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Gene expression profile in the heart of spontaneous dwarf rat: In vivo effects of growth hormone

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Abstract

Excess and deficit of growth hormone (GH) both affect cardiac architecture as well as its function. To date, experimental and clinical studies have reported that GH has an inotropic effect on animal and human heart, however, it remains controversial whether GH is applicable to the treatment for the patients with chronic heart failure. Also, the mechanism by which GH exerts these biological effects on the heart is not well understood. In this study, we attempted to specify the genes regulated by GH in the heart of spontaneous dwarf rat using a microarray analysis. We found that soluble forms of guanylate cyclase, cofilin1, and thymosin β4 mRNA were up-regulated in the heart by GH treatment. On the other hand, acyl-CoA synthetase, aldosterone receptor, myosin regulatory light chain, troponin T, laminA, and β-actin mRNA were down-regulated. These results suggest GH regulates essential molecules that regulate structural, contractile, remodeling, and regenerative functions. Collectively, our data indicate a new integrative understanding for the biological effects of GH on cardiac function.

Keywords: Expression profile; Growth hormone; Spontaneous dwarf rat; Microarray analysis; Heart

Accumulating evidence indicates that an excess of growth hormone (GH) gives a significant effect on cardiac function [1–3]. Impaired cardiovascular function has been demonstrated to potentially reduce life expectancy of patients with acromegaly [1]. Hypertension, diabetes mellitus, cardiac hypertrophy, and hyperlipidemia are common in the patients with acromegaly, and they have an increased risk of cardiac failure [4], which is partially reversed after normalization of GH and IGF-I levels by octreotide treatment [5]. On the other hand, long-standing GH deficiency is also known to cause abnormalities in cardiac performance and structure, which increase the risk of cardiovascular mortality [6], and GH replacement therapy appears to correct such abnormalities [7–10]. Furthermore, both animal and human studies revealed that GH administration improved cardiac function of heart failure secondary to idiopathic and ischemic cardiomyopathy in both experimental [11–14]) and clinical trials [15–18] despite some adverse effects of GH such as water and mineral retention.

Spontaneous dwarf rat (SDR) harbors a mutation in the GH gene yielding undetectable levels of GH, indicating that SDR is an excellent animal model for isolated growth hormone deficiency (GHD) [19].

In this study, using a cDNA microarray technique, we have attempted to identify genes regulated by GH in the SDR heart to clarify the mechanism of GH effect on cardiac function.

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Materials and methods

Animals. Five-week-old male spontaneous dwarf rats were purchased from Japan SLC (Shizuoka, Japan). Recombinant human GH (1 mg/kg), dissolved by saline was injected intra-peritoneally. Three, 24, and 72 h after GH injection, three rats for each group were sacrificed and the heart was dissected immediately. Rats with the vehicle (PBS) injection were used as the control. The isolated heart was cut, washed with PBS to remove the blood, and frozen immediately by liquid nitrogen. The left ventricular muscle was used for RNA extraction. All experiments were conducted according to the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Kobe University Graduate School of Medicine (Kobe, Japan) Animal Care Committee.

RNA extraction. Total RNA was extracted from each group using Trizol LS reagent (Gibco-BRL) according to the manufacturer's protocol. Trace of DNA contamination in RNA preparations was removed by DNase I (Sigma) digestion.

Microarray technique. Probe was synthesized using sample RNA, ^{33}P-labeled dATP (Amersham Biosciences), 10× dNTP mix, 100 mM DTT, Power Script Reverse Transcriptase, random primer mix, and 5× Power Script reaction buffer. After purified by column chromatography, labeled cDNA was hybridized with Clontech rat 4k plastic microarray (Clontech) film overnight at 60 °C, and the film was washed by SSC containing 0.1% SDS as manufacturer's protocol. Then it was exposed to BAS2040 (FUJIFILM) for two days and each signal was detected by FLA8000 (FUJIFILM). Data were analyzed by Image Reader software (FUJIFILM) and Array Gauge software (FUJIFILM). Each signal intensity was corrected by local background. We selected the genes, which were up-regulated in SDR heart by rhGH more than twice higher than control levels. On the other hand, we also selected the genes, which were down-regulated less than a half of control levels.

Real-time quantitative PCR. For precise quantitative analysis of mRNA, real-time quantitative PCR was carried out on ABI Prism 7000 Sequence Detector (Applied Biosystems). Taqman probe, sense and antisense primers were designed by primer express software (Applied Biosystems) (Table 1). Data were collected using ABI Prism 7000 Sequence Detection System software (Applied Biosystems). cDNA was generated as described before. For each experiment, 2 μl of the reverse transcription reaction was used with PCR master mix (Applied Biosystems) for PCR as manufacturer's protocol. Cycling conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Each reaction was repeated three times on a MicroAmp Optical 96-Well Reaction Plate and Optical Caps (applied biosystems). GAPDH was used as an internal standard. Threshold cycles, in which PCR products were reached the given amount, were determined to indicate the initial copies of each mRNA.

Data analysis. The results were shown as means ± SD. Data analysis was performed using Student's t test. A p value less than 0.05 was considered as statistically significant.

Table 1
Nucleotide sequences of the primers and probes for quantitative real-time PCR (forward primer, **TaqMan probe**, reverse primer, respectively)

Acyl-CoA synthetase	(5′-CAGACAAACCCGGAAGTCCAT-3′ **5′-TCGCTCTGTCACGCACTTCGACTCA-3′** 5′-TCTGCTCCAGGGATGTCTATGA-3′)
Aldosterone receptor	(5′-GCTTGAGTGGGTCAGCGTTT-3′ **5′-TCACCATGCAGGCAACATTACCGTG-3′** 5′-GGCAGGCGTCGTCTGAGA-3′)
Troponin T	(5′-TGTTCGACAAAGCTCTGTTCCTT-3′ **5′-TGCCCTTGCCCTGTGAATCCCA-3′** 5′-CGGGTGCCTGGCAAGA-3′)
Myosin regulatory light chain	(5′-GAAACGCCTTCGCTTGCTT-3′ **5′-TGAGGAAGCCACAGGCACCATCC-3′** 5′-AGCAGCTCCCTCAGGTAATCC-3′)
β-Actin	(5′-ACTGGTGAAGGCTGGCTTTG-3′ **5′-TGATGATGCTCCCAGAGCTGTCTTCC-3′** 5′-GGCGACCCACGATGGA-3′)
Cofilin1	(5′-TGCACCCTGGCAGAGAAAC-3′ **5′-TGGCAGCGCCGTCATTTCCC-3′** 5′-TGGAGGTGGCTCACAAAGG-3′)

Nucleotide sequences of primers and TaqMan probes design for quantitative real-time PCR.

Results

The treatment with GH in SDR significantly altered the profile of gene expression in the heart. Three hours after GH administration, the expression of mRNA of guanylate cyclase, soluble α1 was increased by 2.29-folds. The expression of mRNA of acyl-CoA synthetase, aldosterone receptor, troponin T, and myosin regulatory light chain was decreased by 0.27-, 0.38-, 0.38-, and 0.46-folds, respectively (Table 2). After 24 h, the expression of mRNA of A-kinase-anchoring-protein, thymosin β4, olfactory protein, and cofilin 1 was increased by 2.35-, 2.35-, 2.36-, and 2.86-folds, respectively, and the expression of mRNA of β-actin and zinc finger (OCZF) protein were decreased by 0.32- and 0.41-folds, respectively (Table 2). After 72 h, the expression of mRNA of transcription factor HES-3, late gestation lung protein 2 (Lgl2), N^G-dimethylarginine dimethylaminohydrolase, small proline-rich protein (spr), and lamin A was decreased by 0.37-, 0.37-, 0.45-, 0.48-, and 0.49-folds, respectively (Table 2).

To assure the reliability of the results of the microarray analysis, we made a validation using quantitative real-time PCR. We successfully confirmed the changes in the expression of mRNA of acyl-CoA synthetase, aldosterone receptor, troponin T, myosin regulatory light chain, β-actin, and cofilin1 as were indicated by microarray analysis (Table 3).

Next, we analyzed the time-dependent alternations in the expression of these genes using quantitative real-time PCR (Fig. 1). GH-induced increment of guanylate cyclase mRNA reached the peak at three hours after GH administration and returned to the basal level at 24 h. In contrast, the expression of mRNA of cofilin 1 and thymosin β4 reached the peak at 24 h. The mRNA of acyl-CoA synthetase, aldosterone receptor, troponin T, myosin regulatory light chain, and lamin A decreased with troughs at three hours after rhGH administration. Besides, the nadir of β-actin expression appeared at 24 h.

Discussion

It is well known that in acromegaly, cardiac function and structure are markedly affected and these changes influence the prognosis significantly [1]. It is also reported that adult GHD patients show impaired cardiac function and GH treatment improves its dysfunction in these patients [8–10]. GH exerts its effects either directly or

Table 2
Up- or down-regulated genes in the heart of SDR after GH treatment

	Index	Locus link/GenBank ID	Ratio
3 h			
Up-regulated genes			
Guanylate cyclase, soluble, α1	L15a4/b4	25201/U60835	2.30
Down-regulated genes			
Acyl-CoA synthetase	E20a8/b8	113976/D85189	0.27
Aldosterone receptor	F14c2/d2	25672/M36074	0.38
Troponin T cardiac	I06a2/b2	24837/M26052	0.38
Myosin regulatory light chain	Na6/b6	50685/X52840	0.46
24 h			
Up-regulated genes			
A-kinase-anchoring-protein	P19a7/b7	60332/AJ002474	2.35
Thymosin β4	O03c4/d4	81814/M34043	2.35
Olfactory protein	O23c4/d4	/M64388	2.36
Cofilin1	P23c3/d3	29271/X62908	2.86
Down-regulated genes			
β-Actin	L07a4/b4	29275/X00306	0.32
Zinc finger (OCZF) protein	24a8/b8	117107/D88450	0.41
72 h			
Down-regulated genes			
Transcription factor HES-3	E09a8/b8	64628/D13418	0.37
Late gestation lung protein 2	b5	116458/AF110195	0.37
N^G,N^G-Dimethylarginine	G02a8/b8	64157/D86041	0.45
Small proline-rich protein (spr)	F14a8/b8	60461/L46593	0.48
Lamin A	L04a6/b6	60374/X66870	0.49

Effect of GH treatment on cardiac gene expression in SDR. Index of the Clontech rat 4k plastic microarray sheet, Locus link/GenBank ID, and the ratio (the average spot intensity of GH-treated group/the average spot intensity of control group) is shown.

Table 3
Comparison of the threshold cycle numbers of the genes in the heart of control and GH-treated SDR using quantitative real-time PCR analysis

	Control C_t	GH C_t
Acyl-CoA synthetase (3 h)	31.29 ± 0.59	33.45 ± 0.30
Aldosterone receptor (3 h)	31.80 ± 0.54	33.29 ± 0.23
Troponin T cardiac (3 h)	26.80 ± 0.12	28.93 ± 0.18
Myosin regulatory light chain (3 h)	29.78 ± 0.16	31.59 ± 0.27
β-Actin (24 h)	25.83 ± 0.22	26.81 ± 0.07
Cofilin1 (24 h)	29.38 ± 0.45	27.92 ± 0.52

Comparison of the threshold cycle numbers of the genes in the heart of control and GH-treated SDR. The numbers of the threshold cycle, in which PCR products reached the given amount, were determined to assess the initial copies of each mRNA. Data were calculated using ABI Prism 7000 Sequence Detection System software (Applied Biosystems). Threshold cycles (C_t) are shown as means ± the standard deviation (SD) ($n = 3$). The changes in the threshold cycles of the genes affected by GH treatment were compatible with the results of the microarray analysis.

indirectly via IGF-I. Some of these effects have been explained, however, the molecular basis of its effects is largely unknown. In the present study, we have demonstrated that GH regulates the expression of some of the key molecules that play roles in the structural, contractile, remodeling, and regenerative functions in the heart.

Accumulating evidence indicates that renin–angiotensin–aldosterone (R-A-A) system plays a critical role in cardiac function and remodeling. It is known that GH induces retention of sodium and its mechanism may involve the activation of the renin–angiotensin–aldosterone (R-A-A) system [20–23]. In this study, we demonstrated GH reduced the expression of aldosterone receptor mRNA in the heart of SDR. The decreased expression of aldosterone receptor could reduce the sensitivity to aldosterone. It is possible that GH-regulated reduction in the expression level of aldosterone receptor contributes to the impaired cardiac function in AGHD.

Acyl-CoA synthetase is a regulatory enzyme of β-oxidation in mitochondria. In terms of lipid metabolism, AGHD patients are often accompanied with hyperlipidemia and hepatic steatosis [6]. In the bovine GH-transgenic mouse, mitochondria in myocytes were swollen with dissolution of the regular cristae arrangements [11]. In this study, GH treatment decreased the expression of acyl-CoA synthetase in the SDR heart. In the heart, fatty acid is considered as the main energy source. These results suggest that GH regulates cardiac lipid oxidation pathways by affecting β-oxidation and is comparable to the fact that GH regulates β-oxidation pathway decreasing the expression level of PPARα [24].

Soluble guanylate cyclase (sGC) is a main receptor for nitric oxide (NO) in cytosol, and the binding of NO to sGC results in increasing cellular cGMP concentration [25,26]. The increase of cellular cGMP level is responsible for the decrease in intracellular calcium level. It is well known that the decrease in calcium level by NO causes

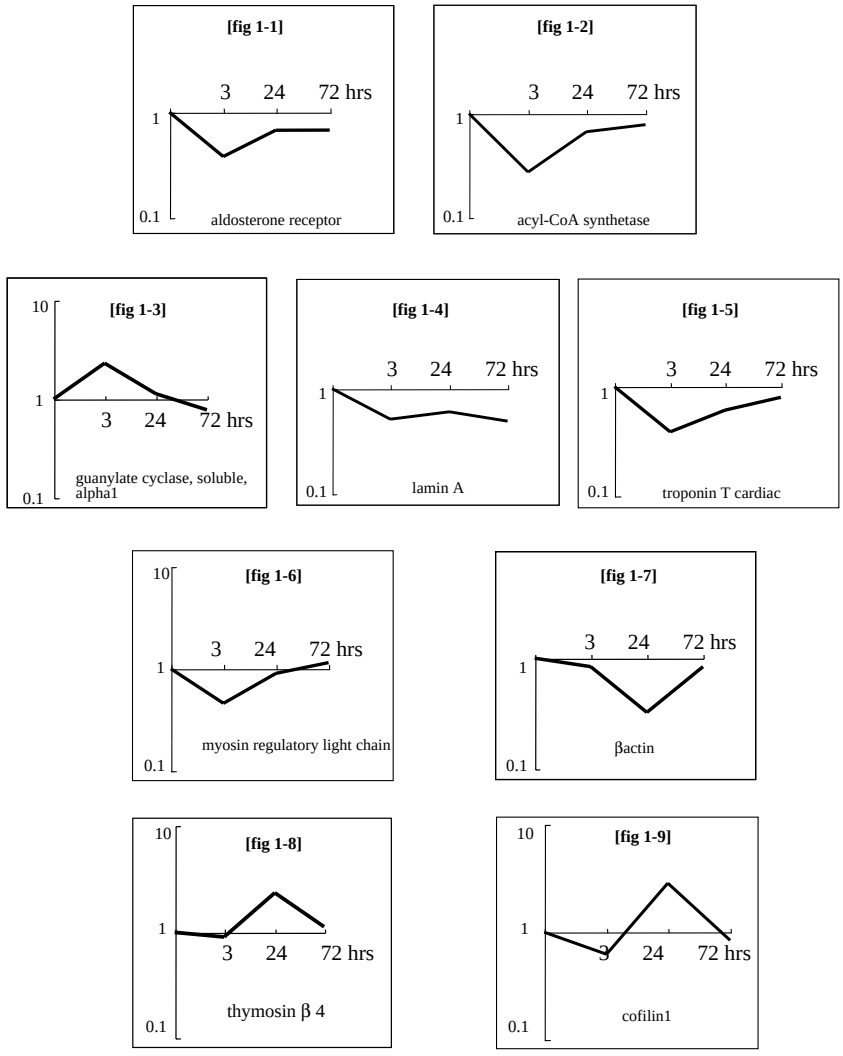

Fig. 1. Time-dependent changes of the cardiac gene expressions in the GH-treated SDR analyzed by quantitative real-time PCR. (1) A line graph indicates the expression change of aldosterone receptor. The horizontal axis represents the time after GH injection, and the vertical axis represents the expression ratio compared to control level. (2) A line graph for acyl-CoA synthetase. (3) A line graph for soluble guanylate cyclase α1. (4) A line graph for lamin A. (5) A line graph for troponin T. (6) A line graph for myosin regulatory light chain. (7) A line graph for β-actin. (8) A line graph for thymosin β4. (9) A line graph for cofilin1.

the improvement of endothelial function. On the other hand, it is also known that myocyte function is suppressed by the same mechanism [26]. Our results indicate NO receptor, sGC, was up-regulated by GH. This could cause an increased sensitivity to NO in myocardium and regulate cardiac function as well as enhancing GC activity [27,28].

Lamin A/C, the nuclear envelope protein which lines the inner nuclear membrane is encoded by the LMNA gene and its defect is responsible for the familial dilated cardiomyopathies (DCM) associated with atrioventricular block, such as autosomal dominant Emery–Dreifuss muscular dystrophy [29,30]. In this study, the expression of Lamin A was decreased by GH. Our result also shows contractile proteins, such as myosin regulatory light chain, troponin T, and β-actin, were down-regulated by GH treatment. These results suggest that GH regulates cardiac contractility via alternation of the expression level of these proteins.

Thymosin β4 is a main intracellular G-actin sequestering peptide [31,32]. It binds monomer acting in a 1:1 complex and acts as acting buffer, preventing polymerization into acting filaments but supplying a pool of acting monomers when the cell needs filaments. It is thought to be an important mediator in myocyte proliferation, migration, and differentiation. On the other hand, cofilin1 is also a small acting binding protein and belongs to a member of the cofilin1/ADF family, and it enhances acting filament turnover by increasing the rate of acting depolymerization from the pointed ends [33,34]. In contrast to thymosin β4, cofilin binds more efficiently to ADP-actin subunits in filaments and promotes filament disassembly. Recently, it is reported that thymosin β4 expression is abundant mainly in proliferative region during heart development and it promotes cardiac cell migration, survival, and cardiac repair in adult mice after myocardial infarction and improves cardiac

function [35]. In this study, we demonstrated that GH increases the expression of both genes. Given the fact that in patients with dilated or ischemic cardiomyopathy, the administration of rhGH results in significant improvement in hemodynamics and cardiac function [16–18], it is considerable that GH improves cardiac function via up-regulating the expression of thymosin β4 and cofilin.

Previous microarray analysis of GH effect indicated that myosin light chain is up-regulated in the heart of hypophysectomized rat [36]. These results are compatible to our data. On the other hand, several genes related with fatty acid metabolism such as long-chain acyl-CoA synthetase in hypophysectomized rat heart were reported to be decreased [36], however, in our study, GH treatment suppressed acyl-CoA synthetase mRNA expression in the heart of SDR. It is not clear as to the reason for this discrepancy, however, it is considerable that SDR is deficient only in GH, however, hypophysectomized rat is deficient for all pituitary hormones even some hormones are replaced. In this aspect, SDR is more appropriate to analyze specific changes depending on GH.

Our study demonstrated that the expression of sGC, acyl-CoA synthetase, aldosterone receptor, myosin regulatory light chain, and troponin T in SDR heart was changed in 3 h. Together with the fact that the GH receptor is expressed in myocardium [37], it is possible that GH directly regulates the expression of these genes. On the other hand, the expression of cofilin1, thymosin β4, and β-actin was altered in 24 h. These late-phase changes in the expression levels suggest indirect action of GH via such as IGF-I.

In conclusion, GH significantly changed gene expression levels of sGC, acyl-CoA synthetase, aldosterone receptor, troponin T, cardiac myosin regulatory light chain, thymosin β4, β-actin, and cofilin1 in SDR heart. These changes in the gene expression profiles seem to explain some of the physiological and pharmacological effects of GH in the heart. Although, further studies are required to gain an insight into the precise mechanisms of GH action, this approach shows effective to obtain a comprehensive understanding for GH action in vivo.

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References

[1] B.A. Bengtsson, S. Eden, I. Ernest, A. Oden, B. Sjogren, Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984, Acta Med. Scand. 223 (1988) 327–335.

[2] P. Chanson, J. Timsit, C. Masquet, A. Warnet, P.J. Guillausseau, P. Birman, A.G. Harris, J. Lubetzki, Cardiovascular effects of the somatostatin analog octreotide in acromegaly, Ann. Intern. Med. 113 (1990) 921–925.

[3] A. Colao, G. Vitale, R. Pivonello, A. Ciccarelli, C. Di Somma, G. Lombardi, The heart: an end-organ of GH action, Eur. J. Endocrinol. 151 (Suppl. 1) (2004) S93–S101.

[4] W.L. McGuffin Jr., B.M. Sherman, F. Roth, P. Gorden, C.R. Kahn, W.C. Roberts, P.L. Frommer, Acromegaly and cardiovascular disorders. A prospective study, Ann. Intern. Med. 81 (1974) 11–18.

[5] M. Nishiki, Y. Murakami, M. Sohmiya, K. Koshimura, K. Inoue, Y. Goto, N. Nakamura, Y. Kato, Histopathological improvement of acromegalic cardiomyopathy by intermittent subcutaneous infusion of octreotide, Endocr. J. 44 (1997) 655–660.

[6] T. Rosen, B.A. Bengtsson, Premature mortality due to cardiovascular disease in hypopituitarism, Lancet 336 (1990) 285–288.

[7] B.A. Bengtsson, G. Johannsson, Effect of growth-hormone therapy on early atherosclerotic changes in GH-deficient adults, Lancet 353 (1999) 1898–1899.

[8] S. Longobardi, A. Cuocolo, B. Merola, F. Di Rella, A. Colao, E. Nicolai, S. Cardei, M. Salvatore, G. Lombardi, Left ventricular function in young adults with childhood and adulthood onset growth hormone deficiency, Clin. Endocrinol. (Oxf.) 48 (1998) 137–143.

[9] A. Frustaci, G.A. Perrone, N. Gentiloni, M.A. Russo, Reversible dilated cardiomyopathy due to growth hormone deficiency, Am. J. Clin. Pathol. 97 (1992) 503–511.

[10] A. Colao, C. Di Somma, A. Cuocolo, L. Spinelli, W. Acampa, S. Spiezia, F. Rota, M.C. Savanelli, G. Lombardi, Does a gender-related effect of growth hormone (GH) replacement exist on cardiovascular risk factors, cardiac morphology, and performance and atherosclerosis? Results of a two-year open, prospective study in young adult men and women with severe GH deficiency, J. Clin. Endocrinol. Metab. 90 (2005) 5146–5155.

[11] J. Isgaard, V. Kujacic, E. Jennische, A. Holmang, X.Y. Sun, T. Hedner, A. Hjalmarson, B.A. Bengtsson, Growth hormone improves cardiac function in rats with experimental myocardial infarction, Eur. J. Clin. Invest. 27 (1997) 517–525.

[12] H. bJin, R. Yang, N. Gillett, R.G. Clark, A. Ko, N.F. Paoni, Beneficial effects of growth hormone and insulin-like growth factor-1 in experimental heart failure in rats treated with chronic ACE inhibition, J. Cardiovasc. Pharmacol. 26 (1995) 420–425.

[13] R. Yang, S. Bunting, N. Gillett, R. Clark, H. Jin, Growth hormone improves cardiac performance in experimental heart failure, Circulation 92 (1995) 262–267.

[14] M. Hongo, T. Ryoke, J. Schoenfeld, J. Hunter, N. Dalton, R. Clark, D. Lowe, K. Chien, J. Ross Jr, Effects of growth hormone on cardiac dysfunction and gene expression in genetic murine dilated cardiomyopathy, Basic Res. Cardiol. 95 (2000) 431–441.

[15] S. Fazio, D. Sabatini, B. Capaldo, C. Vigorito, A. Giordano, R. Guida, F. Pardo, B. Biondi, L. Sacca, A preliminary study of growth hormone in the treatment of dilated cardiomyopathy, N. Engl. J. Med. 334 (1996) 809–814.

[16] S. Genth-Zotz, R. Zotz, S. Geil, T. Voigtlander, J. Meyer, H. Darius, Recombinant growth hormone therapy in patients with ischemic cardiomyopathy: effects on hemodynamics, left ventricular function, and cardiopulmonary exercise capacity, Circulation 99 (1999) 18–21.

[17] W.V. Houck, L.C. Pan, S.B. Kribbs, M.J. Clair, G.M. McDaniel, R.S. Krombach, W.M. Merritt, C. Pirie, J.P. Iannini, R. Mukherjee, F.G. Spinale, Effects of growth hormone supplementation on left ventricular morphology and myocyte function with the development of congestive heart failure, Circulation 100 (1999) 2003–2009.

[18] R.C. Cuneo, P. Wilmshurst, C. Lowy, G. McGauley, P.H. Sonksen, Cardiac failure responding to growth hormone, Lancet 1 (1989) 838–839.

[19] T. Takeuchi, H. Suzuki, S. Sakurai, H. Nogami, S. Okuma, H. Ishikawa, Molecular mechanism of growth hormone (GH) deficiency

in the spontaneous dwarf rat: detection of abnormal splicing of GH messenger ribonucleic acid by the polymerase chain reaction, Endocrinology 126 (1990) 31–38.
[20] F. Mantero, G. Opocher, D. Armanini, G. Paviotti, M. Boscaro, M. Muggeo, Plasma renin activity and urinary aldosterone in acromegaly, J. Endocrinol. Invest. 2 (1979) 13–18.
[21] R.C. Cuneo, F. Salomon, P. Wilmshurst, C. Byrne, C.M. Wiles, R. Hesp, P.H. Sonksen, Cardiovascular effects of growth hormone treatment in growth-hormone-deficient adults: stimulation of the renin-aldosterone system, Clin. Sci. (Lond.) 81 (1991) 587–592.
[22] J. Moller, N. Moller, E. Frandsen, T. Wolthers, J.O. Jorgensen, J.S. Christiansen, Blockade of the renin-angiotensin-aldosterone system prevents growth hormone-induced fluid retention in humans, Am. J. Physiol. 272 (1997) E803–E808.
[23] G. Strauch, M.B. Vallotton, Y. Touitou, H. Bricaire, The renin–angiotensin–aldosterone system in normotensive and hypertensive patients with acromegaly, N. Engl. J. Med. 287 (1972) 795–799.
[24] P. Tollet-Egnell, A. Flores-Morales, N. Stahlberg, R.L. Malek, N. Lee, G. Norstedt, Gene expression profile of the aging process in rat liver: normalizing effects of growth hormone replacement, Mol. Endocrinol. 15 (2001) 308–318.
[25] U. Zabel, M. Weeger, M. La, H.H. Schmidt, Human soluble guanylate cyclase: functional expression and revised isoenzyme family, Biochem. J. 335 (1998) 51–517.
[26] J.P. Stasch, E.M. Becker, C. Alonso-Alija, H. Apeler, K. Dembowsky, A. Feurer, R. Gerzer, T. Minuth, E. Perzborn, U. Pleiss, H. Schroder, W. Schroeder, E. Stahl, W. Steinke, A. Straub, M. Schramm, NO-independent regulatory site on soluble guanylate cyclase, Nature 410 (2001) 212–215.
[27] D.L. Vesely, Human and rat growth hormones enhance guanylate cyclase activity, Am. J. Physiol. 240 (1981) E79–E82.
[28] R.H. Boger, Nitric oxide and the mediation of the hemodynamic effects of growth hormone in humans, J. Endocrinol. Invest. 22 (1999) 75–81.
[29] G. Bonne, M.R. Di Barletta, S. Varnous, H.M. Becane, E.H. Hammouda, L. Merlini, F. Muntoni, C.R. Greenberg, F. Gary, J.A. Urtizberea, D. Duboc, M. Fardeau, D. Toniolo, K. Schwartz, Mutations in the gene encoding lamin A/C cause autosomal dominant Emery–Dreifuss muscular dystrophy, Nat. Genet. 21 (1999) 285–288.
[30] D. Fatkin, C. MacRae, T. Sasaki, M.R. Wolff, M. Porcu, M. Frenneaux, J. Atherton, H.J. Vidaillet Jr., S. Spudich, U. De Girolami, J.G. Seidman, C. Seidman, F. Muntoni, G. Muehle, W. Johnson, B. McDonough, Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease, N. Engl. J. Med. 341 (1999) 1715–1724.
[31] M.C. Sanders, A.L. Goldstein, Y.L. Wang, Thymosin beta 4 (Fx peptide) is a potent regulator of actin polymerization in living cells, Proc. Natl. Acad. Sci. USA 89 (1992) 4678–4682.
[32] F.X. Yu, S.C. Lin, M. Morrison-Bogorad, M.A. Atkinson, H.L. Yin, Thymosin beta 10 and thymosin beta 4 are both actin monomer sequestering proteins, J. Biol. Chem. 268 (1993) 502–509.
[33] P. Lappalainen, D.G. Drubin, Cofilin promotes rapid actin filament turnover in vivo, Nature 388 (1997) 78–82.
[34] E.M. De La Cruz, T.D. Pollard, Structural biology. Actin' up, Science 293 (2001) 616–618.
[35] I. Bock-Marquette, A. Saxena, M.D. White, J.M. Dimaio, D. Srivastava, Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair, Nature 432 (2004) 466–472.
[36] A. Flores-Morales, N. Stahlberg, P. Tollet-Egnell, J. Lundeberg, R.L. Malek, J. Quackenbush, N.H. Lee, Norstedt Microarray analysis of the in vivo effects of hypophysectomy and growth hormone treatment on gene expression in the rat, Endocrinology 142 (2001) 3163–3176.
[37] T.S. Tiong, A.C. Herington, Tissue distribution, characterization, and regulation of messenger ribonucleic acid for growth hormone receptor and serum binding protein in the rat, Endocrinology 129 (1991) 1628–1634.