

rate of ATP splitting necessary for maintenance of isometric force. It is a measure of muscle economy, which has the advantage that it is not influenced by the inaccuracy in the determination of cross-sectional area. The relation between tension cost of the trabeculae and body weight of the guinea-pigs is shown in Fig. 5C. Statistical analysis revealed highly significant correlations ($r = 0.78$ for LV and 0.74 for RV; $P < 0.001$) between tension cost and body weight. This figure clearly shows that the tension cost of cardiac contraction declines with age.

Differences exist between trabeculae from left and right ventricles. Firstly, the slopes of the relations between ATPase activity and between tension cost and body weight differed significantly between LV and RV ($P < 0.05$), although there was no difference in mean ATPase activity per volume and in tension cost between LV and RV (respectively, $0.13 \pm 0.01 \text{ mmol l}^{-1} \text{ s}^{-1}$ (LV) and $0.11 \pm 0.01 \text{ mmol l}^{-1} \text{ s}^{-1}$ (RV), and $3.0 \pm 0.2 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$ (LV) and $2.9 \pm 0.1 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$ (RV)). The differences

in ATPase activity per volume and tension cost between young and mature guinea-pigs were highly significant ($P < 0.001$). These differences were significant in both left and right ventricular trabeculae (Table 1). The regression coefficients of the relations between force per cross-sectional area and body weight did not differ between LV and RV. However, there was a small but significant difference in the mean force per cross-sectional area between LV ($43.8 \pm 1.7 \text{ kN m}^{-2}$) and RV ($38.6 \pm 1.6 \text{ kN m}^{-2}$) ($P < 0.05$). This is mainly due to the difference between LV and RV in mature guinea-pigs. The mean force per cross-sectional area between young and mature guinea-pigs did not vary significantly, in both LVs and RVs (Table 1).

Age dependence of MHC composition

To see if the age-dependent changes in ATP consumption and tension cost are accompanied by a change in the myosin isoform pattern, we determined the MHC composition of the ventricular tissues by means of ELISA. Freeze-dried hearts were weighed first and total heart weights were

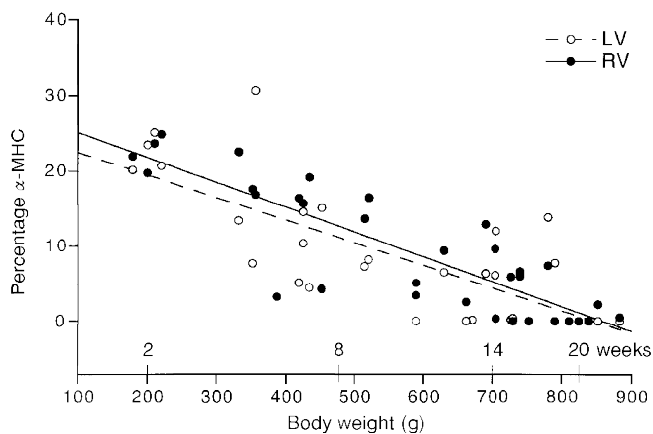


Figure 6. The α -MHC fraction of total MHC content plotted as a function of body weight

A negative correlation exists between the percentage of α -MHC and body weight. LV: percentage of α -MHC = $(25.5 \pm 2.8) - (3.0 \pm 0.5) \times 10^{-2} \times \text{BW}$ ($r = 0.76$; $P < 0.001$). RV: percentage of α -MHC = $(28.4 \pm 2.2) - (3.3 \pm 0.4) \times 10^{-2} \times \text{BW}$ ($r = 0.86$; $P < 0.001$).

Table 2. ATP consumption per volume and tension cost for V1 and V3

Parameter	V1 ($\alpha\alpha$)	V3 ($\beta\beta$)	Ratio
ATPase activity per volume (mmol l ⁻¹ s ⁻¹)			
LV + RV (<i>n</i> = 63)	0.39 ± 0.06	0.10 ± 0.01	3.9
LV (<i>n</i> = 35)	0.50 ± 0.09	0.10 ± 0.01	5.0
RV (<i>n</i> = 28)	0.33 ± 0.08	0.09 ± 0.01	3.7
Tension cost (mmol kN ⁻¹ m ⁻¹ s ⁻¹)			
LV + RV (<i>n</i> = 63)	10.95 ± 1.17	2.28 ± 0.13	4.8
LV (<i>n</i> = 35)	12.99 ± 2.03	2.25 ± 0.19	5.8
RV (<i>n</i> = 28)	9.60 ± 1.21	2.27 ± 0.15	4.2

ATPase activity per volume and tension cost of the homodimers V1 ($\alpha\alpha$) and V3 ($\beta\beta$), calculated from the regression lines between ATPase activity per volume and between tension cost and MHC composition. Intercept values of the different regression lines are given ± S.E.M.

normalized for body weight. These results are included in Table 1. The increase in body weight with age was larger than the increase in heart weight, resulting in a significantly lower heart weight to body weight ratio in mature guinea-pigs. Figure 6 illustrates the results obtained with ELISA. The mean percentage of α -MHC from all guinea-pig hearts studied did not differ between LV and RV (8.0 ± 1.4 and $9.3 \pm 1.5\%$, respectively). The fraction of α -MHC decreased with age, while the β -MHC fraction increased. The total ($\alpha + \beta$)-MHC content in the ventricles, expressed relative to the total MHC content in the rat, did not change significantly (MHC ratio in young: 0.77 ± 0.05 , and in mature: 0.85 ± 0.03). This suggests that the changes in α - and β -MHC are complementary (i.e. α -MHC is replaced by β -MHC). It is evident from Fig. 6 that there exists a strong correlation between the MHC composition and body weight (LV: $r = 0.76$, RV: $r = 0.86$). The α -MHC fraction decreased in the pooled data from 0.17 ± 0.02 ($n = 24$) in young to 0.04 ± 0.01 ($n = 43$) in mature guinea-pig hearts. Tension cost decreased significantly from

4.1 ± 0.2 mmol kN⁻¹ m⁻¹ s⁻¹ in young to 2.5 ± 0.1 mmol kN⁻¹ m⁻¹ s⁻¹ in mature adult guinea-pigs ($P < 0.001$; Fig. 7). The α - and β -MHC isoforms from guinea-pig could not be separated by SDS-PAGE, but the shift in MHC composition with age obtained in ELISA could be confirmed with Western immunoblotting (Fig. 2C).

Force and ATPase activity versus MHC composition

Because of this age-dependent shift in MHC composition we also studied isometric force per cross-sectional area and ATPase activity per volume as a function of MHC composition. A significant correlation was found between ATPase activity per volume and MHC composition ($r = 0.60$ for LV and 0.51 for RV; Fig. 8A), but not between isometric force per cross-sectional area and MHC composition (LV: $r = 0.16$, RV: $r = 0.08$; Fig. 8B). The slopes of the relations between ATPase activity and MHC composition for LV and RV were significantly different; however, the mean ATPase activity per volume was not significantly different between LV and RV. The ATPase activity per volume for the homodimers $\alpha\alpha$ (V1) and $\beta\beta$ (V3) can be calculated from the

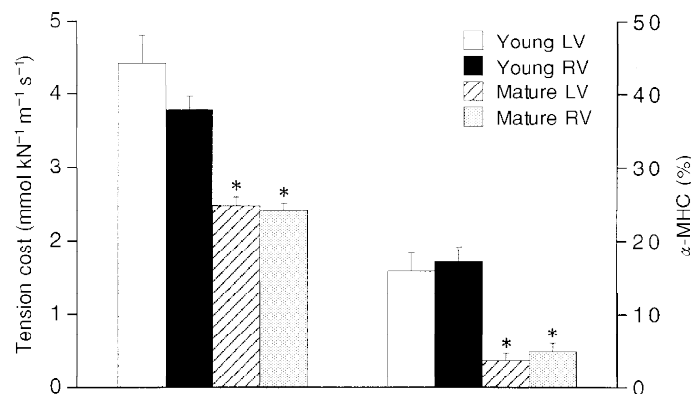


Figure 7. Differences in tension cost (left) and percentage of α -MHC (right) between young (1- to 8-week-old) and mature (9- to 26-week-old) guinea-pigs

Mean values ± S.E.M. * $P < 0.001$, significantly different from young guinea-pigs (Student's *t* test). No significant differences were found between LV and RV.

overall regression lines between ATPase activity per volume and MHC composition for all trabeculae studied. From the intercepts of the regression lines it follows that ATPase activity per volume for V1 ($0.39 \pm 0.06 \text{ mmol l}^{-1} \text{ s}^{-1}$) was larger than that for V3 ($0.10 \pm 0.01 \text{ mmol l}^{-1} \text{ s}^{-1}$). Given the large number of data points ($n = 63$), the 95% confidence limits of these estimates are given by 1.96 times the standard error of the estimate. Values obtained for LV and RV trabeculae separately are given in Table 2.

To confirm that the age-dependent change in ATPase activity is caused by a change in the MHC composition, two mature guinea-pigs were subcutaneously injected daily with thyroid hormone (3,5,3'-triiodothyronine; T_3 , Sigma) for 1 week ($0.1 \text{ mg (kg body weight)}^{-1}$) to increase the fraction of α -MHC. The α -MHC percentage increased in T_3 -treated guinea-pigs to 22.1%. Consequently, the ATPase activity per volume ($0.18 \pm 0.03 \text{ mmol l}^{-1} \text{ s}^{-1}$, $n = 4$) and tension cost ($4.6 \pm 0.4 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$, $n = 4$) increased significantly compared with age-matched guinea-pigs.

The relation between tension cost and MHC composition is shown in Fig. 8C. From this figure it follows that the decrease in tension cost observed with age is correlated with a decrease in the fraction of α -MHC present in the heart ventricles. The slopes of the tension cost–MHC composition relations were significantly different between LV and RV; however, mean tension cost was not significantly different. Tension cost of V1 and V3 for LV and RV trabeculae separately are shown in Table 2. It follows, from the intercepts of the regression lines between tension cost and MHC composition for all trabeculae studied, that the tension cost of V1 is equal to $10.95 \pm 1.17 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$ and that of V3 amounts to $2.28 \pm 0.13 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$. The 95% confidence limits of V1 and V3 were 10.95 ± 2.29 and $2.28 \pm 0.24 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$, respectively. Thus, the slow β -MHC isoform, which is predominantly present in mature guinea-pigs, is about 5 times more economical than the fast α -MHC isoform.

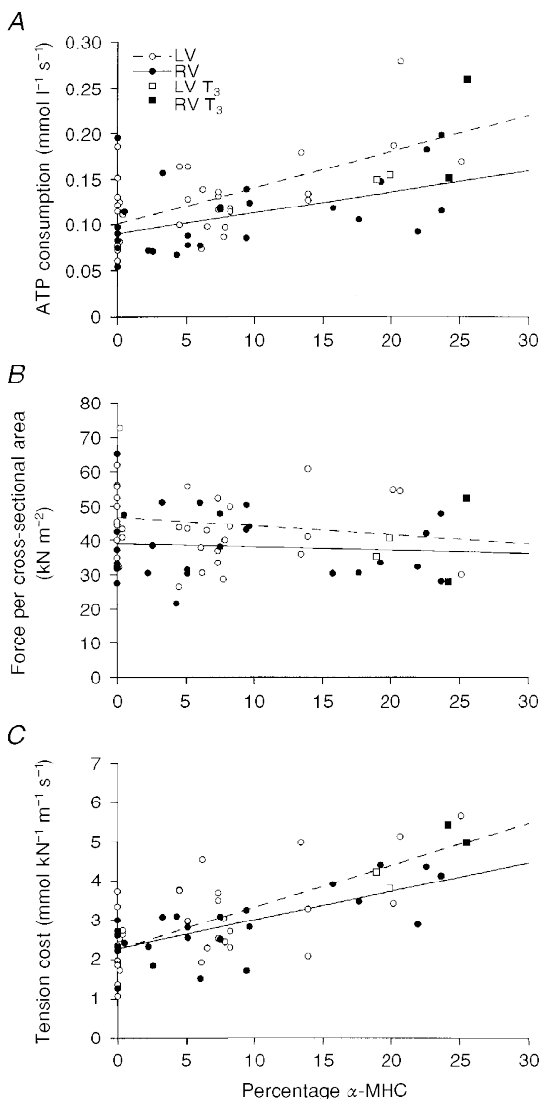


Figure 8. Dependence of ATP consumption, isometric force and tension cost on α -MHC

A, rate of ATP consumption per volume; B, isometric force per cross-sectional area; C, tension cost. % α , α -MHC expressed as a percentage of total MHC concentration; LV T_3 and RV T_3 , respectively, left and right ventricular trabeculae from T_3 -treated guinea-pigs. Regression lines shown: LV: ATPase activity per volume = $(0.10 \pm 0.01) + (4.0 \pm 0.9) \times 10^{-3} \times \% \alpha$ ($r = 0.60$; $P < 0.001$), isometric force = $(46.6 \pm 2.4) - (0.26 \pm 0.28) \times \% \alpha$ ($r = 0.16$), tension cost = $(2.25 \pm 0.19) + (0.11 \pm 0.02) \times \% \alpha$ ($r = 0.66$; $P < 0.001$). RV: ATPase activity per volume = $(0.9 \pm 0.01) + (2.4 \pm 0.8) \times 10^{-3} \times \% \alpha$ ($r = 0.51$; $P < 0.001$), isometric force = $(39.0 \pm 2.6) - (0.09 \pm 0.23) \times \% \alpha$ ($r = 0.08$), tension cost = $(2.27 \pm 0.15) + (0.07 \pm 0.01) \times \% \alpha$ ($r = 0.74$; $P < 0.001$).

Calcium sensitivity versus MHC composition

We also performed regression analysis on the Hill parameters describing calcium sensitivity (n_H and pK) and MHC composition. A significant correlation was obtained for the results from the first experimental group (including very young guinea-pigs; 1–2.5 weeks old), but not for the second experimental group (> 4 weeks old). Since the MHC composition varied substantially in the second group, it is concluded that the MHC composition cannot be a major determinant of the early change in calcium sensitivity observed in the first group.

DISCUSSION

The main findings of this study are that in guinea-pig hearts there is a shift with age towards slow and more economical myosin isoforms. This replacement of fast α -MHCs by slow β -MHCs takes place at a moderate scale, but as β -MHCs are about 5 times more economical than α -MHCs, this causes appreciable changes in cardiac energetics. For guinea-pigs older than 4 weeks, the age-dependent shift in myosin heavy chain composition is not accompanied by a change in calcium sensitivity.

Comparison with previous studies

The mean maximum steady-state force at saturating Ca^{2+} concentration amounted to $41.5 \pm 1.2 \text{ kN m}^{-2}$. The mean rate of ATP consumption accompanying this level of steady-state force was 0.16 and $0.10 \text{ mmol l}^{-1} \text{ s}^{-1}$ in young and in mature hearts, respectively (Table 1). Assuming a myosin head concentration of 0.16 mM (Barsotti & Ferenczi, 1988) this would result in an ATP hydrolysis rate of 1.0 s^{-1} in young and 0.63 s^{-1} in mature hearts. If the difference in temperature during the experiments is taken into account, our values are similar to the value found by Barsotti & Ferenczi (1988) in guinea-pig at 12°C . However, the values in guinea-pig heart are about 4 times lower than those found in the rat (Ebus *et al.* 1994). Even taking into account that the rat hearts used in previous studies contained a mixture of both α - and β -MHC and that guinea-pigs might contain somewhat less myosin in comparison to rats ($\sim 10\%$, see Results), this difference cannot be due entirely to a difference in the myosin isoforms present. By measuring heat production and tension generation in tetanized papillary muscles, Gibbs & Loiselle (1978) found that rats used more energy to maintain tension than guinea-pigs and suggested that this was due to species differences. Our results extend these observations and suggest that corresponding MHC isoforms in rats and guinea-pigs are intrinsically different with respect to their ATPase activity. However, caution should be exerted in extrapolating these results to cardiac performance *in vivo* since the heart does not contract isovolumetrically nor is it maximally activated.

Age-dependent changes in MHC composition

Age-associated changes in the myosin composition have been found previously in other animal models. For instance, in the rat, an isomyosin shift towards fast MHCs was

observed with age (Watras, 1981); the slow V3 disappeared from the ventricles after birth and was replaced by fast V1. Since the *in vitro* ATPase activity of V1 is higher than that of V3 (Pope *et al.* 1980), this shift correlated with an increase in the ATPase activity observed from birth to the adult stage. In rabbit hearts, V1 present in young animals is eventually completely replaced by V3 (Lompré *et al.* 1981). Our results indicate that a shift in the same direction occurs in the guinea-pig. This shift is associated with a decrease in the rate of ATP consumption, while isometric force per cross-sectional area remains unchanged, which consequently results in a functional change in the energetic cost of force maintenance.

It has been suggested that thyroid hormone mediates the shift in isomyosins found with ageing (Hoh *et al.* 1977). Indeed, thyroid hormone is involved in the expression of myosin isoforms (Martin, Pagani & Solaro, 1982; Effron, Bhatnagar, Spurgeon, Ruaño-Arroyo & Lakatta, 1987) and the implications for cardiac energetics are now well established (Leijendekker, van Hardeveld & Elzinga, 1987; Hasenfuss *et al.* 1991). Also, in our study, the T_3 treatment changed the MHC composition in guinea-pig ventricles. The shift in MHC composition is such that it could explain quantitatively the change in economy.

The calcium sensitivity of force production and ATPase activity were highest in very young guinea-pigs (Fig. 4), while they remained the same after 4 weeks of age ($> 300 \text{ g}$). These findings are in agreement with a previous study of force development in enzymatically digested cardiac myocytes (van der Velden, Strang, Stienen & Moss, 1997). Since the MHC composition still changes after this period (Fig. 6), calcium sensitivity of force production and ATPase activity do not seem to be influenced by the type of MHC isoform present. Bhatnagar, Walford, Beard, Humphreys & Lakatta (1984) did not find an altered force–pCa relation with ageing in rat papillary muscle. Reiser *et al.* (1994) did find a comparable change, of about 0.2 – 0.3 pCa units, in the calcium sensitivity of force production in rat hearts during development from neonatal to the adult stage, as we have found. Similar results were obtained in chicken hearts (Godt *et al.* 1990). It can be concluded that the decrease in calcium sensitivity stabilized quickly after birth. The changes found by Reiser *et al.* (1994) were ascribed to shifts in TnT and TnI isoforms during development from neonatal to adult stage. We did not find changes in the TnT and TnI composition between very young and mature guinea-pigs, both in SDS–PAGE and Western immunoblotting. Since only one TnI isoform was found it is possible that our TnI antibody was unable to detect different isoforms. Therefore a change in the TnI composition cannot be completely excluded. Other factors that might be involved in the change in calcium sensitivity of isometric force and ATPase activity could be the state of phosphorylation of troponin isoforms (Solaro & Van Eyk, 1996; Li *et al.* 1997) and myosin light chain 2 (Morano, Rösch, Arner & Rüegg, 1990). An illustration that factors other than MHC composition are

involved in the age-dependent change in calcium sensitivity of force development is given by Gibson, Wendt & Stephenson (1992). A full investigation of these factors in guinea-pig is beyond the scope of this study.

Model calculations and recent results of *in vitro* motility measurements (Alpert, Mulieri & Hasenfuss, 1992; VanBuren, Harris, Alpert & Warshaw, 1995) indicate that the average force per cross-bridge of purified V3 myosin is larger than that of V1 by a factor of 2–4. The force per cross-sectional area in young and mature guinea-pig hearts was the same (Table 1) and also no significant correlation was found between the force per cross-sectional area and MHC composition (Fig. 8B). However, the relatively large error in the estimation of the force per cross-sectional area may obscure possible differences in force per cross-bridge of pure α - and β -MHCs.

Implications for cardiac overload

The myosin composition not only changes during development and with age, but also under pathological conditions. During cardiac hypertrophy in rats, a shift from the fast isomyosin V1 to the slow isomyosin V3 occurs (Mercadier *et al.* 1981; Gorza *et al.* 1981). This change appears to be functional, because with cardiac overload more economical isoforms are expressed. It should be noted that in this respect the rat heart cannot be compared with the human heart, since V3 already predominates in the adult human ventricles. The guinea-pig heart, however, resembles the human heart more closely, since they both contain predominantly V3 during the adult stage.

The fraction of α -MHC decreased from 0.17 ± 0.02 in young to 0.04 ± 0.01 in mature adult guinea-pigs. This age-dependent shift in myosin isoform composition might interfere with changes found after cardiac overload. Malhotra, Siri & Aronson (1992) used an aortic banding technique to induce cardiac hypertrophy in guinea-pigs. A decreased myosin ATPase activity was found in the hypertrophied left heart ventricles. The animals used weighed 225–275 g at the time of the operation and when killed they weighed 300–900 g, which on the basis of our results implies that age-related changes might have interfered with their results. Malhotra *et al.* (1992) have already suggested that the decrease in ATPase activity could be the result of normal growth. Our results suggest that this is the case, and provide evidence that in long-term studies of cardiac overload age dependence of the myosin composition should be taken into account.

Molecular basis of changes in economy

The natural shift in MHC composition with age in guinea-pig hearts can also be used to explore the energetics of 'pure' isomyosins. In our experiments, isometric force and ATP consumption were measured, which have the advantage that they directly provide information on energy utilization and economy of contraction. Furthermore, the measurements at saturating Ca^{2+} concentrations reflect the maximal capacity of the contractile proteins and therefore are not influenced

by factors that modulate activity. From the regression line describing the relation between tension cost and the α -MHC fraction of all trabeculae studied, it follows that the tension cost of pure α -isomyosin (V1) is $10.95 \pm 1.17 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$ and the tension cost of pure β -isomyosin (V3) is $2.28 \pm 0.13 \text{ mmol kN}^{-1} \text{ m}^{-1} \text{ s}^{-1}$. The slow β -MHC, which is predominantly present in mature guinea-pigs, is thus 5 times more economical than the fast α -MHC. This increase in economy is the result of the decrease in ATP consumption, since there is no significant change in isometric force per cross-sectional area with age.

The change in ATP consumption reflects a change in cross-bridge kinetics. In a two-state model of cross-bridge action (Huxley, 1957), in which the rate of detachment of force-generating cross-bridges was rate limiting, the decrease in the rate of ATP turnover is explained by a decrease in the apparent detachment rate. The decrease in the rate-limiting transition in such a model would imply a minor increase in force per cross-sectional area, which is compatible with our results.

Concluding remarks

The human ventricular myocardium predominantly contains β -MHC, and therefore it is unlikely that a shift in the MHC composition is responsible for all observed mechanical and energetic changes which occur during hypertrophy and heart failure. Changes in other contractile proteins might alter the contractile performance of hypertrophied and failing human myocardium (Hirzel, Tuchschnid, Schneider, Krayenbuehl & Schaub, 1985; Henkel, VandeBerg, Shade, Leger & Walsh, 1989; Anderson, Malouf, Oakeley, Pagani & Allen, 1991). However, in the overloaded human ventricles, the small amount of V1 which is normally present seems to be lost (Mercadier *et al.* 1983). The individual variability in the fraction of α -MHC present in human ventricular myocardium, together with the steep relation between MHC isoforms and tension cost found in this study, might be partly responsible for the conflicting findings on human heart myosin ATPase activity (Mercadier *et al.* 1983; Alousi *et al.* 1990) in the literature, because even a minor decrease in the fraction of α -MHC might have a considerable influence on cardiac energetics.

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