Effects of dilated cardiomyopathy on the diaphragm in the Syrian hamster

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ABSTRACT: This study aimed to elucidate changes in respiratory muscles and their mechanism in cardiomyopathy. The contractile properties and histology of the diaphragm, as well as serum levels of insulin-like growth factor (IGF)-1, were examined in 10 hamsters with idiopathic dilated cardiomyopathy (CM) and 10 controls.

At 28 weeks, body weight in CM was reduced compared with controls (114 ± 10 versus 144 ± 14 g, $p < 0.0001$). The ratio of diaphragm to body weight was significantly higher in CM than in controls (0.228 ± 0.015 versus 0.182 ± 0.017 , $p < 0.0001$). *In vitro*, maximal diaphragmatic twitch (303 ± 63 versus 455 ± 119 g·cm⁻²) and tetanic tensions ($1,555 \pm 369$ versus $2,204 \pm 506$ g·cm⁻²) were significantly lower in CM than in controls ($p < 0.005$). The half-relaxation time was significantly shorter in CM (19 ± 1 ms) than in controls (24 ± 3 ms, $p < 0.0005$). Fatiguability at 25 Hz was significantly less in CM (28%) than in controls (42%, $p < 0.0001$). Diaphragm and gastrocnemius adenosine triphosphatase staining showed type I fibre atrophy in CM, associated with an increase in the number of type I fibres in the diaphragm. Histological examination of both muscles revealed an abnormal muscular pattern. Finally, serum levels of IGF-1 were 47% lower in the CM group than in controls ($p < 0.0001$) and were clearly related to the changes in the contractile properties and histology of the diaphragm.

In conclusion, cardiomyopathy in hamsters: 1) depressed the force-generating capacity and shortened the relaxation of the hamster diaphragm; 2) induced type I fibre atrophy in combination with a myopathic pattern; and 3) was associated with a significant reduction in serum levels of insulin-like growth factor-1, related to the diaphragmatic changes. Whether these changes are primary myopathic or secondary to heart failure remains to be elucidated.

Eur Respir J 1999; 13: 391–397.

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Keywords: Cardiac failure
cardiomyopathy
contractile properties
diaphragm
histology

Received: November 20 1997

Accepted after revision October 1 1998

Supported by Fonds voor Wetenschappelijk Onderzoek-Vlaanderen grant No. G0189.97 and Astra Pharmaceuticals.

Muscle fatigue and dyspnoea are the major symptoms reported by patients with congestive heart failure (CHF) [1]. Alterations in the contractile properties, histology and biochemistry of both peripheral and respiratory muscles [1–3] have been proposed as significant contributors to these symptoms. The former include: reduced endurance, variable fibre atrophy, increased percentage of glycolytic type IIb fibres and reduced oxidative enzymatic activity. Different forms of heart failure appear to affect the skeletal muscles in different ways [4]. Therefore, a model of cardiomyopathy (CM) was studied to determine how this type of heart failure affects the diaphragm and peripheral muscles. The model of dilated CM in the Syrian hamster was used since this has been suggested to be more closely related pathophysiologically to human CM than the hypertrophic model [5]. In the dilated model, only data on diaphragm force are available, whereas skeletal muscle histology has only been documented in a model of hypertrophic CM [6]. Diaphragm fatigue has not been described in cardiomyopathic hamsters.

Serum levels of insulin-like growth factor (IGF)-1 were also determined since they have been found to be signi-

ficantly reduced in patients with CHF [7]. In addition, IGF-1 plays an important role in skeletal muscle protein anabolism and growth [8] and could, therefore, be involved in the skeletal muscle alterations seen in CM and CHF.

The purpose of the present study was to examine the effects of CM on force generation and fatiguability as well as on the histopathology of the diaphragm. A further aim was to evaluate whether the finding of reduced serum levels of IGF-1 in patients [7] was reproducible in animals with CM and whether reduced IGF-1 levels were related to the diaphragmatic abnormalities.

Materials and methods

Animals and study design

All experiments were conducted with 28-week-old male Syrian hamsters, with a body weight of 144 ± 14 g (controls) and 114 ± 10 g (CM). The animals were obtained from Bantin and Kingman breeders (Fitchburg, MA, USA). Ten

CM (Bio TO2 strain) and 10 healthy age-matched control golden Syrian hamsters (F1B) were used for the study.

In vitro study

The Syrian hamsters were anaesthetized with sodium pentobarbital (Nembutal, 60 mg·kg⁻¹ *i.p.*) and then tracheostomized to insert a tracheal cannula (polyethylene tubing, PE-200). They were mechanically ventilated during the time of dissection (Harvard pump respirator, South Natick, MA, USA), with an O₂-enriched gas mixture, because the laparotomy, performed to remove the diaphragm, made spontaneous breathing impossible. Following this procedure optimal oxygenation and viability of the diaphragm were obtained.

In vitro contractile properties. After laparotomy, the removed diaphragm was placed in an aerated and cooled Krebs solution containing (in mmol·L⁻¹): NaCl 137, KCl 4, CaCl₂ 2, MgCl₂ 1, KH₂PO₄ 1, NaHCO₃ 12 and glucose 6.5. Two small strips of the diaphragm were then dissected as described in detail elsewhere [9]. Bundles, studied in pairs, were mounted vertically in a tissue bath containing Krebs solution aerated with 95% O₂/5% CO₂ at a temperature of 37°C and stimulated as described elsewhere [9]. Stimulations were performed with 0.2-ms supramaximal square-wave pulses and a train duration of 250 ms. The muscles were first placed at the length at which maximal isometric twitch tension was obtained. This was called the optimal length (*L*_o). The signals were amplified and recorded on computer *via* analogue-to-digital conversion (DT 2801-A) using Labdat software (Labdat/Anadat, RHT-Infodat, Montreal, Canada). Measurements were made with Anadat.

After a 15-min thermoequilibration period, the following measurements were successively performed at *L*_o. 1) Twitch characteristics were determined from two successive measurements: the highest value of peak twitch force (*P*_t) and its corresponding time to peak tension (TPT) and half-relaxation time (1/2 RT) were used for further analysis. 2) Fused tetanic force (*P*_o) was recorded using 160 Hz stimulation. Two successive stimulations were applied at 2-min intervals. The highest value was taken for further analysis. 3) The force–frequency relationship was measured with 2-min intervals between the stimulations, using the following order of frequencies: 25, 160, 50, 160, 80, 160, 120 and 160 Hz (modified from [10]). 4) Fatiguability of the bundles was assessed by using a fatigue run consisting of repeated 25-Hz stimulations in trains of 330-ms duration with one each 3 s during 5 min. 5) Deterioration of the preparation due to the duration of the protocol was evaluated by measuring the tension developed at 160 Hz after each stimulus during the force–frequency protocol.

After completion of the experiment the bundle length, thickness and width were measured at *L*_o using fine calipers. The bundle was dried and fresh muscle weight was determined. Cross-sectional area (CSA) was obtained by dividing weight by specific density (1.056) and muscle length obtained at *L*_o. All forces were expressed per unit CSA [10]. The twitch-to-tetanus ratio (*P*_t/*P*_o) was also calculated for each muscle bundle.

Heart, liver, diaphragm and gastrocnemius were weighed after they had been trimmed and dried.

Histological and histochemical analysis

The gastrocnemius and diaphragm muscles were folded, cut transversely and placed in tissue glue (Tissue-Tek, Elkard, IN, USA) on a cork, such that the longitudinal axis of fibres was perpendicular to the cork. Each muscle specimen was immediately immersed in isopentane cooled with liquid N₂. Subsequently, all muscle biopsies were cut with a cryostat kept at -20°C to obtain cross-sections of 10 µm. Two sections of each muscle were stained using haematoxylin and eosin (H&E). The other serial sections were stained for myofibrillar adenosine triphosphatase (ATPase) after acid preincubation at pH 4.5 and 4.3. For all muscles studied, slides at pH 4.5 offered the better separation of the two fibre types and were, therefore, used for morphometric analysis. Because of the uniform histochemical staining profile of type II fibres in CM no attempt was made to subclassify type II fibres (as IIa, IIb, IIc, or IIx), especially as such classification in hamsters is controversial. According to LEWIS *et al.* [11] there is no objective basis (*i.e.* on the basis of densitometric analysis) for such classification in the hamster diaphragm, although one study, using dystrophic hamsters, subclassified the type II fibre types [6]. Varied stain intensity is, however, mentioned in the diaphragm sections of dystrophic hamsters, making interpretation less reliable. Quantitative measurements of myopathic features were made after H&E staining of the diaphragm, using a microscope (Leitz Laborhama S., Wetzlar, Germany) at 20× magnification (objective), connected to a computerized image analysis system (Quantimet 500; Leica, Cambridge, UK). The following variables were determined in both CM and control animals: number of necrotic cells, number of cells with >5 nuclei and number of cells with central nuclei. Each was expressed as a percentage of the total number of cells.

Fibre size and proportion

Morphometric examination was performed by microscopy at 20× magnification as described above. A minimum of 150 fibres of each muscle was used to calculate the proportion and CSA of all fibre types. Boundaries of individual muscle fibres were delineated and fibre CSA were determined from the number of pixels within the outlined fibre. At the same time, fibre diameters, defined as orthoferret (longest length perpendicular to fibre long axis), were automatically determined. In addition, for the diaphragm the CSA were corrected for the shortening occurring from *L*_o, the mean correction factor being similar in control and CM animals (pooled value for the diaphragm=1.77±0.35). For the gastrocnemius such correction was not performed since the *L*_o of peripheral muscles has generally been found to be between 100 and 120% of their resting length, with the resting length corresponding to the excised length [12].

Measurement of serum levels of insulin-like growth factor-I

Blood was sampled immediately after dissection of the heart from the thorax. Serum was obtained by centrifugation at 1,600 × *g* for 10 min at 4°C and stored at -20°C.

IGF-1 was measured in acid-ethanol-extracted sera by radioimmunoassay as described previously [13], with the use of a guinea-pig polyclonal antiserum (Ciba-Geigy, Basel, Switzerland).

Data analysis

The statistical analysis was performed using the SAS statistical package (SAS Institute, Cary, NC, USA). Data from the two diaphragm strips obtained from each hamster were averaged for further statistical analysis. Differences between means of controls and CM hamsters were assessed using the Student's *t*-test. The Student's *t*-test was also used to evaluate histochemical findings. Correlations were determined using Spearman's rank product correlation.

Statistical significance was defined as a two-tailed *p*-value <0.05. Data are expressed as means±SD.

Results

Body, muscle and organ weight

At 28 weeks, body weight in the CM hamsters was significantly reduced in comparison with the controls: CM 114±10 *versus* controls 144±14 g (*p*<0.0001).

A significantly lower heart (16%) and liver weight (24%) was seen in the CM hamsters than in the control hamsters (*p*<0.0001 and *p*<0.005, respectively). When expressed as a percentage of the body weight, however, there were no significant differences between the groups.

The absolute weight of the diaphragm was similar in both groups (pooled value: 260±31 mg), while after normalization for body weight, the diaphragm to body weight ratio was significantly higher in the CM group (0.228±0.015) than in the controls (0.182±0.017, *p*<0.0001). Gastrocnemius weight was significantly lower in the CM group (225±23 *versus* 263±17 mg, *p*<0.005). However, after normalization for body weight gastrocnemius weight was increased (0.196 in CM *versus* 0.184 in controls, *p*<0.05).

The thickness and width of the dissected diaphragm bundles were comparable in the two groups. The length of the diaphragm bundles, however, was slightly but significantly smaller in the CM group (13.5±1.2 *versus* 14.8±1.1 mm, *p*<0.05).

Contractile properties of the diaphragm

P_t and *P_o* were both comparably reduced in the CM group (by 34% and 29%, respectively, *p*<0.005), so that no significant changes were seen in the twitch to tetanus ratio, *P_t/P_o* (table 1). No statistically significant difference in TPT was seen between the groups. Conversely, the 1/2 RT was significantly reduced by 19% in the CM group (*p*<0.0005) (table 1).

The force–frequency curve showed that the CM diaphragm bundles generated a significantly lower force

Table 1. – Diaphragm contractile properties in control and cardiomyopathic hamsters

	Control Bio F1B (n=10)	Cardiomyopathy Bio TO2 (n=10)
<i>P_t</i> g·cm ⁻²	455±119	303±63 ⁺
<i>P_o</i> kg·cm ⁻²	2.204±506	1.555±369 ⁺
<i>P_t/P_o</i>	0.205±0.021	0.199±0.025
TPT ms	22.6±1.2	21.9±1.9
1/2 RT ms	24.0±2.8	19.5±1.4 [#]

P_t: twitch tension; *P_o*: tetanic tension; *P_t/P_o*: twitch to tetanus ratio; TPT: time to peak tension; 1/2 RT: half-relaxation time. ⁺: *p*<0.005; [#]: *p*<0.0005.

at all frequencies (*p*<0.0005) (fig. 1a). When expressed as a percentage of the interposed 160 Hz stimulations, the force response was still shifted to the right, reaching statistical significance at 25 and 50 Hz (fig. 1b).

The absolute values of decline in tension (160 Hz) during the force–frequency protocol were significantly different (750±205 *versus* 500±225 g·cm⁻², in the CM and control group respectively, *p*<0.05). When these values were expressed as percentage of *P_o*, the decline in tension in the CM group was double that in the control group (48±11 *versus* 24±10%, *p*<0.001).

During the fatigue run, the CM diaphragm strips generated significantly lower force than did the control diaphragm, when force was expressed per unit CSA (fig. 2). Such an effect was already present at the beginning of the fatigue run and persisted during the whole protocol.

Fig. 1. – Force–frequency relationship expressed in a) absolute values and b) as a percentage of interposed 160 Hz stimulations in control (●) and cardiomyopathic (○) hamsters. *P_o*: tetanic tension. *: *p*<0.05; #: *p*<0.0005.

Fig. 2. – Fatigue curve expressed in a) absolute values and b) as a percentage of initial force in control (●) and cardiomyopathic (○) hamsters. *: $p < 0.05$; +: $p < 0.005$; #: $p < 0.0005$.

The decline in force at the end of the fatigue run (fatiguability) was significantly less in the CM (28%) than in the control group (42%, $p < 0.0001$).

Histology and histochemistry

Typical histological features of H&E-stained diaphragm slides of control and CM hamsters are shown in figure 3a and b, respectively. Quantitative analysis of the myopathic features in the diaphragm showed greater proportions of necrotic cells (12 ± 3 versus $2 \pm 1\%$), of cells with >5 nuclei (24 ± 5 versus $6 \pm 3\%$) and of cells with central nuclei (54 ± 6 versus $10 \pm 3\%$) in the CM hamsters than in controls ($p < 0.0001$ for the three variables). H&E staining of the gastrocnemius showed a comparable myopathic pattern. Histochemical analysis of the diaphragm after ATPase staining showed a significantly greater proportion of type I fibres (29 versus 38%, $p < 0.005$) in the CM group, with a moderate reduction in the proportion of type II cells (71 versus 62%, $p < 0.005$). ATPase stainings of the diaphragm are shown in representative control (fig. 4a) and CM (fig. 4b) hamsters. Conversely, in the CM group selective type I fibre atrophy was seen, as was evident from a significant reduction in CSA (-29% , $p < 0.005$) (fig. 5) and in diameter (-17% , $p < 0.0001$), while type II fibre CSA and diameter remained unchanged. The total surface area taken up by type I fibres (defined as the mean surface area of type I fibres $\times$ mean proportion of type I fibres) was comparable in the two groups.

Fig. 3. – Haematoxylin and eosin (H&E) staining of a) control and b) cardiomyopathic diaphragms from representative animals. Note the increase in the number of cells with central nuclei, the number of cells with more than five nuclei and the number of necrotic cells in (b). (Magnification $20 \times$ (objective).)

Histochemical analysis of the gastrocnemius also showed type I atrophy. CSA and diameter were significantly reduced in CM animals (-24% and -16% , $p < 0.01$ and $p < 0.05$, respectively). No fibre shift was seen. The dimensions of the type II fibres were unaltered.

Serum levels of insulin-like growth factor-1

A significant decrease of 47% in IGF-1 serum levels was apparent in the CM group (204 ± 38 ng·mL $^{-1}$) compared with the controls (383 ± 43 ng·mL $^{-1}$, $p < 0.0001$) (fig. 6). There were significant correlations for all animals (both groups taken together) between serum IGF-1 levels on the one hand and 1/2 RT and force during the force–frequency curve of the diaphragm, on the other hand. The correlation coefficients were $r = 0.70$ and $p < 0.001$ for 1/2 RT and r ranging 0.57–0.62 for forces during the force–frequency curve, with p ranging from < 0.005 at stimulation frequencies of 25, 50 and 120 Hz to < 0.01 at 80 Hz. In addition, serum IGF-1 levels were inversely related to fatigue during the fatigue run ($r = -0.67$, $p = 0.001$ at the end of the fatigue run). The type I fibre surface area of the diaphragm was significantly related to the IGF-1 serum levels ($r = 0.51$, $p < 0.05$).

Fig. 4. – Adenosine triphosphatase staining of the diaphragm at pH 4.5 in a) representative control and b) cardiomyopathic hamsters. Note the increase in the number of type I fibres and the atrophy of the type I fibres in (b). (Magnification 20 × (objective).)

Discussion

In the present study, the force-generating capacity of the diaphragm was depressed in CM animals compared with controls, whereas its fatiguability was less. Histochemical analysis showed atrophy of type I fibres in both diaphragm and gastrocnemius, associated with an increased proportion of type I fibres in the diaphragm only. Histological examination of both diaphragm and gastrocnemius showed

Fig. 5. – Diaphragm cross-sectional area (CSA) of type I and type II fibres in control (▨) and cardiomyopathic (□) hamsters. ***: $p = 0.001$.

Fig. 6. – Serum levels of insulin-like growth factor (IGF)-1 in control (●) and cardiomyopathic (CM; ○) hamsters. The lines represent the mean values. ****: $p < 0.0001$.

a myopathic pattern in all fibre types. Finally, a significant decrease in IGF-1 serum levels, related to the diaphragm alterations, was observed in the CM group.

Cardiomyopathy in the Syrian hamster as a model of heart failure

A Syrian hamster model of dilated CM was chosen because pathophysiologically the dilated strain of CM appears to be more closely related to human CM than is the hypertrophic model [5]. Haemodynamic measurements in Syrian hamsters with dilated CM indicate cardiac failure at the age of 8–10 months [14], comparable to the age of the present hamsters. Clinical signs are clear only in the last stage of heart failure when congestion occurs [15].

Body, muscle and organ weight in dilated cardiomyopathy

The reductions found in body weight and heart and liver weight are comparable with the data on the Syrian hamster model in the literature [16, 17]. The unchanged absolute diaphragm weight and the increased diaphragm-to-body weight ratio are in contrast to the muscle wasting expected in dystrophic muscle. The finding is, however, in keeping with the data on diaphragm weight in the model of hypertrophic CM [6, 18]. In that model an increase in absolute diaphragm weight was even documented [6, 18]. It presents, perhaps, a unique expression of the dystrophic process [6]. The finding of an unchanged absolute diaphragm weight is in contrast to the reduction in absolute gastrocnemius weight. This may be explained by the fact that the type I fibre atrophy in the diaphragm was associated with an increase in the number of type I fibres.

Contractile properties of the diaphragm in dilated cardiomyopathy

Several alterations in diaphragmatic contractile properties were noted in the present model of dilated CM. Firstly, at all frequencies a significantly lower force was observed in the diaphragm bundles of the CM group than in controls. Since force remained lower after correction for CSA, these

findings are suggestive of myopathy. This was confirmed by histology (see below). Diaphragm weakness has also been reported with heart failure in humans [19, 20] as well as in different animal models of heart failure [3, 6, 21–23]. LECARPENTIER *et al.* [24] demonstrated a reduction in the number of cross-bridges in the diaphragm of hamsters with dilated CM as an explanation for its reduced force. The tension decline during the force–frequency curve might reflect muscle alterations rather than muscle fatigue. Alternatively, it is possible that the higher decline in tension observed during the force–frequency protocol in the CM group was dependent on the muscle fibre atrophy induced by CM.

Secondly, significantly reduced diaphragm fatiguability was found in the CM group compared with controls, in contrast to the expected results. The present data do not offer a clear explanation for this apparent discrepancy. It is, however, possible that in CM animals the initial force reduction is so important that further decrease during the fatigue run is limited. To the authors' knowledge, diaphragm fatiguability has not been described in CM Syrian hamsters. Data in the literature concerning diaphragm fatiguability in CHF are controversial. On the one hand, in dogs with CEF induced by ventricular pacing [23], an increase in diaphragm fatiguability has been described. On the other hand, in Yucatan minipigs [3] with CHF after supraventricular pacing, it was shown to be unchanged.

Thirdly, the 1/2 RT appeared significantly reduced in CM animals. This is in contrast to the finding of a prolonged isotonic relaxation time in the diaphragm muscles of CM hamsters, demonstrated by COIRAULT *et al.* [18]. Their model, however, concerns a hypertrophic model of CM. Moreover, in their study this impairment was clear only after preloading the muscle at L_0 . The reduced 1/2 RT may be explained by the type I fibre atrophy, knowing that these fibres are characterized by a reduced Ca^{2+} uptake and prolonged twitch relaxation time. An increased rate of sequestration of Ca^{2+} by the sarcoplasmic reticulum by means of a Ca^{2+} ATPase seems less likely. Indeed, ANGER *et al.* [17] found a decreased Ca^{2+} ATPase (SERCA 2) messenger ribonucleic acid (mRNA) level at 6 months of age in the CM Syrian hamster.

It is also possible that peripheral muscle dysfunction was present. No peripheral muscle contractile properties, however, were tested in this study.

Skeletal muscle histology and histochemistry in dilated cardiomyopathy

Histochemical evaluation revealed selective type I fibre atrophy in both gastrocnemius and diaphragm in combination with an increase in the number of type I cells in the diaphragm. Histochemistry has not previously been described in the model of dilated CM. In the model of hypertrophic CM [6] a reduction in fibre size in the diaphragm was clearly seen at 35 days and 180 days in type I and type II fibres, respectively. In contrast to the present findings, a fibre shift towards fast oxidative fibres was present. Differences in results may potentially be explained by differences in the progression of heart failure in the two models since in the hypertrophic model type I fibre atrophy is seen at an earlier stage. The described

differences in histochemical findings between the two CM models confirm the suggestion of LINDSAY *et al.* [4] that different models of heart failure affect the diaphragm in a different way.

The present histological data are in keeping with the myopathic pattern described in the diaphragm and limb muscles of patients with CM undergoing cardiac transplantation [4] and in the hypertrophic Syrian hamster strain [6]. They are also in line with the alterations in contractile properties found in the present study.

When the histological pattern of the diaphragm is compared with the gastrocnemius a similar atrophy pattern but with distinct fibre proportions are noted. This is in line with the findings of TIKUNOV *et al.* [25]. They described fast-to-slow transformations of both myosin and regulatory proteins, such as α -tropomyosin and fast isoforms of troponin-T in the diaphragm of patients with CHF. This is in contrast to the increase in the number of fast fibres described in limb muscles [2]. It is possible that the different fibre proportion is due to the fact that in CHF the work of limb muscles tends to be decreased, whereas that of the diaphragm is increased [26].

Serum insulin-like growth factor-1 in dilated cardiomyopathy

The finding of a reduction in serum IGF-1 is intriguing. IGF-1 is known to cause myofibre hypertrophy and plays an important role in skeletal muscle protein anabolism and growth [8]. The skeletal muscle changes seen in CM could be associated with the noted reduction in IGF-1 serum levels, which is likely to result from liver dysfunction associated with the CHF. Indeed, in the present study diaphragm alterations in contractile properties and histology were clearly related to the IGF-1 serum levels. This study suggests that reduced serum IGF-1 levels appear in heart failure independently of interfering factors such as medication. Whether the decrease in IGF-1 levels is associated with a downregulation of local muscle IGF-1 mRNA has to be examined. No hamster IGF-1 complementary deoxyribonucleic acid (cDNA) probe is, however, available and thus, the determination of IGF-1 expression in skeletal muscle remains problematic in these animals.

Are the myopathic changes documented in this model primary or secondary to heart failure?

A potential criticism of this model is that from the present study it cannot be determined whether the alterations found were primary, related to the congenital myopathy, or whether they were secondary to heart failure. The fact that heart failure could induce myopathic changes is, however, suggested by the study of SUPINSKI *et al.* [23]. They produced heart failure experimentally in dogs by ventricular pacing. In these dogs, the force of the diaphragm strips normalized to their surface area was decreased, suggesting a reduced quality of the diaphragm. Their study demonstrated that myopathic changes are conceptually possible in heart failure. It is, however, possible that some of the skeletal muscle changes found in the present animals are specific to the model of CM and cannot be generalized. Unfortunately, no data are available

on the progression of histological changes in the model of dilated CM. In hypertrophic CM, JASMIN and PROSCHEK [27] described necrotic changes confined to the most active respiratory muscles in newborn animals. These changes extended progressively to the whole musculature with age, reaching their maximum severity in 100-day-old hamsters, and appeared in the heart after 40 days. This suggests that in hypertrophic CM animals at least some of the changes in skeletal muscles are due to the progressively developing myopathy rather than secondary to heart failure.

In conclusion, the present study confirms that heart failure in hamsters with dilated cardiomyopathy has an effect on the contractile and histological properties of the diaphragm. Type I fibre atrophy, in combination with an increase in the number of type I fibres, was associated with myopathic changes. This is in contrast to the model of hypertrophic cardiomyopathy, in which, at a similar age, type II fibre atrophy associated with a fibre shift towards fast oxidative fibres is seen. These differences in histochemical findings confirm the suggestion of LINDSAY *et al.* [4] that different models of heart failure affect the diaphragm in different ways. The reduced systemic production of insulin-like growth factor-1, related to the diaphragm changes, suggests a possible role for this growth factor in skeletal muscle abnormalities in cardiomyopathy.

Acknowledgements. The authors thank N. Butts for expert technical help, E. Van Herck for IGF-1 measurement and A. Bisschop and R. Schepers for their help in statistical analysis.

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