表1　温泉利用期間別連日非連日温泉入浴群間における各疾患の既往頻度

疾患名	入浴頻度	男＋女(%)	男(%)	女(%)
がん	非毎日	10.44	12.08	9.24
	毎日	10.22	12.45	8.42
虚血性心疾患	非毎日	7.16	9.72	5.10
	毎日	6.19	9.32	3.83
脳卒中	非毎日	3.09	4.20	2.25
	毎日	1.97	3.08	1.12
高血圧	非毎日	38.53	40.26	37.17
	毎日	36.24	37.67	35.28
不整脈	非毎日	9.46	10.79	8.37
	毎日	7.89	9.51	6.67
痛風	非毎日	3.81	7.79	0.76
	毎日	3.80	7.97	0.63
喘息	非毎日	4.40	4.03	4.60
	毎日	3.05	3.26	2.88
糖尿病	非毎日	14.25	18.50	10.82
	毎日	12.43	17.48	8.60
高脂質血症	非毎日	11.14	9.81	12.21
	毎日	10.65	8.48	12.36
腎臓病	非毎日	3.87	5.35	2.78
	毎日	2.95	3.78	2.35
うつ病	非毎日	2.70	1.88	3.31
	毎日	1.49	1.35	1.61
慢性肝炎	非毎日	1.83	2.23	1.52
	毎日	1.95	1.96	1.97
膠原病	非毎日	2.55	1.41	3.41
	毎日	2.23	1.40	2.84
アレルギー疾患	非毎日	5.37	3.64	6.69
	毎日	5.38	3.87	6.49

赤字は、連日利用と非連日利用の間で有意差のある場合を示している（以下表4まで同じ）

表2　温泉利用期間１０年未満と４０年以上別の連日利用者と非連日利用者間の疾患既往

疾患	温泉入浴頻度	男性（利用<10y）既往率(%)	男性（利用>40y）既往率(%)	女性（利用<10y）既往率(%)	女性（利用>40y）既往率(%)
がん	非毎日	13.01	11.44	8.58	10.36
	毎日	12.77	11.61	11.89	8.60
虚血性心疾患	非毎日	9.94	10.63	5.11	5.98
	毎日	7.98	9.26	3.78	4.34
脳卒中	非毎日	4.06	4.90	2.46	2.79
	毎日	2.13	3.79	0.00	1.10
高血圧	非毎日	41.34	41.42	38.11	38.05
	毎日	40.96	37.05	31.89	37.78
不整脈	非毎日	11.60	8.72	7.95	10.96
	毎日	9.57	10.16	8.65	6.62
痛風	非毎日	7.87	10.08	0.50	0.80
	毎日	11.17	6.70	0.00	0.79
喘息	非毎日	3.65	5.45	4.04	5.38
	毎日	4.79	2.90	0.00	2.92
糖尿病	非毎日	18.48	20.98	9.97	10.36
	毎日	16.49	17.97	8.65	9.38
高脂質血症	非毎日	11.10	8.99	13.19	10.16
	毎日	13.83	6.47	15.14	11.12
腎臓病	非毎日	4.56	7.36	2.97	2.79
	毎日	3.19	3.57	2.16	2.52
うつ病	非毎日	1.74	1.09	3.60	2.79
	毎日	1.60	0.78	1.08	1.18
慢性肝炎	非毎日	2.40	0.82	1.64	1.20
	毎日	1.60	2.46	3.24	2.60
膠原病	非毎日	1.49	0.54	3.15	4.18
	毎日	1.06	1.23	3.24	2.68
アレルギー疾患	非毎日	3.81	3.81	7.32	5.98
	毎日	2.66	4.35	11.89	6.31

表3　男性既往疾患別の温泉利用条件（浸漬時間　利用期間）ごとの利用割合

	浸漬<10分	浸漬>20分	期間<10年	期間>40年
全体	24.59	31.41	30.30	27.56
がん	23.53	30.88	32.19	26.14
虚血性心疾患	25.81	30.54	31.40	27.74
不整脈	26.65	28.31	33.88	25.83
高血圧	25.71	30.82	32.50	27.39
脳卒中	24.14	37.93	31.03	32.18
痛風	26.72	33.07	32.54	26.98
喘息	26.01	26.59	32.95	27.17
糖尿病	23.91	33.68	30.46	28.39
高脂血症	25.47	32.24	38.32	21.73
腎臓病	18.64	35.00	28.18	27.27
うつ病	34.62	24.36	33.33	14.10
慢性肝炎	22.68	30.93	32.99	27.84
膠原病	22.06	35.29	32.35	22.06
アレルギー	28.89	25.56	29.44	32.22

全体の平均値と比較して有意差がある場合を赤字で示してある（以下表１０まで同じ）

表4　女性既往疾患別の温泉利用条件（浸漬時間　利用期間）ごとの利用割合

	浸漬<10分	浸漬>20分	期間<10年	期間>40年
全体	24.70	32.71	28.79	29.33
がん	26.43	31.48	28.96	30.81
虚血性心疾患	24.30	25.00	32.39	33.10
不整脈	26.53	31.84	30.41	31.22
高血圧	28.20	31.29	29.97	30.61
脳卒中	30.36	32.14	36.61	27.68
痛風	26.00	30.00	16.00	34.00
喘息	28.46	32.11	27.24	29.27
糖尿病	25.12	31.24	29.04	30.30
高脂血症	25.88	31.76	32.42	26.27
腎臓病	24.70	30.12	33.13	30.72
うつ病	22.56	24.39	37.80	20.73
慢性肝炎	21.43	34.82	29.46	36.61
膠原病	24.04	33.65	29.33	26.92
アレルギー	22.82	36.17	34.95	27.67

表5　男性既往疾患別の温泉利用時間帯別の利用割合

	9時前	9～12時	13～18時	19時以降
全体	12.54	10.75	40.46	32.83
がん	9.48	10.95	42.81	33.17
虚血性心疾患	11.18	13.98	41.51	30.75
不整脈	11.78	10.74	44.01	31.40
高血圧	13.21	10.87	43.10	30.11
脳卒中	13.22	17.24	47.13	18.97
痛風	16.40	8.20	43.92	29.10
喘息	13.87	12.14	42.20	28.32
糖尿病	13.22	11.15	41.95	30.46
高脂血症	10.28	9.81	42.76	35.51
腎臓病	12.73	14.55	38.64	29.55
うつ病	8.97	19.23	43.59	24.36
慢性肝炎	12.37	7.22	42.27	35.05
膠原病	16.18	10.29	30.88	41.18
アレルギー	12.22	11.67	37.78	36.67

表6　女性既往疾患別の温泉利用時間帯別の利用割合

	9時前	9～12時	13～18時	19時以降
全体	10.99	10.67	35.61	38.60
がん	10.10	11.78	37.04	37.04
虚血性心疾患	9.15	13.38	37.32	37.68
不整脈	9.18	10.41	39.80	37.35
高血圧	11.39	10.54	37.55	37.04
脳卒中	7.14	18.75	36.61	33.93
痛風	10.00	12.00	44.00	30.00
喘息	11.38	10.98	38.62	34.15
糖尿病	10.68	13.97	36.11	35.16
高脂血症	8.76	9.28	33.99	45.36
腎臓病	9.04	10.84	37.35	39.16
うつ病	10.98	14.63	38.41	27.44
慢性肝炎	13.39	6.25	33.93	42.86
膠原病	15.38	12.02	32.69	34.62
アレルギー	9.47	8.98	31.80	46.84

表7 男性既往疾患別の泉質別の利用割合

	単純温泉	塩化物泉	炭酸水素塩泉
全体	24.80	12.39	3.42
がん	26.14	12.25	2.78
虚血性心疾患	24.95	12.69	4.09
不整脈	24.59	11.36	3.31
高血圧	25.54	11.68	3.04
脳卒中	22.41	7.47	2.30
痛風	24.07	12.17	3.17
喘息	23.70	15.03	2.31
糖尿病	22.99	10.92	2.64
高脂血症	21.50	14.25	1.64
腎臓病	29.09	12.27	3.64
うつ病	19.23	6.41	3.85
慢性肝炎	22.68	10.31	1.03
膠原病	23.53	8.82	4.41
アレルギー	24.44	13.89	2.78

表8 女性既往疾患別の泉質別の利用割合

	単純温泉	塩化物泉	炭酸水素塩泉
全体	23.21	11.65	3.84
がん	25.08	11.78	2.86
虚血性心疾患	19.72	13.38	3.52
不整脈	22.24	12.65	5.31
高血圧	23.45	11.52	3.13
脳卒中	21.43	8.04	5.36
痛風	36.00	10.00	4.00
喘息	25.61	10.98	5.69
糖尿病	24.65	10.36	4.87
高脂血症	24.44	8.63	2.35
腎臓病	24.10	14.46	4.82
うつ病	19.51	7.32	4.88
慢性肝炎	20.54	12.50	2.68
膠原病	27.40	10.10	3.85
アレルギー	24.27	11.41	4.61

表9　温泉連日利用者と非連日利用者の過去1年以内に罹患した疾患数（男性）

	非毎日	毎日
がん	24	24
虚血性心疾患	9	7
脳卒中	0	3
高血圧	16	16
不整脈	3	5
痛風	9	4
喘息	2	1
糖尿病	12	10
高脂血症	3	2
腎臓病	3	2
うつ病	1	0
慢性肝炎	0	0
膠原病	2	1
アレルギー	0	4
全体	2335	2145

表10　温泉連日利用者と非連日利用者の過去1年以内に罹患した疾患数（女性）

	非毎日	毎日
がん	19	13
虚血性心疾患	3	3
脳卒中	1	2
高血圧	45	27
不整脈	5	5
痛風	2	2
喘息	4	3
糖尿病	11	12
高脂血症	8	5
腎臓病	5	1
うつ病	2	5
慢性肝炎	5	0
膠原病	5	4
アレルギー	5	1
全体	3021	2849

表１１　疾病予防効果が期待で切る温泉の利用パターン

	男　性	女　性
<u>高血圧</u>	夜間入浴	毎日 10 分以上
<u>高脂血症</u>	長年の入浴 炭酸水素塩泉	長年の入浴 塩化物泉
<u>うつ病</u>	塩化物泉	塩化物泉
喘息	−	毎日
慢性肝炎	炭酸水素塩泉	−

下線の疾患項目は、年齢により温泉利用の差が生じないような年齢区分でも疾患予防効果が再確認できたもの

表１２　炭酸浴による血圧、脈波の低減効果

	Controls (n=8)	CO_2 bathing (n=16)	
		Pre-CO_2	post-CO_2
s-BP(mmHg)	141±14	134±11	134±14
d-BP(mmHg)	76±5.8	80±5.1	82±6.2
%FMD	5.4± .51	5.9±0.53	5.2±0.48
b-IMT(mm)	0.31±0.03	0.32±0.03	0.30±0.02
ABI(Rt)	1.16±0.06	1.23±0.08	1.17±0.06
ABI(Lt)	1.12±0.04	1.25±0.06	1.16±0.85
rt-PWV(m/sec)	1792±128	1840±125	1749±143[†]
lt-PWV(m/sec)	1821±170	1879±132	1726±122[*†]
CVR-R(%)	2.33±0.17	2.68±0.12	3.65±0.25[*†]
BS(2hr:mg/dl)	148±14	162±12	168±18
HbA1c(%)	7.42 ±0.51	7.71±0.41	7.74±0.38

S-BP: systolic blood pressure, d-BP: diastolic pressure, % FMD: %flow mediated dilation, b-IMT: brachial intima-media thickness, ABI: ankle brachial index, PWV: pulse wave velocity, CVR-R (%): % coefficient of variation of R-R intervals, BS: blood sugar level. Data are means ± SD. *: $p < 0.05$ vs control subjects; †: $p < 0.05$ vs values at before.

表１３　高血圧ラットに対する温浴の効果

	対照	高食塩食	高食塩食＋温浴	高食塩食+温浴 +17-DMAG	対照＋温浴
匹数	10	10	10	10	10
最高血圧 mmHg	126.3±2.2	202.6±5.3	169.9±3.3	179.6±5.3	123.6±3.3

II. 研究成果の刊行に関する一覧表

論文番号	発表者氏名	論文タイトル名	発表誌名	巻号	ペ ー ジ	出 版 年
1	Guan JZ, Guan WP, Maeda T, Makino N.	Different levels of hypoxia regulate telomere length and telomerase activity	Aging Clin Exp Res	24	213-217	2012
2	Maeda T, Guan JZ, Koyanagi M, Higuchi Y, Makino N.	Aging-associated alteration of telomere length and subtelomeric status in female patients with Parkinson's disease.	J Neurogenetics	26	241-251	2012
3	Guan JZ, Guan WP, Maeda T, Makino N.	Alteration of telomere length and subtelomeric methylation in human endothelial cell under different levels of hypoxia.	Arch Med Res	43	15-20	2012
4	Oyama JI, Yamamoto H, Maeda T, Ito A, Node K, Makino N.	Continuous Positive Airway Pressure Therapy Improves Vascular Dysfunction and Decreases Oxidative Stress in Patients With the Metabolic Syndrome and Obstructive Sleep Apnea Syndrome.	Clin Cardiol	35	231-236	2012
5	Oyama JI, Maeda T, Sasaki M, Higuchi Y, Node K, Makino N.	Repetitive hyperthermia attenuates progression of left ventricular hypertrophy and increases telomerase activity in hypertensive rats.	Am J Physiol-Heart Circ Physiol	302	H 2092 – 2101	2012
6	Maeda T, Jing-Zhi Guan, Koyanagi M, Makino N.	The gender-related alterations in the telomere length and subtelomeric methylation status in patients with Parkinson's disease.	Ageing Res	4	61-66	2012
7	Maeda T, Nakamura K, Atsumi K, Hirakawa M, Ueda Y, Makino N.	Radiation-associated telomere length changes of peripheral leukocytes of inpatients with cancer.	Int J Rad Biol	89	106-109	2012
8	Guan JZ, Maeda T, Makino N.	Analysis of telomere length and subtelomeric methylation of circulating leucocytes in female patients with Alzheimer's disease.	Aging Clin Exp Res	25	17-23	2013
9	Oyama J-I, Kudo Y, Maeda T, Node	Hyperthermia by bathing in a hot spring improves the	Heart Vessels	13	774-782	2013

	K, Makino N.	cardiovascular functions and reduces the production of inflammatory cytokines in patients with chronic heart failure.				
10	Maeda T, Guan JZ, Koyanagi M, and Makino N.	Telomerase activity and telomere length distribution in vascular endothelial cell in a short-term culture under the presence of hydrogen peroxide.	Geriatr Gerontol Int	13	774-782	2013
11	Maeda T, Guan JZ, Koyanagi M, Makino N.	Alterations in the telomere length distribution and the subtelomeric methylation status in human vascular endothelial cells under elevated temperature in culture condition.	Ageing Clin Exp Res	25	231-238	2013
12	前田豊樹	別府市高齢者に置ける温泉利用の実態と既往歴との関連の調査について	日本温泉気候物理医学会雑誌	77	26-28	2013
13	Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, Martin-Ruiz C, Shiels P, Maeda T, , von Zglinicki T, Ben-Shlomo Y; Halcyon study team.	Gender and telomere length: systematic review and meta-analysis.	Exp Gerontol	25	15-27	2014
14	Maeda T, Guan JZ, Koyanagi M, Makino N.	Vascular endothelial cell surviving through under prolonged elevated temperature shows persistent or transient up-regulation of telomerase and stress-associated proteins.	Appl Cell Biol	1-8	2	2014
15	Maeda T, Guan JZ, Koyanagi M, Makino N.	X-irradiation alters the telomerase activity and the telomere length distribution of cultured human vascular endothelial cells.	Appl Cell Biol	2-9	3	2014
16	Guan JZ, Guan WP, Maeda T, Makino	Changes in telomere length distribution in low-dose	Mol Cell Biochem.	396	129-135	2014

	N.	X-ray-irradiated human umbilical vein endothelial cells.				
17	Maeda T, Guan JZ, Koyanagi M, Makino N.	Altered expression of genes associated with telomere maintenance and cell function of human vascular endothelial cell at elevated temperature.	Mol Cell Biochem.	397	305-312	2014
18	Maeda T.	The questionnaire about the hot sprong use and the anamnesis for Beppu resitents aged 65 and over.	J Jpn Soc Balneol Climatol Phys Med	77	520-521	2014
19	J-ZGuan, W-P Guan, T Maeda, XG, Wan, N Makino.	Patients with multiple sclerosis show increased oxidative stress markers and somatic telomere length shortening.	Mol Cell Biochem	400	183-187	2015
20	T Maeda, N Makino.	Hot-spring-bathing accelerates wound healing of pressure ulcer of an unconscious patient.	Appl Cell Biol	4	1-3	2015
21	T Maeda, N Makino.	Hot spring footbath is effective for low back pain.	Appl Cell Biol	4	4-5	2015
22	N Makino, T Maeda, N Abe.	Effects of Immersion in Artificial Carbon Dioxide on Endothelial Function Assessed with Flow-Mediated Dilation in Patients with Type 2 Diabetes.	J Jpn Soc Balneol Climatol Phys Med	78	1-9	2015
23	Makino N, Oyama J, Maeda T, Koyanagi M, Higuchi Y, Tsuchida K.	Calorie restriction increases telomerase activity, enhances autophagy, and improves diastolic dysfunction in diabetic rat hearts.	Mol Cell Biochem.	403	1-11	2015

なお、上記のうち特に重要な原著論文を次ページ以降に添付する。(5, 9, 11, 14, 17, 22)

以上

Repetitive hyperthermia attenuates progression of left ventricular hypertrophy and increases telomerase activity in hypertensive rats

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Oyama J, Maeda T, Sasaki M, Higuchi Y, Node K, Makino N. Repetitive hyperthermia attenuates progression of left ventricular hypertrophy and increases telomerase activity in hypertensive rats. *Am J Physiol Heart Circ Physiol* 302: H2092–H2101, 2012. First published March 16, 2012; doi:10.1152/ajpheart.00225.2011.—We investigated the hypothesis that repetitive hyperthermia (RHT) attenuates the progression of cardiac hypertrophy and delays the transition from hypertensive cardiomyopathy to heart failure in Dahl salt-sensitive (DS) hypertensive rats. Six-week-old DS rats were divided into the following five groups: a normal-salt diet (0.4% NaCl) (NS group), a normal-salt diet plus RHT by daily immersion for 10 min in 40°C water (NS+RHT group), a high-salt diet (8% NaCl) (HS group), a high-salt diet (8% NaCl) plus RHT (HS+RHT group), and high-salt diet (8% NaCl) plus RHT with 17-DMAG (HSP90 inhibitor) administration (HS+RHT+17-DMAG group). All rats were killed at 10 wk. Cardiac hypertrophy and fibrosis were noted in the HS group, whereas RHT attenuated salt-induced cardiac hypertrophy, myocardial and perivascular fibrosis, and blood pressure elevation. The phosphorylated endothelial nitric oxide synthase (eNOS) and Akt were decreased in the HS group compared with the NS group, but these changes were not observed in the HS+RHT group. The levels of HSP60, 70, and 90 were elevated by RHT. Moreover, the increased levels of iNOS, nitrotyrosine, Toll-like receptor-4, BNP, PTX3, and TBARS in the HS group were inhibited by RHT. Telomeric DNA length, telomerase activity, and telomere reverse transcriptase (TERT) were reduced in the HS group; however, these changes were partially prevented by hyperthermia. In conclusion, RHT attenuates the development of cardiac hypertrophy and fibrosis and preserves telomerase, TERT activity and the length of telomere DNA in salt-induced hypertensive rats through activation of eNOS and induction of HSPs.

cardiac hypertrophy; endothelial nitric oxide synthase; oxidative stress; telomere; heat shock protein

HYPERTENSION IS ONE OF MAJOR risk factors responsible for cardiovascular disease and may lead to cardiac hypertrophy and diastolic heart failure (DHF). DHF is defined as heart failure with preserved left ventricular (LV) contraction and is characterized by abnormal relaxation and/or increased stiffness of the LV leading to impaired filling during diastole. Typically, diastolic function is evaluated by the E/A ratio using echocardiography. LV diastolic dysfunction is often manifested in individuals with hypertension. About 30–40% of heart failure cases occur in patients with diastolic dysfunction and normal systolic function (28, 36). Cardiac hypertrophy and resultant fibrosis develop as an adaptive response to pressure overload in

hypertension and are commonly associated with DHF. Progressive cardiac remodeling characterized by LV hypertrophy, chamber enlargement, and pump dysfunction occurs in response to hypertension and is accompanied by progressive accumulation of the extracellular matrix (36).

Hyperthermia using dry sauna improves cardiac function and may also improve diastolic dysfunction in patients with congestive heart failure (CHF) (13, 44). However, little is known about possible beneficial effects of hyperthermia on hypertension-induced cardiac hypertrophy. Therefore, we investigated the effects of repetitive hyperthermia (RHT) induced by immersion in a heated water bath on LV remodeling, oxidative stress, inflammation, and telomere biology in Dahl salt-sensitive (DS) rats fed a high-salt (HS) diet, a well-established animal model of hypertensive heart disease.

METHODS

All procedures were carried out according to the protocols approved by the Institutional Committee for the Use and Care of Laboratory Animals of Kyushu University. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the article as it has been written.

Animal preparation. Male DS rats (Kyudo, Fukuoka, Japan) were handled in accordance with the guidelines of Kyushu University, Graduate School of Medicine, as well as with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). The rats were individually housed in a temperature-controlled animal facility and fed a normal diet from weaning until 6 wk of age. At 6 wk of age, the rats were divided into five groups and fed a phytoestrogen-free normal-salt (NS, 0.4% NaCl) or an HS (8% NaCl) diet for the following 4 wk (Fig. 1A). The rats in the NS+RHT, HS+RHT, or HS+RHT+17-DMAG group were treated with RHT for 4 wk. In those groups receiving the treatment of hyperthermia, the rats were placed in the animal holder that was used for tail-cuff plethysmography and immersed in a head-up position at a 30° angle to the horizontal in a polycarbonate water bath. The lower half of the rat's body was immersed in tap water heated to 40°C for 10 min daily for 4 wk (Fig. 1B). All rats tolerated the immersion protocol well throughout the study. In the HS+RHT+17-DMAG group, the rats received 17-DMAG, a novel heat shock protein (HSP) 90 inhibitor (LKT Laboratories, St. Paul, MN) at the dose of 0.5 mg/kg/day by osmotic minipumps (ALZA, Palo Alto, CA) from 6 wk of age for 4 wk subcutaneously. All rats were evaluated 1 day after finishing the whole protocol.

Echocardiographic and hemodynamic measurements. Systolic blood pressure (SBP) and heart rate were measured weekly in conscious animals by tail-cuff plethysmography (Muromachi Kikai, Tokyo, Japan). Transthoracic M-mode and Doppler echocardiographic studies were carried out when the rats were 10 wk of age after they were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg), using an ultrasonographic system (LogiQ 400 Pro; GE Yokogawa Medical Systems, Tokyo, Japan) with a 12-MHz trans-

A

Dahl salt-sensitive rat (n=24)

0.4% NaCl: NS (n=10)

0.4% NaCl + repetitive hyperthermia : NS+RHT (n=10)

8% NaCl : HS (n=10)

8% NaCl + repetitive hyperthermia : HS+RHT (n=10)

8% NaCl + repetitive hyperthermia+17-DMAG : HS+RHT+17-DMAG (n=10)
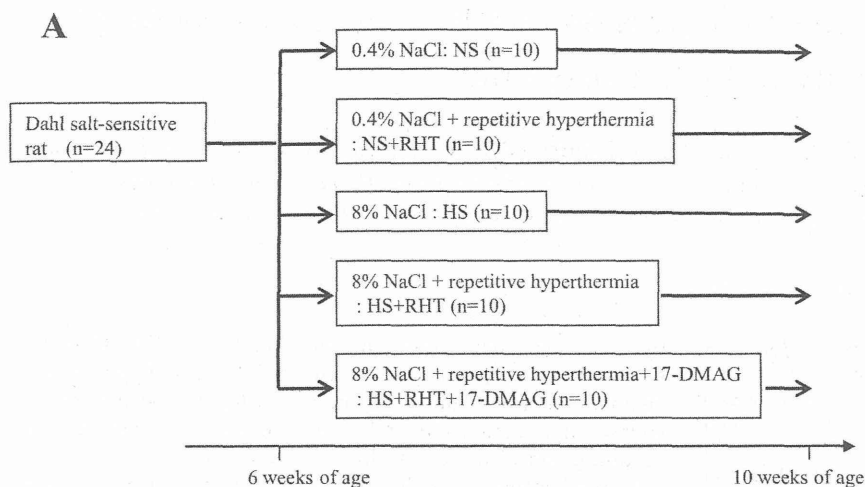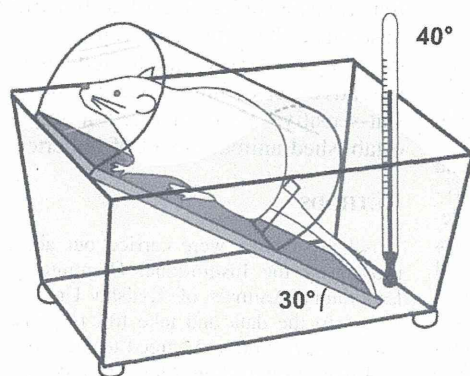
6 weeks of age   10 weeks of age

Fig. 1. *A*: study time line. *B*: scheme of rat immersion in a hot water bath in the HS+RHT group. NS, normal salt; HS, high salt; RHT, repetitive hyperthermia.

B

Therapy of repetitive hyperthermia

40°

30°

ducer. M-mode echocardiograms were recorded in the short-axis view at the papillary muscle level of the LV at a speed of 100 mm/s for measurement of LV end-diastolic (LVDd) and end-systolic (LVDs) diameters, LV fractional shortening (FS), and the thickness of the intraventricular septum (IVS) and LV posterior wall (PW) at end diastole. The following variables were determined as indicators of diastolic ventricular function: peak transmitral flow velocity in early diastole (E), peak transmitral flow velocity in late diastole (A), the E-to-A ratio (E/A), and deceleration time of the E wave. After echocardiography, a 1.4 F micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted into the right carotid artery and then advanced into the LV to measure pressure. After the hemodynamic studies, the heart was immediately harvested. Body weight, heart weight calibrated by tibia length, and the ratio of heart weight to body weight as an index of cardiac hypertrophy were evaluated.

Histological examination. The apical portion of the LV below the papillary muscle was fixed with phosphate-buffered 10% formalin solution for 24 h, embedded in paraffin, sectioned at 1.5 μm, and stained with hematoxylin-eosin (HE), Masson's trichrome (MT), or Sirius red (SR) to evaluate the cardiac fibrosis and hypertrophy of cardiomyocytes. To determine myocyte cross-sectional area (CSA), 30 cardiomyocytes were traced in each section in the slides stained with HE using NIH Image software. The percentage area of perivascular and interstitial fibrosis in the LV at the papillary muscle level in the slides stained with MT and SR was determined (6, 8). The remaining LV was immediately placed in liquid nitrogen and stored at −80°C for Western blot analyses.

Myocardial metalloproteinase activity assay. The SensoLyte 520 Generic MMP Fluorometric Assay Kit (AnaSpec) was used to determine myocardial matrix metalloproteinase (MMP) activity according to the manufacturer's directions. Hearts were homogenized in the assay buffer and incubated with 1 mmol/l of 4-aminophenylmercuric acetate for 24 h at 37°C to activate pro-MMPs. Data were adjusted by protein concentrations in the buffers and normalized to the levels in the NS group.

Analysis of protein expression. Frozen heart tissues were homogenated with 5 volumes of homogenization buffer (RIPA) and centrifuged at 15,000 rpm for 20 min. The protein concentration of the supernatant was determined with bovine serum albumin as a standard protein. The same amount (20 g for each experiment) of extracted protein was loaded for SDS-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes. Membranes were blocked and incubated with antibodies against endothelial nitric oxide synthase (eNOS) (sc-20791, Santa Cruz Biotechnology), phosphor-eNOS (N213220; NOF Medical Department, Tokyo, Japan), Akt (#9272, Cell Signaling Technology), phospho-Akt (#9271, Cell Signaling Technology), HSP60 (SPA-806, Stressgen), HSP70 (SPA-810, Stressgen), HSP90 (SPA-845, Stressgen), brain natriuretic peptide (BNP) (SC-18818, Santa Cruz Biotechnology), inducible nitric oxide synthase (iNOS) (SC-650, Santa Cruz Biotechnology), nitrotyrosin (905-763-100, Stressgen), pentraxin 3 (PTX3) (H00005806-MO2, Abnova), Toll-like receptor (TLR)-4 (IMG-578A, IMGENEX), telomere reverse transcriptase (TERT) (SC-7212, Santa Cruz Biotechnology), Sirt1 (SC-15404, Santa Cruz Biotechnology) and to glyceraldehyde-3-phosphate-dehydrogenase (GAPDH: sc-20357, Santa

Cruz Biotechnology). Detection was performed with secondary horseradish peroxidase-conjugated antibodies (Millipore, Billerica, MA) and the ECL detection system. Subsequently, the signals were normalized to GAPDH expression. To assess the level of myocardial oxidative stress generated in the process of cardiac remodeling, we determined the degree of lipid peroxidation in myocardial tissues through biochemical assay of thiobarbituric acid reactive substances (TBARS) (31). Briefly, LV myocardial tissue was homogenized (10% wt/vol) in 1.15% KCl solution (pH 7.4). The homogenate was mixed with 0.4% SDS, 7.5% acetic acid adjusted to pH 3.5 with NaOH, and 0.3% thiobarbituric acid. Butylated hydroxytoluene (0.01%) was added to the assay mixture to prevent autoxidation of the sample. The mixture was kept at 5°C for 60 min and was heated at 100°C for 60 min. After cooling, the mixture was extracted with distilled water and n-butanol-pyridine (15:1, vol/vol) and centrifuged at 1,600 g for 10 min. The absorbance of the organic phase was measured at 532 nm. The amount of TBARS was determined by absorbance with the molecular extinction coefficient of 156,000 and expressed as mol/g wet wt.

Telomeric biology. To extract the genomic DNA, the tissue samples were lysed by incubation at 55°C for 48 h in 200 μl lysis buffer containing 10 mmol/l Tris·HCl (pH 8.0), 0.1 mmol/l EDTA (pH 8.0), 2% SDS, and 500 μg/ml protease K (Roche Diagnostic, Tokyo, Japan). Genomic DNA extraction was performed using a DNeasy Tissue kit (Qiagen, Tokyo, Japan) according to the manufacturer's recommendations as previously described (6). The length of the telomeric DNA was estimated as the telomeric-to-centromeric DNA content ratio, as previously reported (16). The telomeric DNA contents can be standardized by calculating the relative telomeric DNA content with the centromeric DNA (0.1 μg) content. DNA samples were diluted and denatured. Two microliters of denatured DNA were dotted onto a nylon membrane sheet. The hybridization signal of a digoxigenin (DIG)-labeled probe was converted into a chemiluminescent signal using a DIG Wash and Block buffer set, and a DIG luminescent detection kit (Roche). After the telomere probe had been stripped, the membrane was rehybridized with 2 pmol 5'-DIG-labeled centromere-specific oligonucleotide (5'-GTTTTGAAACACTCTTT-TTGTAGAATCTGC-3') under the same conditions. The telomere probe was visualized by alkaline phosphatase-metabolizing CDP-Star, a highly sensitive chemiluminescence substrate. Telomerase activity was examined by means of a modified telomerase repeat amplification protocol (TRAP) with TeloChaser (Toyobo, Osaka, Japan) according to the manufacturer's instructions (16). Briefly, the substrate oligonu-

cleotide is added to 0.5 g protein extract. If telomerase is present and active, telomeric repeats (GGTTAG) are added to the 3'-end of the oligonucleotide. After amplification, the PCR products were resolved on a 12% polyacrylamide gel, stained with ethidium bromide, and detected using an FLA 5000 system (Fuji Film, Tokyo, Japan). The intensities of the bands were quantified with Image J. Assays were repeated at least twice on LV tissue from each animal to ensure reproducibility. A human cancer cell line overexpressing telomerase was used as the reference in each assay.

Additional methods. The expanded METHODS section in the online-only Data Supplement contains information of the heat-induced lowering effects of blood pressure (BP) by hyperthermia.[1]

Data analysis. Data are presented as means ± SE. The differences in a single parameter among the groups were evaluated by one-way analysis of variance using the Bonferroni post hoc test for multiple comparisons. A P value of < 0.05 was considered to be statistically significant.

RESULTS

Hemodynamic and morphological analyses. Body weight was significantly lower in the HS and HS+RHT groups than in the NS group. Heart weight calibrated by tibia length and the ratio of heart weight to body weight, an index of cardiac hypertrophy, were both significantly increased in the HS group compared with those in the NS and HS+RHT groups (Table 1). At 6 wk of age, SBP was similar among the three groups (NS: 116.0 ± 6.6 mmHg, HS: 118.5 ± 17.7 mmHg, HS+RHT: 119.0 ± 13.5 mmHg, HS+RHT+17-DMAG: 115.7 ± 11.2 mmHg, NS+RHT: 118.4 ± 12.8 mmHg). At 10 wk of age, the BP was elevated in the HS, HS+RHT, and HS+RHT+17-DMAG groups compared with the NS group, but hyperthermia reduced the magnitude of BP elevation (Table 1). Heart rates were comparable in all three groups. LV end-diastolic pressure (LVEDP) in the HS, HS+RHT, and HS+RHT+17-DMAG groups was significantly higher than that in the NS group; however, LVEDP in the HS+RHT group was reduced compared with that in the HS group. In the HS, HS+RHT, and

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[1] The online version of this article contains supplemental material.

Table 1. *Hemodynamic and echocardiographic measurements at 10 wk*

Parameter	NS	HS	HS + RHT	HS + RHT + 17-DMAG	NS + RHT
n	10	10	10	10	10
BW, g	368.3 ± 2.7	291.5 ± 8.6**	299.8 ± 7.9**	302.0 ± 10.8**	360.5 ± 6.4##++$$
HW/tibia length, mg/mm	24.32 ± 1.05	42.11 ± 2.30**	29.24 ± 1.09##	38.05 ± 3.11**+	24.38 ± 1.30##$$
HW/BW, mg/g	3.27 ± 0.12	6.89 ± 0.41**	5.00 ± 0.16**##	6.24 ± 0.54**	3.46 ± 0.13##+$$
Systolic BP, mmHg	126.3 ± 2.2	202.6 ± 5.3**	169.9 ± 3.3**##	179.6 ± 5.3**##	123.6 ± 3.3##++$$
HR, bpm	391.5 ± 6.5	417.5 ± 11.8	392.5 ± 18.9	414.3 ± 17.7	390.8 ± 12.1
LVEDP, mmHg	4.6 ± 0.8	15.5 ± 0.7**	11.4 ± 0.8**#	13.9 ± 0.8**++	5.1 ± 0.9##$$
			Echocardiographic data		
LVDd, mm	6.44 ± 0.08	5.40 ± 0.09**	5.88 ± 0.14*#	5.65 ± 0.13**	6.48 ± 0.10##++$$
LVDs, mm	3.23 ± 0.08	3.29 ± 0.11	3.04 ± 0.12	3.24 ± 0.12	3.22 ± 0.07
IVS, mm	2.03 ± 0.12	3.21 ± 0.06**	2.70 ± 0.12**#	2.93 ± 0.13**	1.98 ± 0.12##$$
PW, mm	1.95 ± 0.13	3.29 ± 0.11**	2.70 ± 0.15**#	2.93 ± 0.13**	2.03 ± 0.10##$$
FS, %	49.91 ± 1.09	38.95 ± 2.49**	48.35 ± 1.67##	42.57 ± 2.25*	50.22 ± 1.23##$
E/A ratio	2.04 ± 0.18	1.24 ± 0.14**	1.71 ± 0.08	1.47 ± 0.11**	2.12 ± 0.12##$

NS, normal salt diet; HS, high salt diet; RHT, repetitive hyperthermia; BW, body weight; HW, heart weight; BP, blood pressure; HR, heart rate; bpm, beats/min; LVEDP, left ventricular end-diastolic pressure; LVDd, diastolic dimension of left ventricle; LVDs, systolic dimension of left ventricle; IVS, thickness of interventricular septum; PW, thickness of posterior wall; FS, ratio of left ventricular fractional shortening; E/A ratio, peak velocity of early transmitral inflow (E)-to-peak velocity of late transmitral inflow (A) ratio. *P < 0.05 vs. NS, **P < 0.01 vs. NS, #P < 0.05 vs. HS, ##P < 0.01 vs. HS, +P < 0.05 vs. HS+RHT, ++P < 0.01 vs. HS+RHT, $$P$ < 0.05 vs. HS+RHT +17-DMAG, $$$P$ < 0.01 vs. HS+RHT +17-DMAG.

HS+RHT+17-DMAG groups, the thicknesses of IVS and PW were significantly increased, and LVDd, LVDs, and FS were reduced at 10 wk of age compared with the values in the NS group (Table 1). However, in the HS+RHT group, the wall thicknesses of the IVS and PW were significantly reduced, and LVDd and FS were increased compared with the values in the HS group. In addition, the E/A ratio was significantly decreased in the HS group at 10 wk of age, compared with that in the NS group, and this decrease was significantly less in the HS+RHT group. To elucidate the BP-lowering effects of RHT

on cardiac morphology and function by hyperthermia, the DS rats receiving HS diet were administered hydralazine for 4 wk (HS+Hy; Supplemental Data). Although BP was similar between HS+RHT and HS+Hy groups, cardiac hypertrophy, including thicker IVS and PW, and reduced cardiac function, including FS and E/A ratio, were recognized in HS+Hy group compared with those in HS+RHT group. (Supplemental Table and Figure).

Histology. Figure 2*B* shows representative histological specimens of myocardial sections demonstrating changes in cell
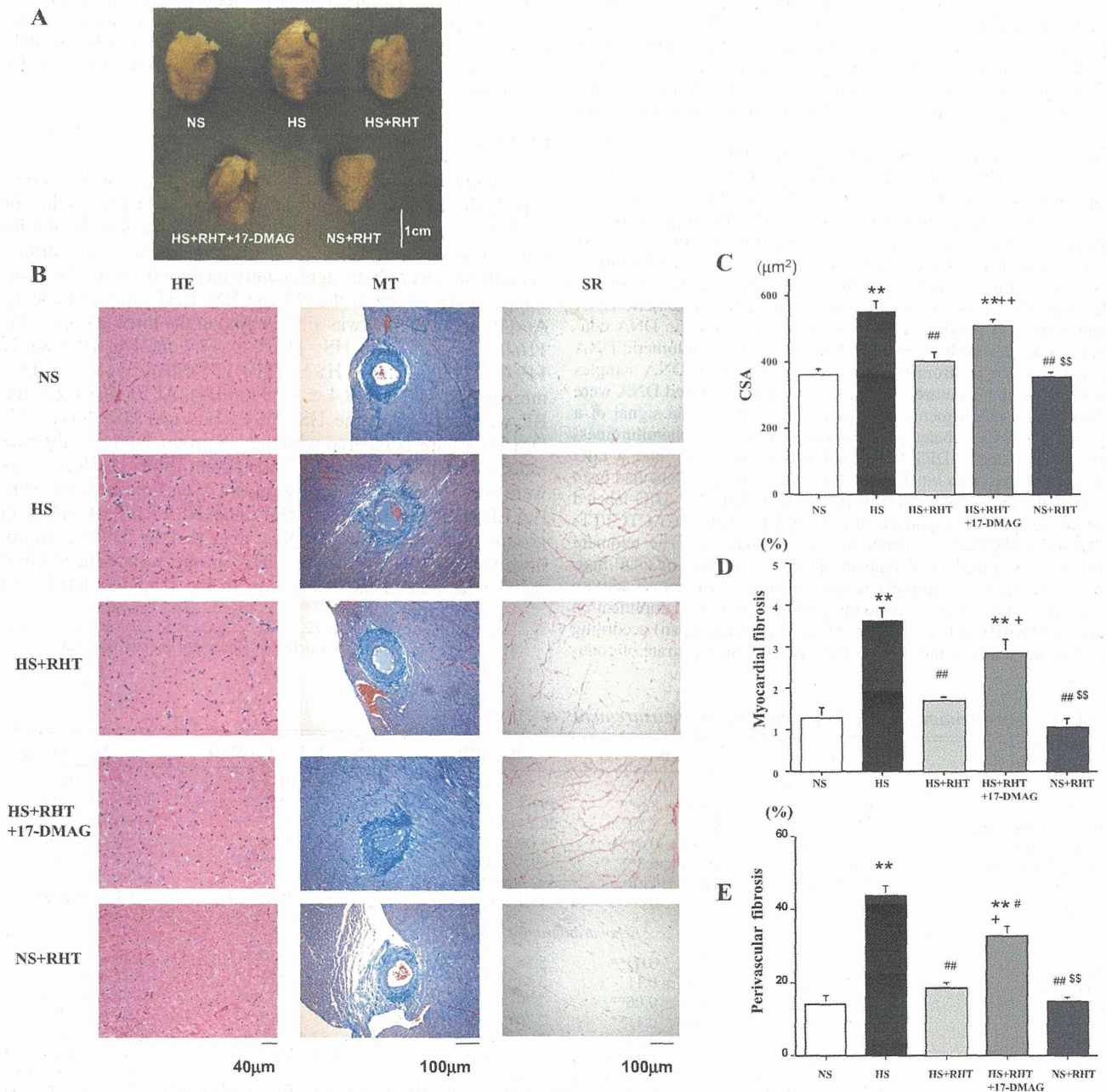
Fig. 2. *A*: representative photos of the hearts in each of the 5 groups. *B*: representative histological examination by hematoxylin-eosin (HE) staining (*left* lane), Masson's trichrome (MT) staining (*middle* lane), Sirius red staining (*right* lane) in each group at 10 wk of age. Cross-sectional area (CSA) of cardiomyocytes (*C*), myocardial interstitial fibrosis (*D*), and perivascular fibrosis (*E*) in each group. **$P < 0.01$ vs. the NS group. #$P < 0.05$, ##$P < 0.01$ vs. the HS group. +$P < 0.05$, ++$P < 0.01$ vs. the HS+RHT group. $$$P < 0.01$ vs. the HS+RHT+17-DMAG group.

size and vascular structure at 10 wk of age in each group. The cardiomyocyte CSA was significantly larger in the HS group than in the NS group; however, hyperthermia significantly ameliorated CSA in the HS+RHT group compared with the HS group (Fig. 2C). The degree of myocardial interstitial or perivascular fibrosis was increased by an HS diet, but was attenuated by hyperthermia (Fig. 2, D and E).

Myocardial MMP activities. Myocardial activities of MMP-1, -2, -3, -9 in the HS group were elevated compared with the values in the NS group. However, RHT suppressed the enzymatic increases in MMP-2, -3, -9 due to salt-induced hypertrophic cardiac remodeling since the values in the HS+RHT group were significantly lower than the values in the HS group (Fig. 3).

Western blotting analysis. Although expressions of eNOS and Akt proteins were comparable among the five groups, phosphorylated eNOS was lower in the HS, HS+RHT, and HS+RHT+17-DMAG groups than in the NS group(Fig. 4, B and C). The expression of phosphorylated Akt in the HS group was reduced compared with that in NS and HS+RHT groups; however, hyperthermia increased the expression of pAkt in the HS+RHT group, and 17-DMAG decreased the level of pAkt increased by hyperthermia in the HS+RHT+17-DMAG group (Fig. 4, D and E).

The expression of HSP60 was lower in the HS group than in the NS group. On the other hand, the level of HSP90 in the HS group was higher than that in NS group. However, the expression of HSP60 was significantly higher in the HS+RHT group

than in the HS group, and the expressions of HSP70 and 90 were significantly higher in the HS+RHT and HS+RHT+17-DMAG groups than in both the HS and NS groups (Fig. 4, F–H). In the NS+RHT group, all HSPs increased compared with those in the NS group.

The expressions of TLR-4, iNOS, nitrotyrosine, PTX3, and BNP increased in the HS group compared with those in the NS and HS+RHT groups. However, RHT inhibited the increase, since the expressions in the HS+RHT group were significantly lower than those in the HS group (Fig. 4, I–M). 17-DMAG partially attenuated the effects of RHT on myocardial inflammation.

Myocardial oxidative stress. Myocardial oxidative stress was expressed as TBARS and was significantly higher in the HS group than in the NS group. However, heat stress attenuated the elevation of oxidative stress since TBARS was significantly lower in the HS+RHT group than in the HS group (Fig. 4N).

Hyperthermia preserved telomere length and telomerase activity. Telomerase activity, quantified using a TRAP assay, was significantly decreased in the HS group compared with that in the NS group but was increased in the HS+RHT group compared with that in the HS group (Fig. 5A). Telomeric DNA length was assessed by dot-blot analysis of 10-wk-old rat heart tissue (Fig. 5B). When all five groups were compared, the length of the telomeric DNA was significantly shorter only in the HS group. Thus, telomere length was preserved by RHT. The protein expression of TERT was attenuated in the HS
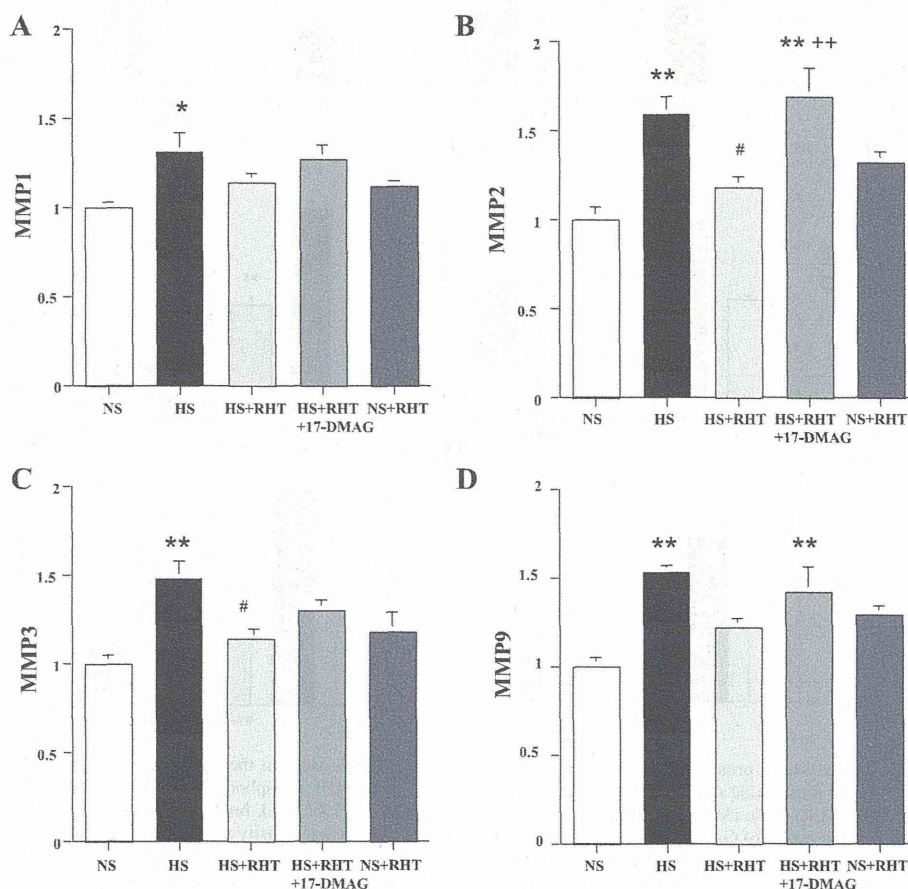

Fig. 3. Cardiac matrix metalloproteinase (MMP) activity in each group. A: MMP-1, B: MMP-2, C: MMP-3, D: MMP-9 in the HS group were higher than the levels in the NS group. The increases in MMP-2, -3, and -6 were suppressed in the HS+RHT group. The activities of MMPs were expressed as a relative ratio to that in the NS group. *P < 0.05, **P < 0.01 vs. the NS group. #P < 0.05 vs. the HS group. ++P < 0.01 vs. the HS+RHT group.
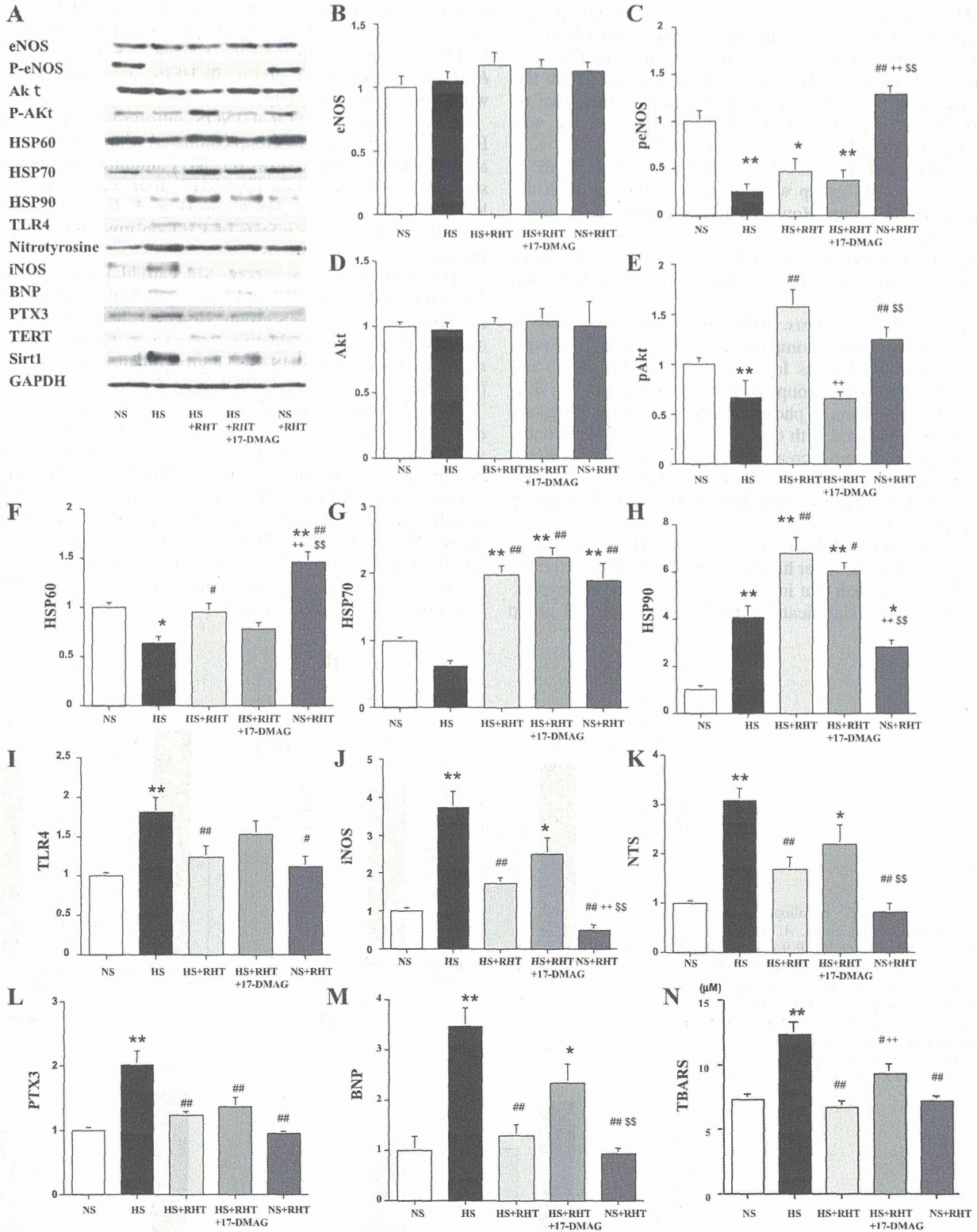
Fig. 4. Analysis of protein expression by Western blotting or ELISA assay in the hearts in each group. Representative data and summarized results (*A*) for endothelial nitric oxide synthase (eNOS, *B*), phosphor-eNOS (*C*), Akt (*D*), phosphor-Akt (*E*), heat shock protein (HSP) 60 (*F*), HSP70 (*G*), HSP90 (*H*), Toll-like receptor (TLR)-4 (*I*), iNOS (*J*), nitrotyrosine (NTS) (*K*), pentraxin (PTX) 3 (*L*), brain natriuretic peptide (*M*), thiobarbituric acid reactive substances (TBARS, *N*). The expression of GAPDH was used as an internal control. The average density value of each protein was expressed as a relative ratio to that in the NS group normalized by GAPDH (*B–M*). The levels of myocardial TBARS are expressed in *N*. *$P < 0.05$, **$P < 0.01$ vs. the NS group. #$P < 0.05$, ##$P < 0.01$ vs. the HS group. +$P < 0.01$ vs. the HS+RHT group. $$$P < 0.01$ vs. the HS+RHT+17-DMAG group.
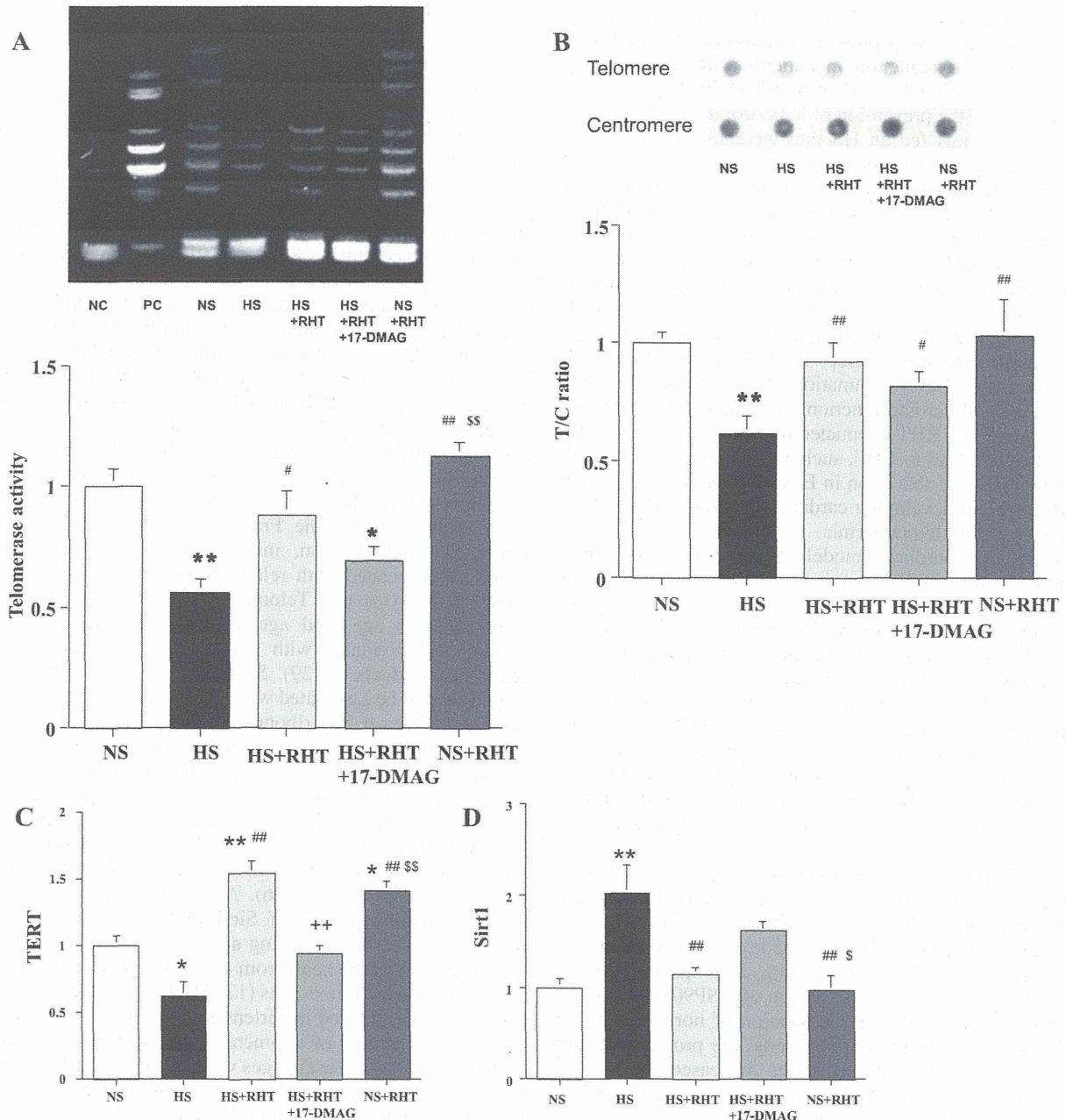
Fig. 5. Telomerase activity in the hearts in each group. Telomerase activity was normalized by that in the NS group (A). PC, positive control; NC, negative control. The length of telomeric DNA in the heart, as assessed by dot-blot analysis, is presented as the telomeric-to-centromeric DNA content (T/C) ratio, which was normalized by that in the NS group (B). The protein levels for telomere reverse transcriptase (TERT) and Sirt1 in the heart were determined by Western blot analysis (C, D). The expressions of proteins are expressed as a relative ratio to that in the NS group normalized by GAPDH. $*P < 0.05$, $**P < 0.01$ vs. the NS group. $\#P < 0.05$, $\#\#P < 0.01$ vs. the HS group. $+P < 0.01$ vs. the HS+RHT group. $\$P < 0.05$, $\$\$P < 0.01$ vs. the HS+RHT+17-DMAG group.

group compared with that in the NS group, but this attenuation was reversed by RHT. Although the level of Sirt1 was significantly increased in the HS group compared with the NS group, this increase was not observed in the HS+RHT group (Fig. 5, C and D).

DISCUSSION

The novel findings of this study are that salt-induced cardiac hypertrophy causes increased cardiac inflammation, shortening

of telomere length, and reduced TERT and telomerase activity. Furthermore, RHT prevents the development of cardiac remodeling, suppresses cardiac inflammation and oxidative stress, and preserves telomere length. To the best of our knowledge, this is the first study that shows RHT attenuates the progression of cardiac hypertrophy, which can lead to DHF.

Hyperthermia, cardiac hypertrophy, and ventricular function. Warm water immersion increases cardiac output and stroke volume and decreases systemic vascular resistance (18, 35).

Tei and coworkers (11, 13, 14) demonstrated that RHT using dry sauna increases eNOS protein in hamsters with CHF and improves cardiac dysfunction in patients with heart failure. Therefore, a reduction in cardiac afterload by hyperthermia may contribute to the prevention of hypertrophy. In the present study, when DS rats fed an HS diet were subjected to brief daily episodes of mild hyperthermia, the increase in BP and cardiac hypertrophy were attenuated. However, when mice fed an HS diet were received hydralazine to adjust similar BP to that in HS+RHT group, all of the preventive changes that were seen in the HS+RHT group including cardiac hypertrophy and protein expression compared with the HS group could not be inhibited only by BP adjustment. Therefore, the treatment with RHT may perform the cardioprotective effects beyond BP control. In the NS+RHT group, RHT increased HSPs with decreasing myocardial inflammation. Although the additional effects on normal cardiac function and remodeling were recognized apparently, RHT attenuated the changes in ventricular function that can lead to CHF, such as an increase in LVEDP, reduction in FS, and reduction in E/A ratio against the burden of an HS diet and developing cardiac hypertrophy beyond the reduction in BP by hyperthermia.

MMP activity. Cardiac remodeling results from an imbalance between the synthesis and degradation of extracellular matrix proteins and plays a key role in the pathophysiology of heart failure (12). Therefore, MMPs are essential in extracellular matrix degradation (19). In the failing heart, there are increased levels of MMPs, including -2, -3, and -9 (32, 33). Furthermore, the activation of MMP-2 was found to accompany progressive myocardial fibrosis, and MMP-9 was activated in rat heart failure (20, 27). Our data suggested that MMP-1,-2,-3, and -9 were all increased by salt-induced hypertension. However, RHT attenuated the activation of MMPs, which may be involved in the improvement of cardiac remodeling.

HSP. HSP70 and 72 have cytoprotective properties against ischemia/reperfusion injury in vitro and in vivo (12, 22), and HSP90 upregulates eNOS activity in vivo (25, 30).

In pacing-induced CHF in dogs, the levels of HSP60 and 70 rapidly increased by 24 h after pacing, although HSP90 was still low. On the other hand, induction of HSP90 was overt at 2 wk after pacing despite the restoration of normal levels of HSP60 and 70 (4). In the present study, the protein levels of HSP60 and 70 were only modestly decreased after 4 wk of hypertension, despite a rather dramatic increase in HSP90. Importantly, all three HSPs were significantly increased by RHT. Therefore, HSPs induced by hyperthermia may protect against cardiac injury and oxidative stress. HSP60 and 70 are possible ligands for TLR4 (1, 21). Exogenous HSPs including microbial HSPs as foreign bodies and extracellular HSPs released from injured cell activate TLR4 signaling. However, endogenous HSP induced by heat shock and drug have cardioprotective effects against myocardial injury (12, 22). RHT suppressed myocardial inflammation, which activated TLR4 induction, resulting in decreased expression of TLR4.

In this study, HSP90 is involved in the effects of RHT partially as 17-DMAG diminished the effect of RHT slightly.

Oxidative stress and inflammation. Evidence from experimental models of heart failure show that oxidative stress is increased in the failing heart and contributes to the pathophysiology of heart failure (5). iNOS, oxidative stress, and nitrotyrosine, which is the footprint of peroxynitrite, were induced by inflammation caused by heart failure (23).

TLR-4 expression is upregulated in patients or animal models with cardiac dysfunction (7, 24) and thought to be a toll gate to the various inflammatory responses to immunological stress. In this study, upregulated signaling of TLR-4 was suppressed by RHT.

PTX-3, which is a prototypic member of the long pentraxin family, is structurally related to, but distinct from the classic short pentraxin, C-reactive protein or serum amyloid proteins. PTX3 is produced by a variety of cell types, including monocytes/macrophages, vascular endothelial cells, vascular smooth muscle cells, adipocytes, fibroblasts, and dendritic cells in response to primary inflammatory stimuli (10). The plasma levels of PTX3 are increased in patients with CHF (15).

Our data suggest that elevated TLR4, iNOS, TBARS, nitrotyrosine, PTX3, and BNP in salt-induced hypertension were prevented by RHT. The beneficial effects of hyperthermia might be attributable in part to a reduction in myocardial oxidative stress in addition to a decrease in myocardial inflammation, which resulted in preserved cardiac function.

Telomere biology. The Framingham heart study demonstrates that hypertension, insulin resistance, and oxidative stress are associated with relatively short telomere length in human leukocytes (3). Telomere length was found to vary inversely with age, and age-adjusted telomere length was shorter in individuals with cardiovascular disease than in healthy individuals (9, 29). Shortening of the telomeres has been shown to be associated with an increased rate of mortality from heart disease. The ribonuclear complex telomerase is the central component of the telomere complex. The enzyme complex of telomerase contains a catalytic subunit, TERT. In isolated cells, TERT gene transfer reduces replicative senescence and extends the lifespan of numerous cell types including cardiomyocytes (2). Thus, telomere-associated proteins are thought to be important for the regulation of cardiac muscle cell growth and survival (26). A recent study suggested that moderate overexpression of Sirt1 protects the heart against oxidative stress. The antiaging and stress-resistance effects of Sirt1 may protect the heart from oxidative stress by facilitating the expression of antioxidants (17, 34). In the present study, we found that salt-induced hypertension caused telomere shortening, reduced activity of telomerase, and decreased expression of TERT and that these changes were prevented by RHT. In the present study, telomerase activity was inhibited and downregulated in the HS group compared with that in the NS group. However, RHT led to a recovery of the expression of TERT and preservation of the length of telomeric DNA.

Limitations

There are few limitations of this study. First, systemic BP was reduced by RHT. However, the lowering BP did not improve the cardiac remodeling, function, and protein expressions in the group in which BP was adjusted by hydralazine (HS+Hy) to the extent to that in the HS+RHT group in the supplemental study. As the whole effects of RHT contain not only the slight BP-lowering effect but also a preventive effect of increasing BP probably due to increased eNOS by increased shear stress and HSP, the combined mechanisms of RHT including the above-mentioned prevent cardiac hypertrophy in

Dahl hypertensive rat. Second, Dahl hypertensive rats receiving the HS diet developed cardiac hypertrophy but still preserved cardiac systolic function at the compensated stage in this study. The follow-up period was not enough for CHF to develop, and thus we could not show a beneficial effect of RHT on the development of systolic CHF sufficiently. Further longer observation would be needed. However, it is very likely that hyperthermia would reduce mortality in this experimental model.

In conclusion, RHT attenuates the development of cardiac hypertrophy and fibrosis and preserves telomerase, TERT activity, and the length of telomere DNA in salt-induced hypertensive rats through activation of eNOS and induction of HSPs.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.-i.O. and N.M. conception and design of research; J.-i.O. and M.S. performed experiments; T.M. interpreted results of experiments; Y.H. prepared figures; K.N. edited and revised manuscript; N.M. approved final version of manuscript.

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