[27] Yang JH, Qi YX, Jia YX, et al. Protective effect of intermedin/adrenomedullin-2 on ischemia/reperfusion injury in isolated rat hearts. Peptides 2005;26:501–7.
[28] Jia YX, Yang JH, Pan CS, et al. Intermedin 1–53 protects the heart against isoproterenol-induced ischemic injury in rats. Eur J Pharmacol 2006;549:117–23.
[29] Taylor MM, Bagley SL, Samson WK. Intermedin/Adrenomedullin-2 inhibits growth hormone release from cultured, primary anterior pituitary cells. Endocrinology 2006;147:859–64.
[30] Nakamura Y, Shimatsu A, Murabe H, Mizuta H, Ihara C, Nakao K. Calcitonin gene-related peptide as a GH secretagogue in human and rat pituitary somatotrophs. Brain Res 1998;807:203–7.

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Feasibility of cardiac MR examination during quantitative isometric muscular exercise

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Objective — MR examination during quantitative muscular exercise is currently unavailable because the MR instrument has a long gantry and exercise machines are made from ferromagnetic materials such as iron. The purpose of this study was to develop a quantitative, continuously monitored, voluntary isometric exercise device (QIED) for MR examination and to analyse the feasibility of performing MR examination using the new device.

Methods and results — The QIED, which consists of 1) a handgrip (rubber cuff), 2) an extension plastic tube, 3) a pressure transducer, and 4) a pressure digital display that includes the power supply. Components 1 and 2 are non-magnetic. Although components 3 and 4 are ferromagnetic, they can be set up outside the MR examination room using component 2 to prevent them from influencing the MR instrument and examination. We did not observe MR image noise or artifacts in the phantom study using the QIED. MR examination and low sensitivity ^{31}P-MRS could be feasible during quantitative isometric exercise using the QIED.

Conclusion — Exercise MR examination using the QIED will provide useful information for the detailed evaluation of cardiac patients.

Keywords: *magnetic resonance imaging – phosphorus-31 magnetic resonance spectroscopy – stress exercise – ventricular function.*

Introduction

Assessing cardiac performance under application of a circulatory stressor such as muscular exercise is important because patients with major heart disease may show entirely normal haemodynamics when assessed in a resting state[1-4]. The physiologic information obtained from this type of evaluation is often valuable in selecting patients for corrective cardiac surgery, estimating prognosis, and prescribing specific medical therapy[1-4].

Cardiac MRI can provide valuable information about ventricular function and haemodynamics[5-7]. MRI is non-invasive and does not require exposure to ionizing radiation. In addition, cardiac ^{31}P-MRS is unique in its ability to quantify myocardial levels of high-energy phosphate compounds such as adenosine triphosphate (ATP) and phosphocreatine (PCr) in a non-invasive manner[8-10].

There are very few reports regarding MR examination during quantitative muscular exercise because the MR instrument has a long gantry and exercise machines are made from ferromagnetic materials such as iron[11,12]. Therefore today the most used stressor is adenosine, pharmacological stress, when investigating for myocardial ischaemia in cardiac MRI. However, isometric handgrip exercise is reportedly useful for evaluating left ventricular (LV) performance[13].

The purpose of this study was to develop a quantitative, continuously monitored, voluntary isometric exercise device (QIED) for MR examination and to analyse the feasibility of performing MR examination using the new device.

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Methods

Development of a QIED for MR examination

We developed a QIED for MR examination using a handgrip (inflated sphygmomanometer cuff), which allows continuous monitoring of voluntary contractile strength during stress exercise (figure 1). Continuous monitoring of the contractile strength can maintain the stress during exercise, thus providing quantitative isometric exercise. Figure 2 shows a schematic drawing of the QIED, which consists of 1) a handgrip (rubber cuff), 2) an extension plastic tube, 3) a pressure transducer, and 4) a pressure digital display that includes the power supply. Components 1 and 2 are non-magnetic. Although components 3 and 4 are ferromagnetic, they can be set up outside the MR examination room using component 2 to prevent them from influencing the MR instrument and examination.

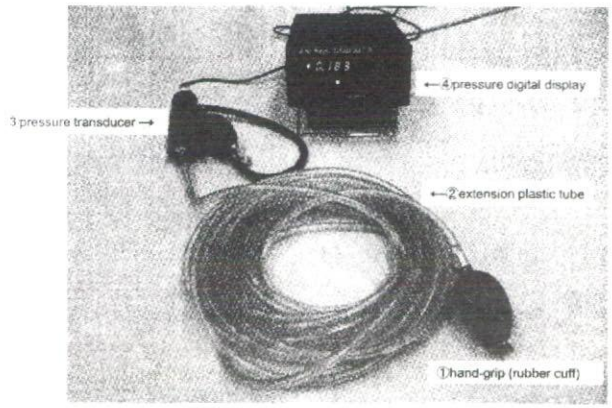

Fig. 1. – Quantitative isometric exercise device for MR examination.

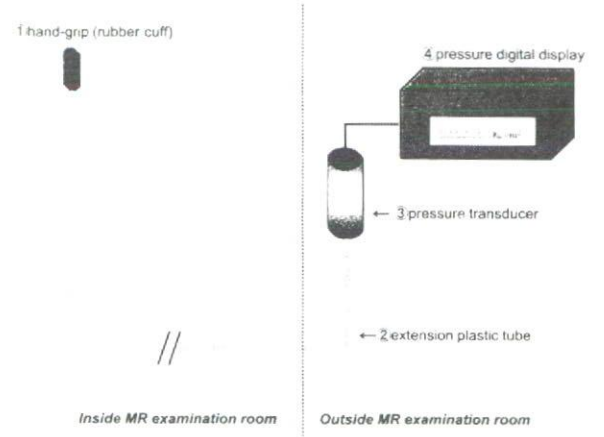

Fig. 2. – Schematic drawing of the developed quantitative isometric exercise device for MR examination.

Phantom study

To evaluate whether the exercise device affects the MR image, we performed MR scans using a phantom with components 3 and 4 placed inside (inside setting) or outside (outside setting) the MR examination room.

A 1.5-T whole-body MR imager was used. Signal transmission and reception were performed with a head coil (250-mm circular polarization coil). A 20-cm NiSO4 solution phantom was used for the phantom study. The phantom was placed inside a load phantom in the head coil. Proton images were obtained for localization in the transverse, coronal, and sagittal orientations using a modified gradient-echo technique.

The signal-to-noise ratio (SNR) was determined according to the method recommended by the National Electrical Manufacturers Association (NEMA). The SD (air), an indicator of noise, and the reproducibility of the MR signals were assessed using ten consecutive measurements and were compared between the inside setting and the outside setting.

Volunteer study

We performed rapid cardiac ^{31}P-MRS using a double-resonant (^{31}P/^{1}H) single-turn surface coil with the same MR instrument.

The experimental protocol for rapid cardiac ^{31}P-MRS was described in detail in our previous study[14,15]. Briefly, using a two-dimensional phosphorus chemical shift imaging sequence (2D-CSI) in combination with 30-mm axial slice-selective excitation, complete 3-D localization was performed. The rapid ^{31}P-MRS procedure was phase-encoded in arrays of 8 * 8 steps with an average of four acquisitions. K-space zero-filling was used, yielding 2 * 2 * 3-cm volume elements. The TR was set to one R-R interval for the phosphorus 2D-CSI measurements. The acquisition time was 3–5 min, depending on the heart rate (HR). The volume element for each 2D-CSI sequence was positioned on the interventricular septum of the anterior wall of the LV. The areas under each peak of the PCr and beta-ATP signal curves from the chosen volume element were evaluated using a built-in curve-fitting programme. The values for the PCr/ATP ratios obtained using rapid cardiac ^{31}P-MRS were compared.

1. Study using a single volunteer

To evaluate whether the QIED can affect human MR examinations, a healthy volunteer (26-years-old man), assessed using five measurements, was examined by rapid cardiac ^{31}P-MRS with both the inside and outside settings.

2. Study using six volunteers

To evaluate the feasibility of performing MR examinations during quantitative muscular exercise using

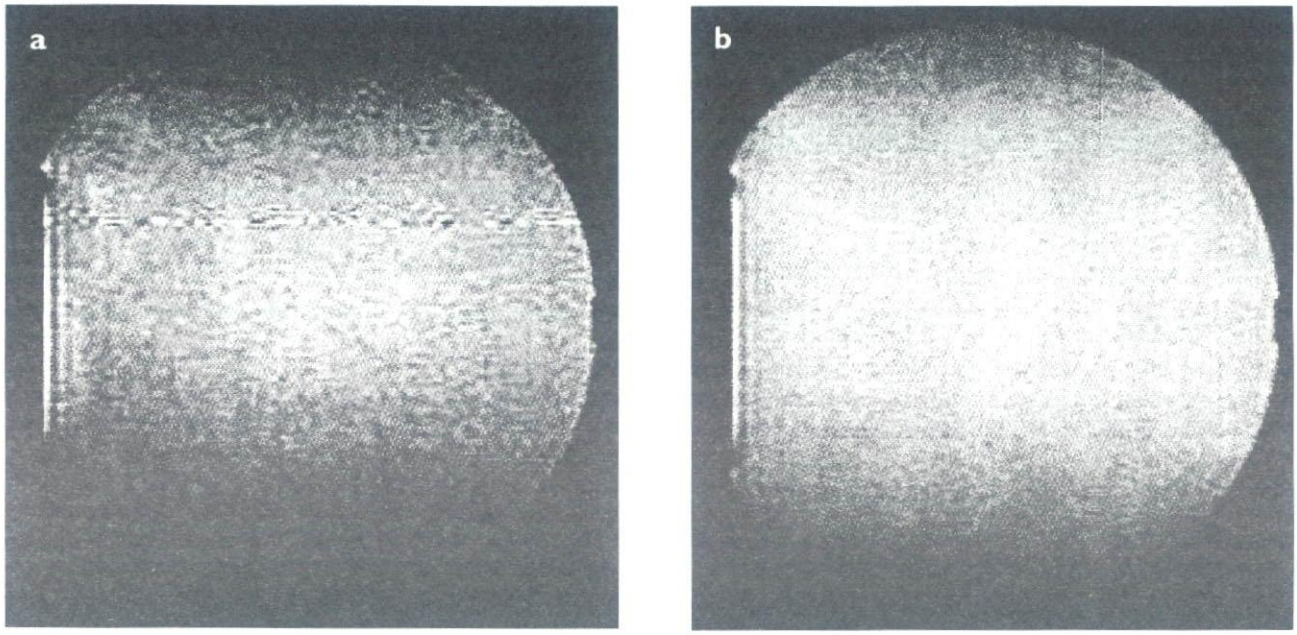

Fig. 3. – MR image of the phantom: (a) inside setting, (b) outside setting.

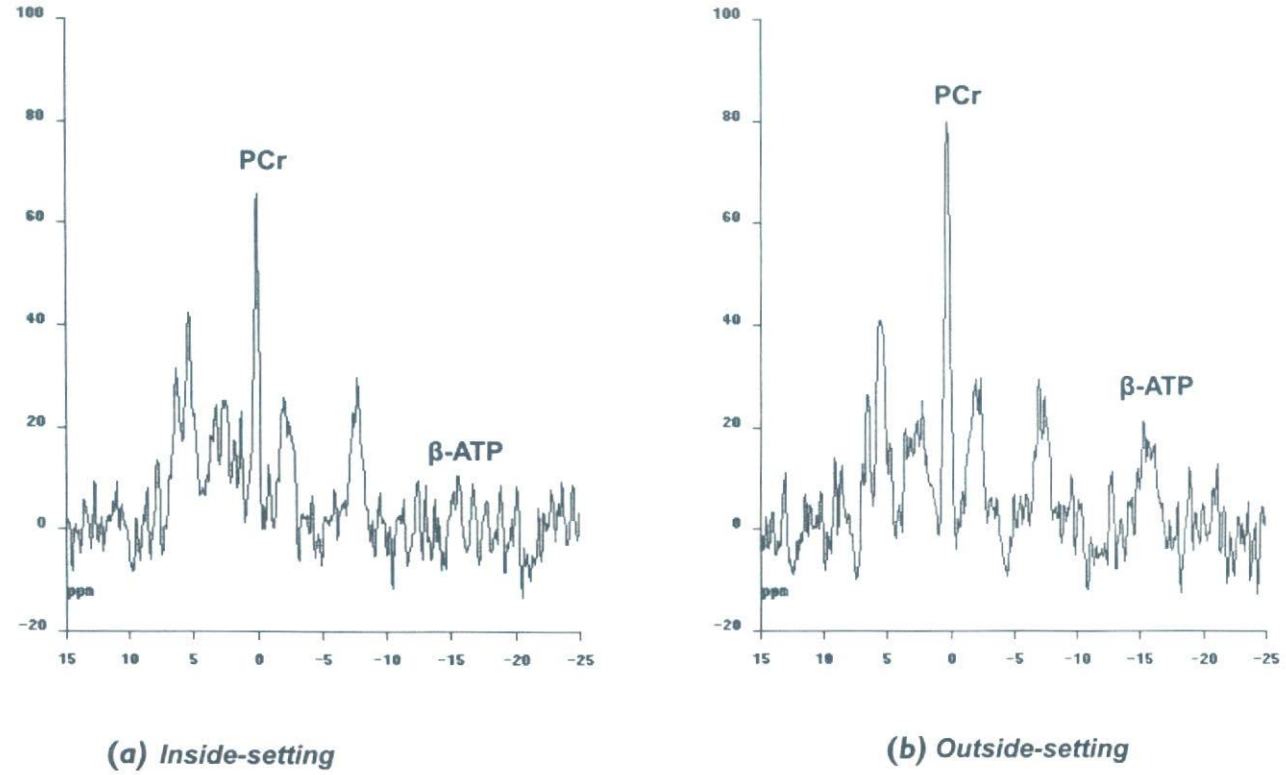

Fig. 4. – Typical outside setting and inside setting ^{31}P -MR spectra from the same volunteer. (a) Inside setting, (b) outside setting.

the QIED (outside setting), six healthy volunteers (male, 22.5 ± 2.0 years old) were examined by rapid cardiac ^{31}P-MRS at rest (resting state = baseline) and while exercising with the QIED. Once baseline ^{31}P-MRS, blood pressure, and HR data were collected, the volunteers were asked to grip the inflated dynamometer at a level that was 30-40% of their previously determined maximal voluntary contractile force for approximately 5 min. Some coaching was required to ensure that the volunteer sustained the grip by referencing the digital display for continuous pressure monitoring. Repeat measurements of rapid cardiac ^{31}P-MRS data, blood pressure, and HR were begun 2 min after the start of muscular exercise so that measurements were completed by approximately 5 minutes. The test was terminated depending on the heart rate.

Statistics. An unpaired Student's t-test was used to compare the two groups (inside setting vs. outside setting, rest vs. exercise). *P*-values < 0.05 were considered statistically significant.

Results

An MR image of the phantom is shown in figure 3. During the inside setting, the MR image had artifacts and noise (figure 3a). When changed to the outside setting (figure 3b), the MR image had good quality as well as a normal condition (without QIED). The SNR obtained from the phantom study was significantly higher ($P < 0.01$) with the outside setting (88.97 ± 2.68) than the inside setting (79.18 ± 1.06). The SD (air) was significantly lower ($P < 0.05$) with the outside setting (2.38 ± 0.08) than the inside setting (2.72 ± 0.35). The coefficient of variation (CV) of the MR signal measurements for the outside and inside settings was 3.3 and 12.9%, respectively; the reproducibility of the inside setting was worse than that of the outside setting.

Typical outside and inside setting ^{31}P-MR spectra from the single-volunteer study are shown in figure 4a,b. The ^{31}P-MR spectra for the inside setting was noisier than for the outside setting. The myocardial PCr/ATP ratio tended to be higher in the inside setting (4.45 ± 5.18) than the outside setting (2.07 ± 0.29). Although the difference was not statistically significant ($P = 0.33$), the myocardial PCr/ATP ratio for the inside setting was abnormal.

Figure 5 shows a cardiac ^{31}P-MRS examination of a volunteer during quantitative isometric exercise using the QIED (outside setting). The results for the study using six volunteers are summarized in table 1. The systolic and diastolic blood pressures and HRs during the exercise increased 14.2, 33.5, and 22.5%, respectively. Typical rapid ^{31}P-MR spectrums of a healthy volunteer in the resting state and during the exercise test are shown in figure 6a and b. The myocardial PCr/ATP ratios of the six volunteers were identical for the resting state (2.08 ± 0.31) and during the exercise test (2.00 ± 0.26).

Discussion

This manuscript reports the development of a quantitative, continuously monitored, voluntary isometric exercise device that can be used during an MR examination, particularly the ^{31}P magnetic resonance study.

We developed a new exercise device using a handgrip to augment MR examinations. The ferromagnetic components (3 and 4) were placed outside the MR examination room using component 2 to prevent these components from affecting the MR image and instrument (no artifacts, smaller noise, and increased safety). In the phantom study, the QIED exercise device had good SNR and SD values and good reproducibility.

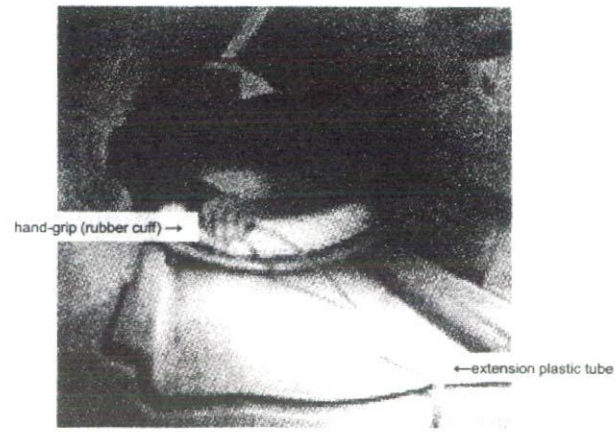

Fig. 5. – Cardiac ^{31}P -MRS examination of the volunteer during quantitative muscular exercise using the developed quantitative exercise device.

Table 1. – *Results of our exercise study using the developed quantitative exercise device (with six volunteer subjects)*

	Rest	Exercise	*P* value (rest vs. exercise)
Systolic blood pressure (mm Hg)	116.2 ± 17.8	132.7 ± 22.9	*0.194*
Diastolic blood pressure (mm Hg)	48.2 ± 6.4	64.3 ± 6.6	< 0.01
Heart rate (bpm)	63.5 ± 13.7	77.8 ± 13.0	*0.09*
PCr/ATP	2.08 ± 0.31	2.00 ± 0.26	*0.645*

Average ± SD.

In the study using a single volunteer, the myocardial PCr/ATP ratio had an abnormally high value with the inside setting; however, the PCr/ATP ratio was normal with the outside setting. One of the most likely reasons for this is that the ATP signal was not detected well, resulting in an artificially low value and thereby producing a high PCr/ATP ratio using the routine fitting programme, because the spectra (inside setting) were noisier and the ATP signal peak was small and not sharp.

Understanding MR examinations during exercise will be important not only for cardiologists but also for radiologists.

A handgrip exercise is suitable for the long gantry of an MR imager, unlike a bicycle ergometer. In the study with the six volunteers, cardiac ^{31}P-MRS examinations during quantitative muscular exercise using the QIED were feasible, and the blood pressure and HR measurements increased during the exercise. In addition, the QIED did not affect the ^{31}P-MRS, although ^{31}P-MRS has a very low sensitivity (S/N

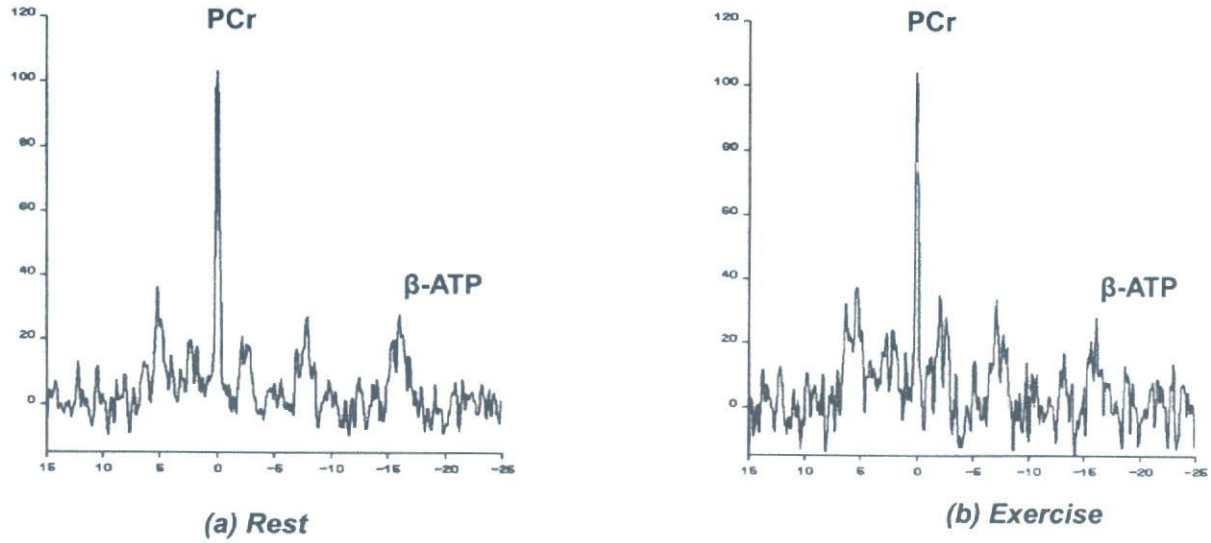

Fig. 6. – Typical rest and exercise ^{31}P-MR spectra. (a) Rest, (b) exercise.

ratio). The myocardial PCr/ATP ratios were identical between the resting state and during the exercise test because the six volunteers were healthy. If cardiac patients are tested, the myocardial PCr/ATP ratio obtained by rapid ^{31}P-MRS may be significantly lower during the exercise test than the resting state.

This study has some limitations: the small number of subjects studied and that the study was limited to healthy volunteers; the fact that they only performed MRS (did not perform other MRI sequences) and the fact the haemodynamic response to isometric exercise was only mild.

In summary, we developed the QIED for MR examinations. Exercise MR examination could be employed in the evaluation of cardiac patients, and the examination using the QIED will be a useful diagnostic tool of cardiac disease for both cardiologists and radiologists.

Conclusion

We developed the QIED using a handgrip (inflated sphygmomanometer cuff) for MR examination. We did not observe MR image noise or artifacts in the phantom study using the QIED. MR examination and low sensitivity ^{31}P-MRS could be feasible during quantitative isometric exercise using the QIED, and exercise MR examination using the QIED will provide useful information for the detailed evaluation of cardiac patients.

Acknowledgment

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Abbreviations

MRI; magnetic resonance imaging, MRS; magnetic resonance spectroscopy, QIED; quantitative voluntary isometric exercise device, ATP; adenosine triphosphate, PCr; phosphocreatine, 2D-CSI; two-dimensional phosphorus chemical shift imaging sequence,

Conflict of interest: none declared.

References

1. Fletcher GF, Mills WC, Taylor WC. Update on exercise stress testing. *Am Fam Physician* 2006; **74**: 1749-54.
2. Grossman W. Stress testing during cardiac catheterization. In: Baim DS and Grossman W, eds. *Grossman's cardiac catheterization, angiography, and intervention*, 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA: 2000: 325-51.
3. Kitzman DW, Higginbotham MB, Cobb FR, Sheikh KH, Sullivan MJ. Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism. *J Am Coll Cardiol* 1991; **17**: 1065-72.
4. Carroll JD, Hess OM, Hirzel HO, Krayenbuehl HP. Dynamics of left ventricular filling at rest and during exercise. *Circulation* 1983; **68**: 59-67.
5. Sodickson DK. Clinical cardiovascular magnetic resonance imaging techniques: In Manning WJ and Pennell DJ, eds: *Cardiovascular Magnetic Resonance*. Churchill Livingstone. New York: 2002: 18-30.

6. Jauhiainen T, Jarvinen VM, Hekali PE. Evaluation of methods for MR imaging of human right ventricular heart volumes and mass. *Acta Radiol* 2002; **43**: 587-92.
7. Triantafyllidi H, Rizos I, Arvaniti C, Stefanadis C. Incidental aneurysms of aorta and basilar artery in patients with coronary artery ectasia. A magnetic resonance angiography study. *Acta Cardiol* 2005; **60**: 619-23.
8. Chida K, Otani H, Kohzuki M, Saito H, Kagaya Y, Takai Y, Takahashi S, Yamada S, Zuguchi M. The relationship between plasma BNP level and the myocardial phosphocreatine/adenosine triphosphate ratio determined by phosphorus-31 magnetic resonance spectroscopy in patients with dilated cardiomyopathy. *Cardiology* 2006; **106**: 132-6.
9. Weiss RG, Kalil-Filho R, Bottomley PA. Clinical cardiac magnetic spectroscopy: In Manning WJ and Pennell DJ, eds: *Cardiovascular Magnetic Resonance*. Churchill Livingstone. New York: 2002: 437-46.
10. Jung W-I, Sieverding L, Breuer J, Hoess T, Widmaier S, Schmidt O, Bunse M, van Erckelens F, Apitz J, Lutz O, Dietze GJ. 31P NMR spectroscopy detects metabolic abnormalities in asymptomatic patients with hypertrophic cardiomyopathy. *Circulation* 1998; **97**: 2536-2542.
11. Roest AA, Lamb HJ, van der Wall EE, Vliegen HW, van den Aardweg JG, Kunz P, de Roos A, Helbing WA. Cardiovascular response to physical exercise in adult patients after atrial correction for transposition of the great arteries assessed with magnetic resonance imaging. *Heart* 2004; **90**: 678-84.
12. Tops LF, Roest AA, Lamb HJ, Vliegen HW, Helbing WA, van der Wall EE, de Roos A. Intraatrial repair of transposition of the great arteries: use of MR imaging after exercise to evaluate regional systemic right ventricular function. *Radiology* 2005; **237**: 861-7.
13. Grossman W, McLaurin LP, Saltz SB, Paraskos JA, Dalen JE, Dexter L. Changes in the inotropic state of the left ventricle during isometric exercise. *Br Heart J* 1973; **35**: 697-704.
14. Chida K, Saito H, Nagasaka T, Otani H, Kohzuki M, Zuguchi M. Rapid-sequence phosphorus-31 magnetic resonance spectroscopy of the human heart using a 1.5 T clinical system. *Acta Radiol* 2004; **45**: 30-7.
15. Chida K, Otani H, Saito H, Nagasaka T, Kagaya Y, Kohzuki M, Zuguchi M, Shirato K. Feasibility of rapid-sequence ^{31}P magnetic resonance spectroscopy in cardiac patients. *Acta Radiol* 2005; **46**: 386-40.

Outpatient Phase III Cardiac Rehabilitation and the Training System of the Masters of Cardiac Rehabilitation in Japan

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KOHZUKI ET AL.: ***Outpatient Phase III Cardiac Rehabilitation and the Training System of the Masters of Cardiac Rehabilitation in Japan.*** *Cardiac rehabilitation (CR) is an integral component of the continuum of care for patients with cardiovascular diseases. The CR program usually consists of three stages: the acute stage (phase I), subacute stage (stage II) and maintenance stage (phase III). Phase III CR is recognized as a community or home-based program committed to encourage exercise and a healthful lifestyle with the goal of minimizing the risk of recurring cardiac problems (secondary prevention). The Japanese Association of Cardiac Rehabilitation (JACR) established a certification program for the Master of Cardiac Rehabilitation (MCR) to improve quality of cardiac rehabilitation services and to educate the professionals playing a pivotal role in a primary CVD prevention programs in Japan. The number of certified MCR has increased to 1835 by 2008. JACR also established a non-profit organization Japan Heart Club (JHC) in 2004 and started to publish learning materials for health promotion and prevention of CVD. JHC also provides opportunities to participate in CR programs in the community by the activities of "MedEX Club", a multidisciplinary facility provides MCR-supervised exercise sessions, education for patients, and training classes for citizens and health professionals. There are seven MedEx Club branches and 11 classes are being offered nationwide. The MedEx Club can offer convenient, affordable, safe and enjoyable phase III programs and, in the near future, it may be recognized as. a standard model of phase III CR service in Japan. (J HK Coll Cardiol 2008;16 (Suppl 1):A23-A28)*

Cardiac rehabilitation, master, phase III, Japan Heart Club, MedEX Club

摘要

心臟康復是心血管疾病長期治療不可分割的部分。心臟康復療程通常由三個階段組成：急性期（第一期），亞急性期（第二期）和恢復期（第三期）。第三期心臟康復以社區或家庭爲基礎，提倡運動和健康生活方式，從而使心臟病復發的危險性最小化（二級預防）。日本心臟康復協會（JACR）建立了心臟康復碩士的資格培訓計畫，目的在於提高心臟康復醫療品質以及培養在預防原發性心血管疾病中起關鍵作用的專業人員。心臟康復碩士合格人數至２００８年已達1835人。JACR在2004年創立了非盈利組織日本心臟學會（JHC）並出版了關於改善健康狀況及預防心血管疾病的學習資料。JHC也通過MedEX協會提供參加社區心臟康復培訓的機會。MedEX協會是一個提供心臟康復碩士監督課程、患者宣教及市民和健康專業人員培訓等課程的等多學科機構，目前全球有7個MedEX分會和11個班級。MedEX協會提供便捷、經濟、安全而有趣的三期培訓，不久它可能成爲日本第三期心臟康復的標準模式。

關鍵詞：心臟康復　碩士　第三期　日本心臟協會　MedEX協會

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Cardiac Rehabilitation Program As a Lifelong, Multidisciplinary Approach

Over the past two decades, risk factor modification programs for cardiac patients, commonly referred to as cardiac rehabilitation (CR), have evolved into a comprehensive management strategy. The

American Heart Association (AHA) and the American Association of Cardiovascular and Pulmonary Rehabilitation (AACVPR) define CR programs as, "Coordinated, multifaceted interventions designed to optimize a cardiac patient's physical, psychological, and social functioning, in addition to stabilizing, slowing, or even reversing the progression of the underlying atherosclerotic processes, thereby reducing morbidity and mortality".[1]

Core components for CR recommend a multidisciplinary approach to CR consisting of trained and competent staffs which include physician, cardiac specialist nurse, physical therapist, dietician, occupational therapist, administrator, social worker, and so on.[2] The team should also include, where appropriate, pharmacist, physical activity/exercise specialist, and psychologist. The core components of CR are (1) lifestyle management: (physical activity, diet, weight management, and smoking cessation; (2) education; (3) risk factor management; (4) psychosocial counseling; (5) cardio-protective drug therapy and implantable devices; and (6) long-term management strategy. The content of these components should be developed collaboratively by the core CR team and should be delivered by competent, appropriately skilled professionals.

The CR program usually consists of three stages: the acute stage (phase I), subacute stage (stage II) and maintenance stage (phase III). Phase III CR is recognized as a community or home-based program committed to encourage exercise and a healthful lifestyle with the goal of minimizing the risk of recurring cardiac problems (secondary prevention). For the patients' benefit, phase III CR programs should be convenient, affordable, safe and enjoyable. The European Society of Cardiology also recommends that cardiac patients should be oriented to a long-term maintenance regimen with the use of support systems such as coronary clubs, gymnasiums or other facilities to promote long-term prevention strategies in the community.[3] In Germany, a close network of currently approximately 6600 heart groups has been established,[4] the concept of cardiac reconditioning centers for the prevention and rehabilitation of coronary patients has been tremendously successful.[5]

Japan Heart Club and the Certification Program for the Masters of CR

With support of the Japanese Association of Cardiac Rehabilitation (JACR), the Japan Heart Club (JHC), a non-profit organization, was established in 2004. The missions of JHC are to (1) organize scientific meetings and workshops for health promotion and prevention of cardiovascular diseases (CVD); (2) publish journals and learning materials for health promotion and prevention of CVD, (3) conduct research for health promotion and prevention of CVD; (4) organize facilities and develop programs for primary and secondary CVD prevention; (5) offer education programs and certification for the Master of Cardiac Rehabilitation (MCR), and other health-related professionals; (6) collaborate with national and international research institutes.

Certification program for MCR started in 2000. The objectives of the certification program are to improve quality of cardiac rehabilitation services and to educate the professionals playing a pivotal role in a primary CVD prevention programs in Japan. The JACR certifies those who understand the purpose of CR and have knowledge, skills and abilities for providing comprehensive CR program through a comprehensive team approach. Referring to American College of Sports Medicine certification objectives,[6] the MCR certification examination is based upon the knowledge, skills and abilities (KSA's) in each of the 11 categories below:

1. Anatomy and Biomechanics (4 KSAs)
2. Exercise Physiology (8 KSAs)
3. Electrocardiology (7 KSAs)
4. Human behavior and psychology (6 KSAs)
5. Pathophysiology (13 KSAs)
6. Clinical diagnosis and treatment (7 KSAs)
7. Health appraisal and fitness testing (10 KSAs)
8. Cardiac rehabilitation (3 KSAs)
9. Secondary prevention and patient education for CAD (11 KSAs)
10. Exercise programing (14 KSAs)
11. Safety, injury prevention and emergency care (3 KSAs)

Minimum requirements for candidates are as follows:

- Candidates must possess any of the following certifications or degrees: physician, registered nurse, physical therapist, occupational therapist, clinical laboratory technician, medical engineer, clinical psychologist, and/or exercise trainer.
- Have been a member of the JACR for more than 2 years.
- Have a minimum of 1 year of experience in a CR program or equivalent, and submit 10 case reports about the diagnosis, tests, treatment, and rehabilitation for patients with CVD.

The number of certified MCR has increased to 1835 by 2008 (Figure 1). As shown in Figure 2, the MCR attracts health-related professionals with various backgrounds some of which include physical therapists (39%), physicians (24%), nurses (18%), and clinical labolatory technicians (13%).

Community-based Phase III CR and Primary Prevention Programs in Japan

One of the missions for JHC is providing opportunities to participate in a CR program in the community. MedEX Club, a multidisciplinary facility provides MCR-supervised exercise sessions, education for patients, and training classes for citizens and health professionals. There are seven MedEx Club branches and 11 classes are being offered nationwide (Figure 3). The purpose of the MedEx club is to promote regular physical activity in CR patients and prevent cardiac disease and the recurrence of coronary events. Exercise training classes are held in various settings, some of which include hospitals, community centers, fitness facilities, and schools. The classes are typically held once or twice a week under the supervision of the MCRs. In the MedEx Club branch in Sendai, each session lasts 70 minutes and has a capacity of 12 people (Table 1).

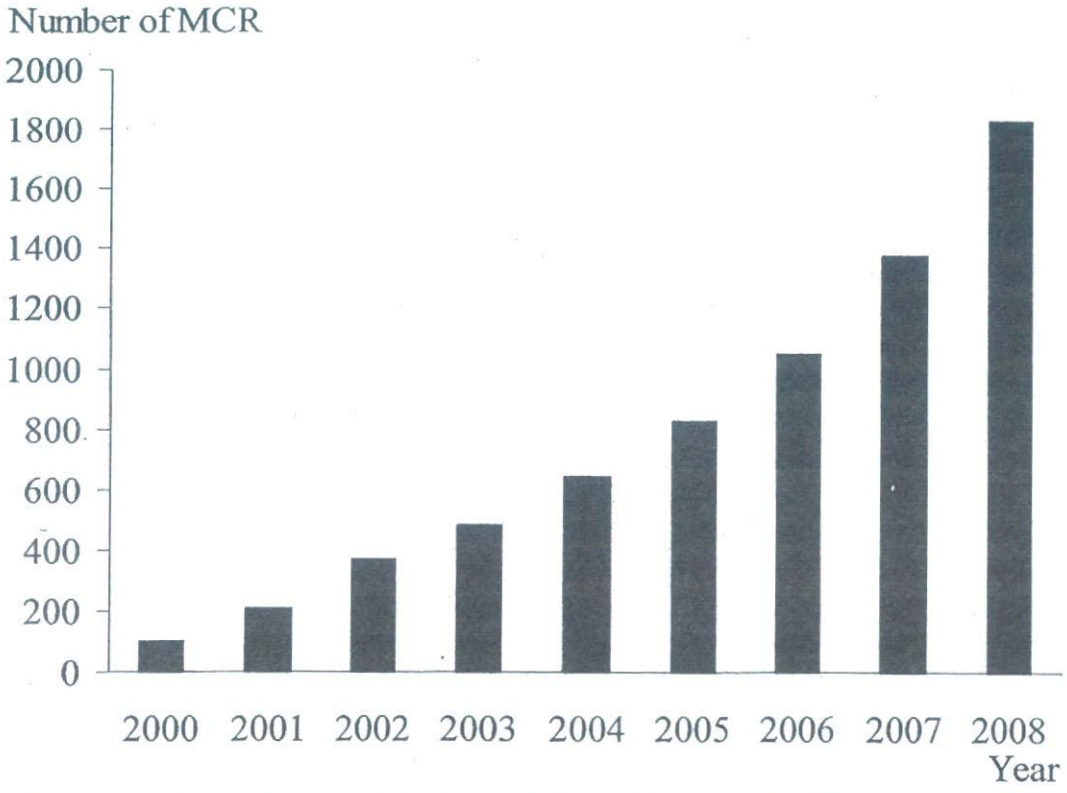

Figure 1. *The number of certified MCR from 2000-2008 (data from reference 7).*

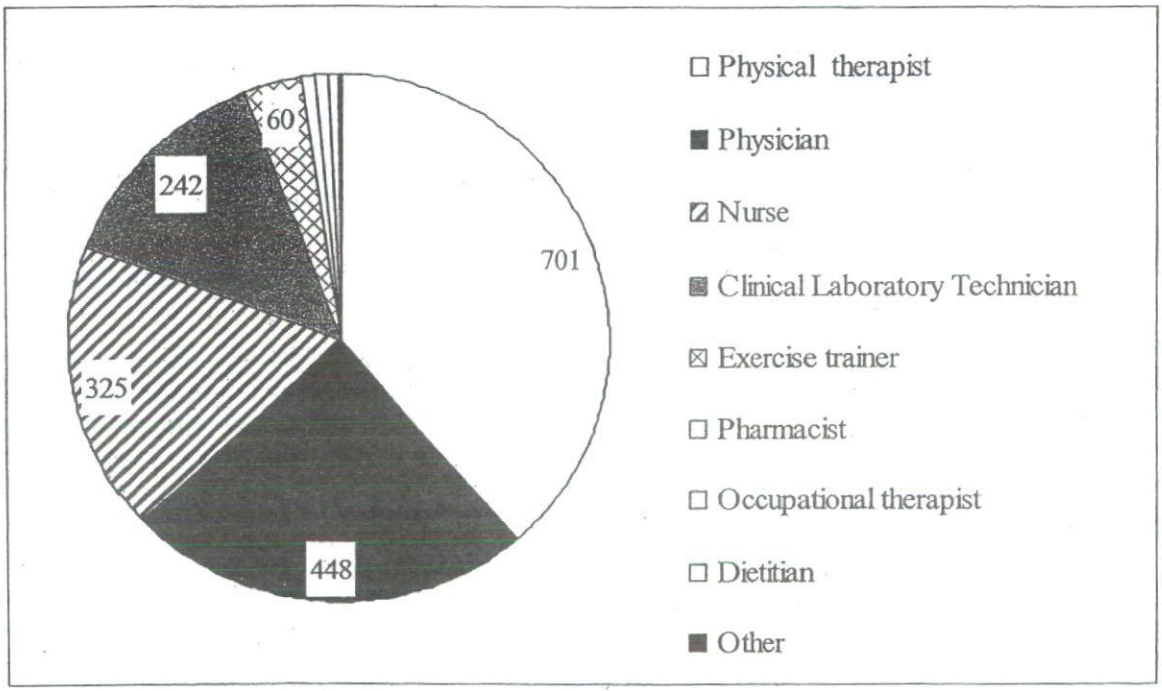

Figure 2. *The professional backgrounds of MCR (data from reference 7).*

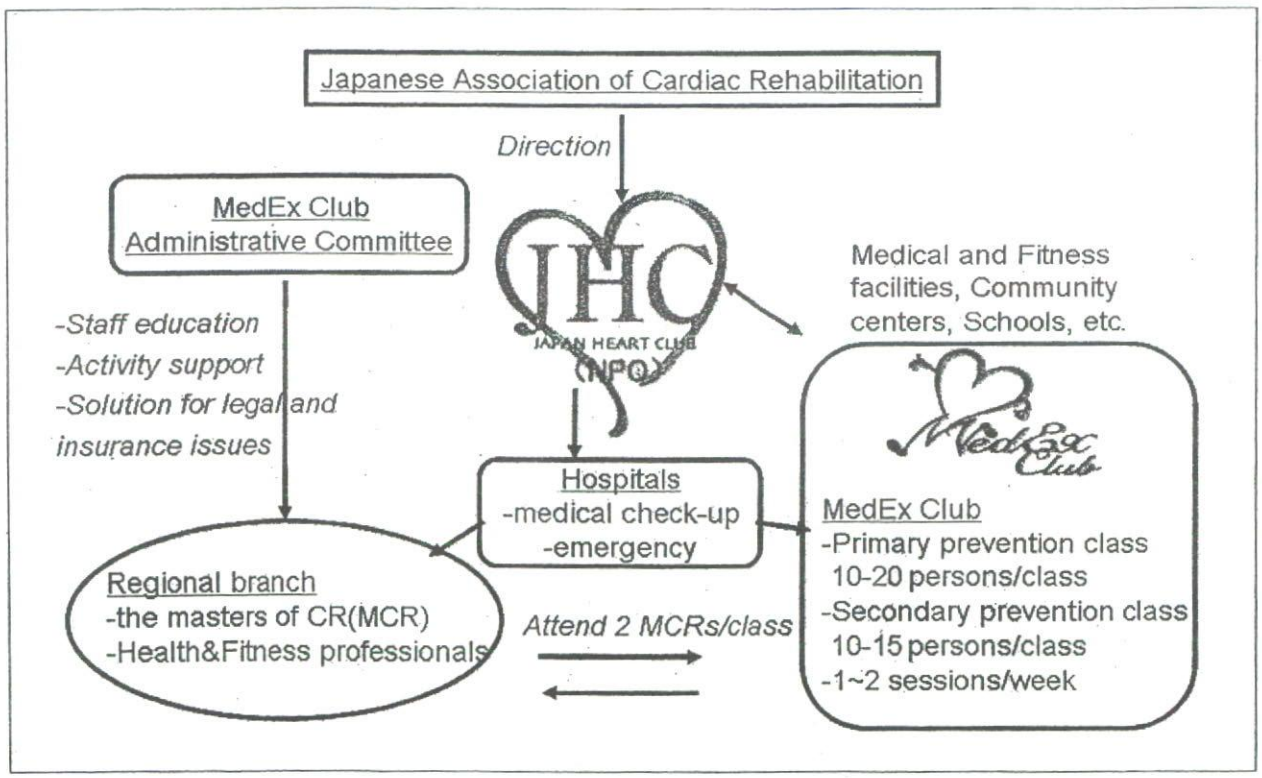

Figure 3. *MedEx Club, the community-based CR programs in Japan (data from references 8 and 9).*

Table 1. Current participants in MedEx Club at Sendai Branch

No	Age	Sex	CVD	Type of procedure	Coronary risk factors	Weight (kg)	BMI (kg/m^2)	VO_2@VT (ml/kg/min)	VO_2@Peak (ml/kg/min)
1	76	F	AP	stent	DL	54	23.1	12.1	16.0
2	74	M	MI	stent	HT	63.5	23.6	9.1	18.0
3	56	M	MI	CABG	DM, HT	67	23.2	15.6	23.3
4	70	M	MI	Stent	HT	56	19.2	7.0	19.3
5	66	M	MI	stent	HT, DL	62	23.3	18.8	28.2
6	61	M	MI	stent	HT	65	23.0	18.8	30.2
7	78	M	MI	stent	IGT	53	20.4	11.2	20.8
8	64	M	DCM	-	HT, DM	66	24.6	14.2	23.8
9	61	F	Paf	ablation	Ob	82	32.8	10.8	18.2
10	74	M	-	-	DM, HT, DL, Ob	87	33.2	7.9	16.4
11	60	F	MI	stent	HT, DL	53	22.6	15.6	19.6
12	66	F	MI	CABG	DM, HT, DL, Ob	69	30.3	9.7	18.1

Abbreviations: AP: angina pectoris, MI: myocardial infarction, DCM: dilated cardiomyopathy, Paf: paroxysmal atrial fibrillation, VT: ventilatory threshold, HT: hypertension, DL: dyslipidemia, DM: diabetes mellitus, IGT: impaired glucose tolerance, Ob: obesity.

Prior to and post-exercise session, participants measure their blood pressure and body weight and fill in the self-health check sheet. The exercise session starts with a 15 minute warm-up, either sitting or standing, followed by 15 minutes of aerobic exercise and 15 minutes of resistance training using elastic bands or their own body weight. The intensity of the aerobic exercise is determined by the cardiopulmonary exercise test measured upon entry to the club. Each session ends with cool down for 15 minutes which includes stretching of the major muscle groups. In addition to weekly exercise sessions, each patient keeps a log for blood pressure and body weight in the morning and night as well as step counts and exercise energy expenditure measured by an accelerometer. The log is submitted to the MCR program every 2 weeks. The MedEx club mainly offers exercise-based CR program, but patients also learn about physical activity, lifestyle modification, psychological management from the MCRs and other participants.

Cardiac rehabilitation is an integral component of the continuum of care for patients with CVD, the MedEx club can offer convenient, affordable, safe and enjoyable phase III programs and, in the near future, it may be recognized as a standard model of phase III CR service in Japan.

References

1. Leon AS, Franklin BA, Costa F, et al. Cardiac rehabilitation and secondary prevention of coronary heart disease: an American Heart Association scientific statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Cardiac Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity), in collaboration with the American association of Cardiovascular and Pulmonary Rehabilitation. Circulation 2005;111:369-76.
2. Balady GJ, Williams MA, Ades PA, et al. Core components of cardiac rehabilitation/secondary prevention programs: 2007 update: a scientific statement from the American Heart Association Exercise, Cardiac Rehabilitation, and Prevention Committee, the Council on Clinical Cardiology; the Councils on Cardiovascular Nursing, Epidemiology and Prevention, and Nutrition, Physical Activity, and Metabolism; and the American Association of Cardiovascular and Pulmonary Rehabilitation. Circulation 2007;115:2675-82.
3. Giannuzzia P, Sanerb H, Björnstadc H, et al. Secondary prevention through cardiac rehabilitation: position paper of the Working Group on Cardiac Rehabilitation and Exercise Physiology of the European Society of Cardiology. Eur Heart J 2003;24:1273-8.
4. Karoffa M, Held K, Bjarnason-Wehrens B. Cardiac

rehabilitation in Germany. Eur J Cardiovasc Prev Rehabil 2007; 14:18-27.
5. Buchwalsky G, Buchwalsky R, Held K. Long-term effects of rehabilitation of an outpatient 'heart group'. A case control study. Z Kardiol 2002;91:139-46.
6. American College of Sports Medicine. ACSM's Guidelines for Exercise Testing and Prescription, 7th edition. Philadelphia, PA, Lippincott Williams &Wilkins, 2006, pp. 315-49.
7. Master of Japanese Association of Cardiac Rehabilitation. Available from URL: http://square.umin.ac.jp/jacrreha/ (cited Oct 27, 2008).
8. MedEx Club. Available from URL: http://www.npo-jhc.org/medex_club/index.htm (cited Oct 27, 2008).
9. Itoh H. Maintenance phase cardiac rehabilitation. J Clin Rehabil 2006;15:738-44 (in Japanese).

Electrical Stimulation of Skeletal Muscles in Patients with Heart Failure: An Alternative to Aerobic Training?

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KOHZUKI ET AL.: ***Electrical Stimulation of Skeletal Muscles in Patients with Heart Failure: An Alternative to Aerobic Training?*** *The exercise training has been shown to improve the functional capacity, quality of life and also the patterns of strength muscles in patients with chronic heart failure (CHF). Most of actual training types, however, are based on the systemic exercises resulting in increased cardiac workload. This could lead to onset of life-threatening side effects such as fatal dysrhythmias. Moreover, most of patients with CHF have low exercise tolerance and poor motivation to exercise. Low-frequency electrical stimulation (ES) has been shown to increase oxidative capacity in the skeletal muscle fibers, to enhance muscular regeneration and to prevent atrophy. We aimed to evaluate the possible benefit of ES in patients with mild to severe CHF, and to compare the results with the conventional bicycle training. In our data, 6 weeks ES (60 min/day) of quadriceps and calf muscles of both legs significantly improved muscle strength and blood flow in patients with advanced CHF. Moreover, a similar improvement of exercise capacity in patients with CHF can be achieved either by aerobic bicycle training or by local ES of the strength muscles of the lower limbs in patients with mild to moderate CHF. Although the effectiveness of conventional exercise protocols in cardiovascular rehabilitation is beyond doubt, the safety and easy application of ES could be of great benefit in the rehabilitation of patients with CHF, especially those with a severe grade of the disease. Future studies should also address the possibility of combining ES with some type of classical exercise training. (J HK Coll Cardiol 2008;16 (Suppl 1):A38-A42)*

Electrical stimulation, exercise, heart failure, skeletal muscle

摘要

運動被證明能夠增強身體的機能、生活品質，並且在慢性心衰竭病人中增強肌肉力量。然而，大多數的運動方式是基於系統訓練並導致心臟負荷加重。這將會引起威脅生命的副作用如致死性的心律失常。不僅如此，絕大多數的慢性心衰竭病人的運動耐受性差，且運動意願低下。低頻率的電刺激能夠增加骨骼肌纖維的氧耗能力，促進肌肉增生，防止肌肉萎縮。我們旨在評估在輕度至重度慢性心衰患者中使用電刺激的可能性，並且與傳統的自行車訓練加以比較。在我們的資料中，6 周的電刺激（每天60 分鐘）進展期的心衰竭患者雙下肢股四頭肌和腓腸肌的肌肉力量和血流狀況得以明顯改善。不僅如此，在輕度至中度慢性心衰竭病人中不論通過需氧的自行車運動或者電刺激，運動能力的改善和下肢肌肉力量恢復狀況相似。儘管傳統運動的有效性在心血管病人的康復期中的作用毋庸置疑，但電刺激的安全性和易操作性對於慢性心衰竭病人的康復治療總顯示出巨大的益處，尤其在那些疾病程度嚴重的患者中。我們需要進一步研究電刺激聯合一些經典運動訓練的可能性。

關鍵詞：電刺激　運動　心衰竭　骨骼肌

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Chronic Heart Failure and Exercise Training

Dyspnea and premature fatigue of skeletal muscles are common symptoms of reduced exercise capacity in subjects with chronic heart failure (CHF). CHF is a complex metabolic syndrome with impaired left ventricular function and poor prognosis.[1] The exercise training has been shown to improve the functional capacity, quality of life and also the patterns of strength muscles, and therefore should be considered as an integral part of therapeutic standards in such patients.[2-5] Most of actual training types, however, are based on the systemic exercises resulting in increased cardiac workload.[6-7] This could lead to onset of life-threatening side effects such as fatal dysrhythmias, and for this reason classical rehabilitation programs need medical supervision and assistance. Other problems related to the choice of adequate exercise therapy for these patients are the grade of disease, low exercise tolerance and poor motivation to exercise.

Low-frequency electrical stimulation (ES) of strength muscles has been shown to increase oxidative capacity in the skeletal muscle fibers, to enhance muscular regeneration and to prevent atrophy.[8] Nuhr et al.[9] reported that ES increased functional capacity in patients with advanced CHF compared with sedentary controls. Recently, results from the first randomized trial comparing home-based ES and classical exercise training have demonstrated that both methods could significantly influence functional capacity, muscle strength of patients with mild to moderate CHF.[10] However, chronic effects of ES on systemic blood pressure, heart rate in mild to moderate CHF, or chronic effects of ES on systemic blood pressure, heart rate serum enzymes and functional capacity in severe CHF have not been elucidated. Regarding to the dramatic pathophysiologic changes which deteriorate the organism in conditions of chronic heart failure (including strength muscle mass) there is enough reasons to take the profit from ES also in conditions of CHF. We aimed to evaluate the possible benefit of ES in patients with mild to severe CHF, and to compare the results with the conventional bicycle training.

Electrical Stimulation: Animal Hindlimb Ischaemia Model Study[11]

ES in skeletal muscle at a level far below the threshold of muscle contraction has been reported to promote local angiogenesis in a hindlimb ischaemia model of rats. We completely excised bilateral femoral arteries of male Sprague-Dawley rats. After the operation, electrodes were implanted onto the centre of the fascia of the bilateral tibialis anterior (TA) muscles, tunnelled subcutaneously and exteriorized at the level of the scapulae. The right TA muscles of rats were stimulated continuously at a stimulus frequency of 50 Hz, with a 0.1 V stimulus strength and no interval, for 5 days. The left TA muscles served as controls. We found that angiogenic factors, such as vascular endotherial growth factor (VEGF), and hepatocyte growth factor (HGF) were significantly increased by ES in stimulated muscles compared with control rats.[11] It is concluded that both VEGF and HGF may contribute to the local angiogenesis produced by ES in a hindlimb ischaemia model of rats.[11]

Electrical Stimulation in Patients with Severe CHF[12]

We evaluated 15 patients with advanced grade of CHF (mean age 52±7 years; NYHA class III-IV; EF 19±3%) admitted at hospital for heart graft (Table 1). ES was performed 60 min/day, 7 days a week for 5 consecutive weeks, using dual-channel battery-powered stimulator.[12] The stimulator delivered a biphasic current of 10 Hz frequency. The electrical stimulated muscles were quadriceps and calf muscles of both legs. Self-

Table 1. Characteristics of patients included in the study[12]

Women/men	1/14
Age (years)	56.5±5.2
Heart failure aetiology (ischaemic/nonischaemic)	10/5
NYHA class (III/IV)	4/11
Left-ventricular ejection fraction (%)	18.7± 3.3

adhesive surface electrodes were positioned on the thighs approx. 5 cm under inguinal fold and 3 cm over the upper patella border; in the calf muscles the electrodes position was the area approx. 2 cm under the knee joint and just over the proximal end of Achilles tendon. The current characteristics were set up as follows: "on-off" mode stimulus (20s stimulation, 20s rest), pulse width 200 msec, rise and fall time 1s, and maximal stimulation amplitude 60 mA. The stimulation was performed in supine position, at the same day period and under supervision of medical staff. The first session of stimulation was started with lower amplitudes (around 30 mA). In the following days this value was gradually increased (by 10-15 mA/day) until the final value of 60 mA was achieved and well tolerated by the patient (in 2-4 days).[12]

Effects of ES on the muscle strength and blood flow in patients with advanced CHF were examined before and after 6 weeks of 10 Hz ES of quadriceps and calf muscles of both legs (1 h/day, 7 days/week). Dynamometry was performed weekly to determine maximal muscle strength (F_{max}; N) and isokinetic peak torque (PT_{max}; Nm); blood flow velocity (BFV) was registered before and after 6 weeks of ES using pulsed-wave Doppler velocimetry of right femoral artery. Six weeks of ES increased significantly F_{max} ($p<0.001$), and also PT_{max} ($p<0.01$). Mean BFV in femoral artery increased after 6 weeks ($p<0.05$); BFV values at rest before and after 6 weeks of ES did not differ significantly.[12]

After 6 weeks of LFES, all patients had less dyspnea and a marked decrease in subjective feelings of fatigue during everyday activities, although this change was not statistically significant. Similar results were observed for NYHA classification: 4 patients moved from NYHA IV to NYHA III after 6 weeks of stimulation and the remainder were unchanged but not (e.g., from NYHA III to NYHA IV).[12]

ES did not cause any significant changes of systolic blood pressure and diastolic blood pressure, nor were there significant changes in the recorded values of heart rate. In order to evaluate the possible risk of damage to muscle fiber by stimulation, the activity of both CK and LDH was monitored at the beginning, after 1 week and after 6 weeks of stimulation and only insignificant increases after 1 week of stimulation was observed and after 6 weeks of stimulation, the serum levels of both enzymes returned to baseline.[12] It is concluded that ES may improve the skeletal muscle strength and the blood supply. Thus, this method could be recommended in the treatment of patients with severe CHF.

Electrical Stimulation and Aerobic Exercise Training in Patients with Mild to Moderate CHF[13]

Thirty patients with stabilized form of CHF (mean age 56±6 years, NYHA class II-III, mean EF 35 ±5%) were randomly assigned to a rehabilitation program using either electrical stimulation of skeletal muscles or bicycle training (Table 2).[13] Patients in the first group had 8 weeks of home-based ES applied simultaneously to the quadriceps and calf muscles of both legs (1 h/day for 7 days/week); patients in the second group underwent 8 weeks of 40 min aerobic exercise (3 times a week; 5 min warm-up without workload, 30 min of training alternating 1 min of work - 2 min of relaxation, and 5 min of cool down without workload), The realization of the exercise training was strongly individual, performed at the level of anaerobic threshold determined by spiroergometry, and under supervision of medical staff (doctor, physiotherapist and nurse).

After the 8-week period significant increases in several functional parameters were observed in both groups: maximal VO2 uptake (ES group: $p<0.05$; bicycle group: $p<0.01$), maximal workload (ES group: $p<0.05$; bicycle group: $p<0.01$), distance walked in 6 minutes (ES group: $p<0.05$; bicycle group: $p<0.05$), and exercise duration (ES group: $p<0.05$; bicycle group:

Table 2. Characteristics of patients included in the study[13]

Women/men	7/23
Age (years)	56.3±6.0
Heart failure aetiology (ischaemic/nonischaemic)	24/6
NYHA class (II/III)	22/8
Left-ventricular ejection fraction (%)	34.7±5.0

p<0.05) (Figure 1). The QoL score assessed using the Minnesota Living with Heart Failure. Questionnaire was significantly improved in the bicycle group (from 41.4±5.3 to 27.3±6.3; p<0.03), whereas there was only a slight improvement in the ES group (from 39.6±2.9 to 31.4±4.8; NS). The present results demonstrated the good tolerance and significant improvement of functional capacity after 8 weeks of ES. The increases in VO2peak, Wpeak, and distance walked in 6 minutes, and also the exercise duration after 8 weeks of LFES were very similar to the increases in these parameters in the bicycle group. Other parameters (HRpeak, VO2AT and QoL) significantly increased in the bicycle group but not in the LFES group (only slight improvement was observed). Despite the differences between the 2 methods (myostimulation activity is local, whereas bicycle exercise training challenges the entire body), LFES could be regarded as an acceptable analogue of endurance training which can improve the physiological condition of CHF patients in a period of several weeks, and can be easily performed at home without medical supervision.[13]

Future Perspectives of Electrical Stimulation in Patients with CHF

Electrical stimulation of skeletal muscles in humans has been shown to be a useful therapeutic tool in neurology,[14] postoperative treatment, and in cases of long-term immobilization.[15] In a recent study, LFES was

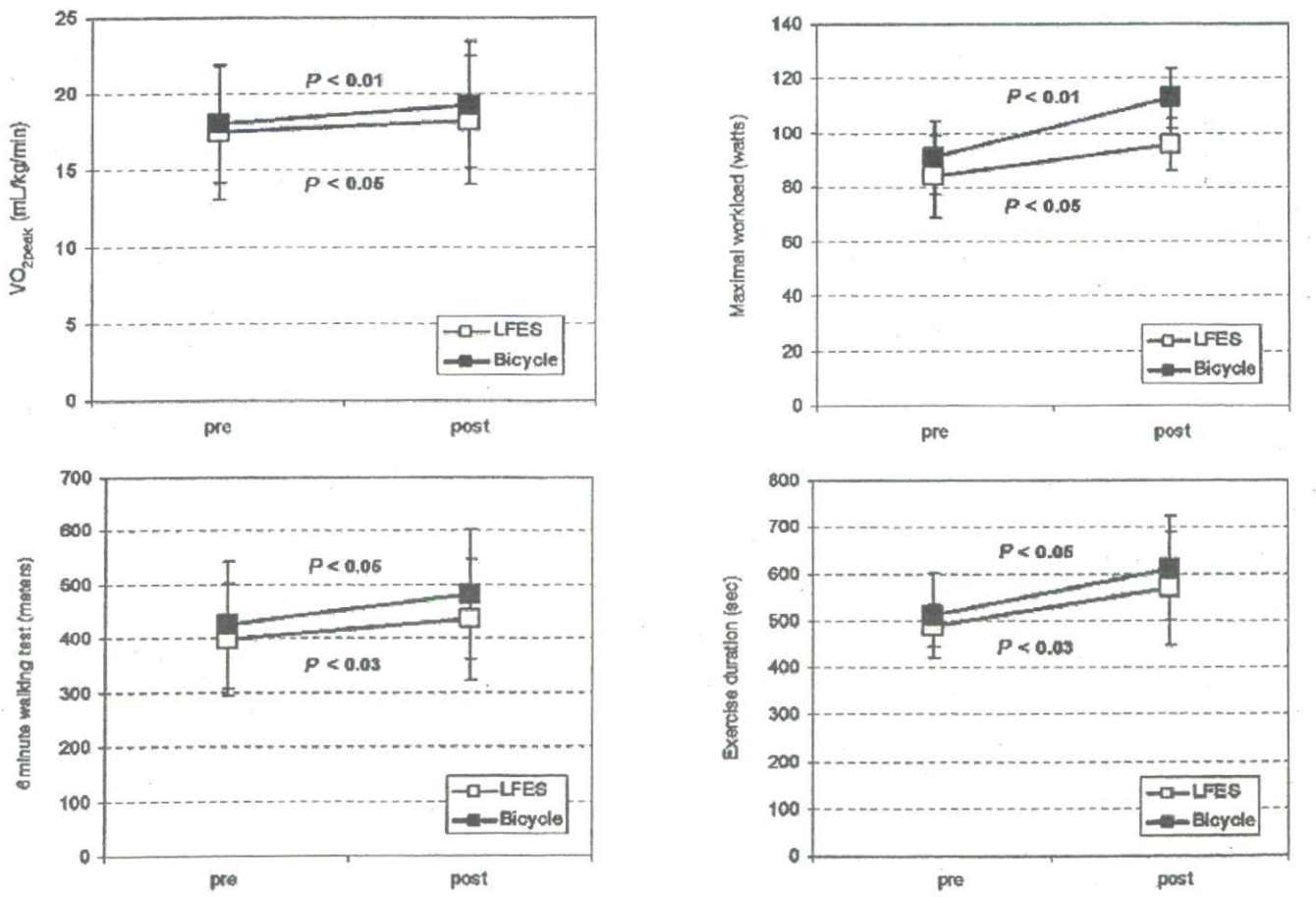

Figure 1. *Results of peak oxygen uptake, peak workload, 6 minutes walking test, and exercise duration in both groups at baseline and after 8 weeks of the given type of rehabilitation.*[13]

reported to improve the functional capacity in claudicants.[16] Hamada, et al.[17] observed enhanced energy consumption, carbohydrate oxidation, and whole body glucose uptake after low-frequency electrical stimulation of the lower limbs, a finding that suggests the possibility of therapeutic application of LFES for diabetic subjects. However, the number of studies concerning the effects of LFES in cardiovascular rehabilitation is still very low. The effectiveness of conventional exercise training in cardiovascular rehabilitation has been sufficiently proven,[18] and LFES is not likely to replace it. But the safety and ease of application could be especially beneficial in patients with advanced CHF (III-IV).

ES should be considered as a valuable alternative to classical exercise training in patients with CHF. Recently, results from the first randomized trial comparing home-based ES and classical exercise training have demonstrated that both methods could significantly influence functional capacity, muscle strength of patients with CHF.[10] Similar results were shown by Nuhr et al in a group of patients with advanced CHF, and an increase of slow myosin heavy chain isoforms at the expense of the fast ones and increased intensity of oxidative enzymatic activity were also found after LFES.[9]

Although the effectiveness of conventional exercise protocols in cardiovascular rehabilitation is beyond doubt, the safety and easy application of ES could be of great benefit in the rehabilitation of patients with CHF, especially those with a severe grade of the disease.

The effectiveness of conventional exercise protocols in cardiovascular rehabilitation is beyond doubt and the patients with mild heart failure would adopt exercise. But the patients with a severe grade of CHF who can not exercise would adopt ES for the safety and easy application. There are scarcely limitations except the allergies of the electrode. Further investigations should yield more detailed data, including information about possible interactions between the central and peripheral cardiovascular mechanisms during muscle stimulation. Future studies should also address the possibility of combining LFES with some type of classical exercise training.

References

1. Böhm M. Pathophysiology of heart failure today. Herz 2002; 27:75-91.
2. Kohzuki M. Cardiac rehabilitation in Japan: prevalence, safety and future plans. J HK Coll Cardiol 2006;14:B43-5.
3. Coats AJ, Adamopoulos S, Radaelli A, et al. Controlled trial of physical training in chronic heart failure. Exercise performance, hemodynamics, ventilation, and autonomic function. Circulation 1992;85:2119-31.
4. Adamopoulos S, Parissis J, Kroupis C, et al. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. Eur Heart J 2001;22:791-7.
5. Schulze PC, Gielen S, Schuler G, et al. Chronic heart failureand skeletal muscle catabolism: effects of exercise training. Int J Cardiol 2002;85:141-9.
6. Wielenga RP, Coats AJ, Mosterd W, et al. The role of exercise training in chronic heart failure. Heart 1997;78:431-6.
7. Piepoli MF, Davos C, Francis DP, et al. ExTraMATCH Collaborative. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH). BMJ 2004; 328:189.
8. Pette D, Staron RS. Mammalian skeletal muscle fiber type transitions. Int Rev Cytol 1997;170:143-223.
9. Nuhr MJ, Pette D, Berger R, et al. Beneficial effects of chronic low-frequency stimulation of thigh muscles in patients with advanced chronic heart failure. Eur Heart J 2004;25:136-43.
10. Harris S, LeMaitre JP, Mackenzie G, et al. A randomised study of home-based electrical stimulation of the legs and conventional bicycle exercise training for patients with chronic heart failure. Eur Heart J 2003;24:871-8.
11. Nagasaka M, Kohzuki M, Fujii T, et al. Effect of low-voltage electrical stimulation on angiogenic growth factors in ischaemic rat skeletal muscle.Clin Exp Pharmacol Physiol 2006;133:623-7.
12. Dobsák P, Nováková M, Siegelová J, et al. Low-frequency electrical stimulation increases muscle strength and improves blood supply in patients with chronic heart failure. Circ J 2006; 70:75-82.
13. Dobsák P, Nováková M, Fiser B, et al. Electrical stimulation of skeletal muscles. an alternative to aerobic exercise training in patients with chronic heart failure? Int Heart J 2006;47:441-53.
14. Hillegass EA, Dudley GA. Surface electrical stimulation of skeletal muscle after spinal cord injury. Spinal Cord 1999;37: 251-7.
15. Lewek M, Stevens J, Snyder-Mackler L. The use of electrical stimulation to increase quadriceps femoris muscle force in an elderly patient following a total knee arthroplasty. Phys Ther 2001;81:1565-71.
16. Anderson SI, Whatling P, Hudlicka O, et al. Chronic transcutaneous electrical stimulation of calf muscles improves functional capacity without inducing systemic inflammation in claudicants. Eur J Vasc Endovasc Surg 2004;27:201-9.
17. Hamada T, Hayashi T, Kimura T, et al. Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation and whole body glucose uptake. J Appl Physiol 2004;96:911-6.
18. Hambrecht R, Gielen S, Linke A, et al. Effects of exercise training on left ventricular function and peripheral resistance in patients with chronic heart failure: a randomized trial. JAMA 2000;283: 3095-101.

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Increased expression of urotensin II-related peptide and its receptor in kidney with hypertension or renal failure

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ABSTRACT

Urotensin II-related peptide (URP) is a novel vasoactive peptide that shares urotensin II receptor (UT) with urotensin II. In order to clarify possible changes of URP expression in hypertension and chronic renal failure (CRF), the expressions of URP and UT were studied by quantitative RT-PCR and immunohistochemistry in kidneys obtained from spontaneous hypertensive rats (SHR), Wistar-Kyoto rats (WKY), and WKY with CRF due to 5/6 nephrectomy. Expression levels of URP mRNA and UT mRNA were significantly higher in the kidneys obtained from SHR compared with age-matched WKY (at 5–16 and 16 weeks old, respectively). A dissection study of the kidney into three portions (inner medulla, outer medulla and cortex) showed that the expression levels of URP mRNA and UT mRNA were highest in the inner medulla and the outer medulla, respectively, in both SHR and WKY. The expression levels of URP and UT mRNAs were greatly elevated in the remnant kidneys of CRF rats at day 56 after nephrectomy, compared with sham-operated rats (about 6.5- and 11.9-fold, respectively). Immunohistochemistry showed that URP immunostaining was found mainly in the renal tubules, vascular smooth muscle cells and vascular endothelial cells. UT immunoreactivity was localized in the renal tubules and vascular endothelial cells. These findings suggest that the expressions of URP and UT mRNAs in the kidney are enhanced in hypertension and CRF, and that URP and its receptor have important pathophysiological roles in these diseases.

1. Introduction

Urotensin II (UII), initially isolated from the caudal neurosecretory system of teleost fish [18], is present in mammals including human [1,8]. Human UII was identified as an endogenous ligand for the orphan G-protein-coupled receptor GPR14 (urotensin II receptor, UT) [1], and shown to be a potent mammalian vasoconstrictor [1]. This peptide also

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has a vasodilatory effect on the small arteries through the release of endothelium-derived hyperpolarizing factor and nitric oxide (NO) [4,26]. Another ligand for UT, urotensin II-related peptide (URP), was discovered from rat brain [27,28]. The genes encoding URP and UII belong to the same superfamily as those encoding somatostatin 1 and cortistatin [34]. URP is an octapeptide with a high degree of homology with the cyclic region of the UII molecule, which is critical for the biological activity of UII.

UII has various biological actions, such as the stimulation of cell proliferation [31,41,42] and positive inotropic action [21]. Injection of UII induced a reduction in the glomerular filtration rate, urine out flow, and sodium excretion in normal rats, whereas injection of Urantide, a UII antagonist, resulted in increases in these variables [25]. URP has been shown to bind to UT [28], and has approximately the same binding affinity, but less potency than UII in contracting rat aortic strips in vitro [5]. URP dilated rat coronary arteries with 10-fold less potency compared to rat UII [19].

UII and UT are highly expressed in the kidney, which may be the principal site of UII synthesis in humans [14,23,25,38]. Immunohistochemistry of the kidney showed positive UII staining in the renal tubular cells, blood vessels and epithelial cells [23]. UT is expressed in glomerular arterioles, thin ascending limbs, and inner medullary collecting ducts [25]. High-affinity binding of human ^{125}I-UII has been reported in the human kidney [13]. Increased expression of UII and UT mRNAs was found in the kidney of spontaneous hypertensive rats (SHR) [25], and patients with diabetic nephropathy [12]. High concentrations of immunoreactive (IR)-UII are found in the urine of human and rat, and are presumably derived from renal tubular cells [14,25,37]. Plasma concentrations of UII are elevated in patients with chronic renal failure (CRF) [38], congestive heart failure [9,20], hypertension [6], and diabetes mellitus [36,37]. Increased excretion of UII in urine was observed in hypertensive patients [14], and patients with reduced renal function due to diabetic nephropathy [37]. UII may therefore contribute to cardiovascular regulation and be involved in the pathophysiology of cardiovascular and renal diseases.

URP mRNA is also expressed in human and rat kidney [25,28]. In contrast to UII, the expression levels of URP mRNA in the kidney were lower in SHR than in control rats (Wistar-Kyoto rats; WKY) [25]. We have recently reported that the gene expressions of URP and UT are upregulated in the heart of rats with congestive heart failure due to coronary ligation [16]. However, information on URP expression in various pathological conditions is limited. URP expression in the kidney has not been studied in CRF.

The aim of present study is to clarify possible changes of URP expression in the kidney, particularly in hypertension and CRF. The expression of URP, UII and UT in the kidney were studied in kidney tissues obtained from rats with hypertension or CRF (5/6 nephrectomized rats) by quantitative reverse-transcriptase polymerase chain reaction (RT-PCR). The localization of URP and UT in the rat kidney was then investigated by immunohistochemistry.

2. Methods

2.1. Animals

The present study was approved by the Ethics Committee for Animal Experimentation of Tohoku University School of Medicine, and performed in accordance with the guidelines of animal experimentation of Tohoku University. WKY and SHR were obtained from Charles River Japan Ltd. (Yokohama, Japan). Rats were housed in a humidity- and temperature-controlled room (30 ± 10% and 22 ± 2 °C, respectively) with a 12-h light/dark cycle. The rats were fed a regular diet and had free access to tap water.

Male SHR at 5, 8, 12 and 16 weeks of age and age-matched WKY (n = 6, all groups) were used to study age-related changes in the expression of URP, UII and UT mRNAs in the kidney. Rats were anesthetized with sodium pentobarbital. After cannulating the abdominal aorta, both kidneys were flushed with ice-cold saline. The kidney tissues were collected and stored at −80 °C until RNA extraction. The expression levels of URP and UT mRNAs were compared among the renal inner medulla, outer medulla and cortex using 8-week-old male WKY and SHR (n = 6). After papilla were removed, inner stripes of the outer medulla and a part of the cortex were collected, as previously reported [10]. Each part of the kidney tissue was stored at −80 °C until RNA extraction.

Renal mass ablation experiments were performed using male WKY as previously reported [11]. Briefly, 8-week-old male WKY were subjected to 5/6 nephrectomy by two-thirds infarction of the right and removal of the left kidney under ether anesthesia. In the first session, the right kidney was exposed via flank incision, and the two poles were encircled with loops of ligatures. After tightening the loops, the incision was closed. Seven days later, the left kidney was exposed via flank incision, and removed in total. The flank wound was then closed. Age-matched male WKY with sham operations were used as the control group. Rats were killed under anesthesia 3, 14 or 56 days after the last operation. Kidneys were harvested and stored at −80 °C until RNA extraction (n = 5, all groups). Kidneys of sham-operated rats and remnant kidneys of 5/6 nephrectomized rats at day 56 were fixed in 10% neutral buffered formalin and embedded into paraffin for immunohistochemistry.

2.2. Competitive and quantitative PCR of URP, UII and UT

Total RNAs were extracted by the guanidinium isothiocyanate/CsCl method. 4 μg of total RNA were reverse transcribed with 400 U of Moloney Murine Leukemia Virus reverse transcriptase (ReverTra Ace, Toyobo, Osaka, Japan) using an oligo(dT) primer, as previously described [16,35,39,40].

Expression levels of URP, UII, UT and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were determined using competitive, quantitative RT-PCR methods, as previously reported [16,35,39,40]. The primers for UII, URP, UT and GAPDH are shown in Table 1. The competitive reference standards (CRS)-DNA for URP, UII, UT and GAPDH were prepared with PCR as previously reported [16,35,39,40]. In the competitive, quantitative RT-PCR, a constant amount of wild-type cDNA and increasing amounts of CRS-DNA ($8–10^3$